Protective Role of Alpha Lipoic Acid Against Disorders Induced by Gamma Radiation

Thesis
Submitted in Partial Fulfillment of the Requirements for the Award of Degree of Master in Science (Zoology).

Presented by
Khalid Nady Mohammed Abd El Azeem
B.Sc., Zoology Department,
Faculty of Science, Minia University

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Protective Role of Alpha Lipoic Acid Against Disorders Induced by Gamma Radiation

THESIS ADVISORS

Prof. Wafaa M. Zahran
Prof. of Immunohistology,
Faculty of Science,
Minia University

Prof. Soad Abdel Salam
Prof. of Physiological Biochemistry,
National Center for Radiation Research and Technology,
Atomic Energy Authority.

Prof. Mahmud H. Ayoub
Prof. of physiology,
Faculty of Medicine,
Ain Shams University.

THESIS APPROVED
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(سَنَّرِيْهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ أَوَّلَمْ يَكْفُ بَرِيَّكَ أَنَّهُ عَلَى كُلِّ شَيْءٍ شَهِيدٌ

سورة فصلت الآية (53)

(وَفِي أَنفُسِكُمْ أَفَلا تَبْصِرُونَ

سورة الذاريات الآية (21)

صدق الله العظيم
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*Khalid Nady Mohamed Abd El Azeem*
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<tr>
<td>ALA</td>
<td>Alpha lipoic acid</td>
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<td>ALAT or ALT</td>
<td>Alanine aminotransferase</td>
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<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>ATP</td>
<td>Adenosine tri-phosphate</td>
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<td>CK-MB</td>
<td>Creatine kinase</td>
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<td>CPK</td>
<td>Creatine phosphokinase</td>
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<td>DHLA</td>
<td>Dihydrolipoic acid</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>EDTA</td>
<td>Ethylene Diamine Tetraacetic acid</td>
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<td>Food and drug administration</td>
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<td>GPx</td>
<td>Glutathione peroxidase</td>
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<td>Gray</td>
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<td>H&amp;E</td>
<td>Haematoxyline and Eosin</td>
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<td>H, hydrogen radical</td>
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<tr>
<td>H⁺, hydrogen ion</td>
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<tr>
<td>H₂O₂, hydrogen peroxide</td>
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<tr>
<td>IU/L</td>
<td>International unit/liter</td>
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<td>LD₅₀</td>
<td>Lethal dose</td>
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<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<td>LFTs or LF₅₀</td>
<td>Liver function tests</td>
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<td>MTS</td>
<td>Mallory's Tripple Stain</td>
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<tr>
<td>NCRRT</td>
<td>National Center for Radiation Research and Technology</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>OH⁻, hydroxyl radical</td>
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<tr>
<td>PAS</td>
<td>Periodic acid Schiff's</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
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<tr>
<td>PUN</td>
<td>Plasma urea nitrogen</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>Description</td>
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<tr>
<td>RNS</td>
<td>reactive nitrogen species</td>
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<td>Alkoxyl radicals</td>
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<td>UV</td>
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Fig. (58): Electron micrograph of hepatocyte of albino rat irradiated with 2 Gy after 3 days from the exposure showing degeneration in cytoplasmic components of the hepatocyte and active lysosome (arrow) near the dilated bile canal (Bc). Also, rough endoplasmic reticulum (rER) was still seen (X 2600).

Fig. (59): Electron micrograph of section of a hepatocyte of albino rat irradiated with 6 Gy after 3 days from the exposure showing swollen spaces of degenerated mitochondria (SM) and dilated
blood sinusoid (BS) in between 5 parts of hepatocytes and fragmentation of endoplasmic reticulum (arrow). (X 1600).

Fig. (60): Electron micrograph of a binucleated hepatocyte of albino rat treated with ALA for 3 days post irradiation (2 Gy), showing regeneration of mitochondria (M). The bile canal was observed (Bc). (X 2600).

Fig. (61): Electron micrograph of a hepatocyte in liver of albino rat treated with ALA for 3 days post irradiation (6 Gy), showing regenerated nucleus (N), rough endoplasmic reticulum (rER) and elongated mitochondria (M). The bile canal (Bc) and active lysosome (arrows) were observed. (X 2000).

Fig. (62): Electron micrograph of a binucleated hepatocyte of pre-irradiated ALA rat after 2 Gy, rich with mitochondria (M) and regenerated bile canals (Bc). Desmosomes can be also seen (arrow). (X 2000).

Fig. (63): Electron micrograph of a hepatocyte in liver of pre-irradiated ALA rat after 6 Gy, showing active nucleus (N) with peripheral nucleolus and numerous electron dense mitochondria (M). Lipid droplets were observed (L). (X 2000).

Fig. (64). A photomicrograph of a section in the cortex of the kidney of a normal control rat, illustrating the normal appearance of Bowman's capsule (thin arrows), urinary space (thick arrows) and glomerular tuft (g). Note the proximal convoluted tubules (pt) and distal convoluted tubules (dt) (H&E., x 400).

Fig. (65). A photomicrograph of a section in the medulla of the kidney of a normal control rat, illustrating the normal appearance of renal tubules. Note the ascending (al) and the descending (dl) loop of Henel (H&E., x 400).

Fig. (66). A photomicrograph of the cortex of the kidney of an ALA treated rat, showing the proximal convoluted tubules (pt), distal convoluted tubules (dt), Bowman's capsule (thin arrows), urinary space (thick arrows) and glomerular tuft (g) (H&E., x 400).
Fig. (67). A photomicrograph of the medulla of the kidney of an ALA treated rat, showing the medullary tubules (the ascending (al) and the descending (dl) loop of Henel) and few pyknotic nuclei can be also detected (arrows) (H&E., x 400).

Fig. (68). A photomicrograph of a section in the cortex of the kidney of a 1st irradiated rat (1st 2 Gy), showing ruptured wall of Bowman's capsule, obvious shrunken glomerular tuft (g) as well as the widened urinary space (thick arrows) and Cellular damaged of both distal (dt) and proximal (pt) tubules were also seen. Note also some fibroblasts (arrow head) and few pyknotic nuclei (thin arrows) (H&E., x 400).

Fig. (69). A photomicrograph of the medulla of the above mentioned section, showing pale cytoplasm in both ascending (al) and descending (dl) loop of Henel. Interstitial hemorrhage (arrows) and pyknotic nuclei (arrow heads) were detected (H&E., x 400).

Fig. (70). A photomicrograph of the cortex of the kidney of a 2nd irradiated rat (2nd 2 Gy), showing pale cytoplasm, some cells of renal tubule (pt & dt) lost their nuclei and others with pyknotic ones (thin arrows). The glomeruli appeared swollen with diffused and fragmented tufts (g) and congested with blood. Interstitial hemorrhages (arrow heads) were also detected surrounding Bowman's capsule (H&E., x 400).

Fig. (71). A photomicrograph in the medulla of the above mentioned section, showing cells of medullary tubules (al & dl) with degenerated cytoplasm, pyknotic nuclei (thin arrows), dilation of ascending (al) and descending (dl) loop of Henel. Also, signs of interstitial hemorrhage (thick arrows) were detected (H&E., x 400).

Fig. (72). A photomicrograph of the cortex of the kidney of a 3rd irradiated rat (3rd 2 Gy), showing damaged cellular wall of the proximal (pt) and distal (dt) tubules. The glomeruli showed obvious degree of shrinkage with rupture glomerular tufts (g) and dilated urinary spaces (thick arrows). Pyknotic nuclei were detected (thin arrows) (H&E., x 400).
Fig. (73). A photomicrograph of the medulla of the previous section, showing most cellular wall of both ascending (al) and descending (dl) loop of Henel with degenerated cytoplasm (thick arrows) and many pyknotic nuclei (thin arrows) (H&E., x 400).

Fig. (74). A photomicrograph of the cortex of a kidney of rat left for one month after 3rd 2 Gy, showing the degenerated renal tubules. Swollen and fragmented glomerular tufts (g) as well as widened urinary space (thick arrows) were also detected (H&E., x 400).

Fig. (75). A photomicrograph of the medulla of the above mentioned section, showing most cells of the ascending (al) and descending (dl) loop of Henel with degenerated cytoplasm (thick arrows), pyknotic nuclei (thin arrows) (H&E., x 400).

Fig. (76). A photomicrograph of the cortex of the kidney of a 1st simultaneously irradiated ALA rat, showing most proximal (pt) and distal (dt) tubules with destructed cellular wall and a glomerulus with fragmented tufts (g) (H&E., x 400).

Fig. (77). A photomicrograph of the medulla of the above section, showing many ascending (al) and descending (dl) loop of Henel having pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).

Fig. (78). A photomicrograph of the cortex of the kidney of a 2nd simultaneously irradiated ALA rat, showing nearly normal renal tubules, glomerulus (g) and narrow urinary space (thick arrows). Both types of renal tubules (pt & dt) had narrow lumen filled with amyloid threads (H&E., x 400).

Fig. (79). A photomicrograph of the medulla of the above section, showing the ascending (al) and descending (dl) loop of Henel with few pyknotic nuclei (arrows) (H&E., x 400).

Fig. (80). A photomicrograph of the cortex of the kidney of a 3rd simultaneously irradiated ALA rat, showing shrunken and fragmented glomerular tufts (g) and widened urinary space (thick arrows). Some pyknotic nuclei in the damaged wall of renal tubules were also present (thin arrows) (H&E., x 400).
Fig. (81). A photomicrograph of the medulla of the above section, showing some cells in ascending (al) and descending (dl) loop of Henel with pyknotic nuclei (arrows) (H&E., x 400).

Fig. (82). A photomicrograph of the cortex of the kidney of a simultaneously irradiated ALA rat left for one month after 3rd 2 Gy, showing the swollen glomerulus (g). Many pyknotic nuclei (thin arrows) and fibroblasts (arrow heads) were present (H&E., x 400).

Fig. (83). A photomicrograph of the medulla of the above section, showing the ascending (al) and descending (dl) loop of Henel with pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).

Fig. (84). A photomicrograph of the cortex of the kidney of a 1st pre irradiated ALA rat, showing few nearly normal renal tubules, fibroblasts (thin arrow) and interstitial hemorrhage (thick arrows). Glomerulus was also congested with blood (g) (H&E., x 400).

Fig. (85). A photomicrograph of the medulla of the above section, showing few ascending (al) and descending (dl) loop of Henel with pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).

Fig. (86). A photomicrograph of the cortex of the kidney of a 2nd pre irradiated ALA rat, showing damaged and ill-defined renal tubules. The glomerulus was slightly shrunken (g) (H&E., x 400).

Fig. (87). A photomicrograph of the medulla of the above section, showing both ascending (al) and descending (dl) loop of Henel with many pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).

Fig. (88). A photomicrograph of the cortex of the kidney of a 3rd pre irradiated ALA rat, showing the fragmented and vacuolated glomerulus (g). Some pyknotic nuclei (arrows) and destructed renal tubules (pt & dt) were detected (H&E., x 400).
Fig. (89). A photomicrograph of the medulla of the above section, showing both ascending (al) and descending (dl) loop of Henel with some pyknotic nuclei (thin arrows). Interstitial hemorrhage was detected (thick arrows) (H&E., x 400).

Fig. (90). A photomicrograph of the cortex of the kidney of a pre irradiated ALA rat left for one month after 3rd 2 Gy, showing obvious shrunken and vacuolated glomerulus (g). Damaged renal tubules with many pyknotic nuclei were observed (thin arrows) (H&E., x 400).

Fig. (91). A photomicrograph of the medulla of the above section, showing some widened ascending (al) and descending (dl) loop of Henel with many pyknotic nuclei (arrows) (H&E., x 400).

Fig. (92). A photomicrograph of the cortex of the kidney of a normal control rat, showing the little amount of collagenous fibers around the renal tubules and the wall of Bowman's capsule (thin arrows). A moderate amount of these fibers was detected in the glomerular tuft (thick arrows) (MTS., x 400).

Fig. (93). A photomicrograph of the medulla of the previous section, showing little amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (94). A photomicrograph of the cortex of the kidney of an ALA treated rat, showing little increase in the amount of collagenous fibers around the renal tubules and glomerular tuft (arrows) (MTS., x 400).

Fig. (95). A photomicrograph of the medulla of the previous section, showing slight increase in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (96). A photomicrograph of the cortex of the kidney of a 1st irradiated rat (1st 2 Gy), showing obviously increase in the amount of collagenous fibers around the renal tubules and inside glomerular tuft (arrows). (MTS., x 400).
Fig. (97). A photomicrograph of the medulla of the previous section, showing little increase in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (98). A photomicrograph of the cortex of the kidney of a 2nd irradiated rat (2nd 2 Gy), showing the obviously decreased amount of collagenous fibers around the renal tubules and inside the glomerular tuft (arrows) (MTS., x 400).

Fig. (99). A photomicrograph of the medulla of the previous section, showing the rare amount of collagenous fibers except, little amount around some ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (100). A photomicrograph of the cortex of the kidney of a 3rd irradiated rat (3rd 2 Gy), showing the marked decrease in the amount of collagenous fibers around the renal tubules and inside the glomerulus (arrows). (MTS., x 400).

Fig. (101). A photomicrograph of the medulla of the previous section, showing a slight reincrease in the amount of collagenous fibers around some ascending (al) and descending (dl) loop of Henel (arrows). Interstitial hemorrhage was prominently seen (MTS., x 400).

Fig. (102). A photomicrograph of the cortex of the kidney of irradiated rat left for one month after 3rd 2 Gy, showing the increased amount of collagenous fibers around the renal tubules as well as glomerular tuft (arrows). (MTS., x 400).

Fig. (103). A photomicrograph of the medulla of the previous section, showing obvious increase in the amount of collagenous fibers around some ascending (al) and descending (dl) loop of Henel (thin arrows). Interstitial blood cells and amyloid substances (thick arrows) can be seen (MTS., x 400).
Fig. (104). A photomicrograph of the cortex of the kidney of a 1st simultaneously irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules and the glomerular tufts (arrows) (MTS., x 400).

Fig. (105). A photomicrograph of the medulla of the previous section, showing moderate increased in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (106). A photomicrograph of the cortex of the kidney of a 2nd simultaneously irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules that was also increased in the glomerular tufts (arrows) (MTS., x 400).

Fig. (107). A photomicrograph of the medulla of the previous section, showing moderate increased in the amount of collagenous fibers around the ascending (al) and descending loop of Henel (dl) (arrows) (MTS., x 400).

Fig. (108). A photomicrograph of the cortex of the kidney of a 3rd simultaneously irradiated ALA rat, showing the obviously increased amount of collagenous fibers around the renal tubules and glomerular tufts (arrows) (MTS., x 400).

Fig. (109). A photomicrograph of the medulla of the previous section, showing obviously the increased amount of collagenous fibers around the ascending (al) and descending loop of Henel (dl) (arrows) (MTS., x 400).

Fig. (110). A photomicrograph of the cortex of the kidney of simultaneously irradiated ALA rat left for one month after 3rd 2 Gy, showing the little amount of collagenous fibers around the renal tubules (arrows) and rarely detected in glomerular tufts (MTS., x 400).

Fig. (111). A photomicrograph of the medulla of the previous section, showing the obviously increased amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).
Fig. (112). A photomicrograph of the cortex of the kidney of a 1st pre irradiated ALA rat, showing the moderate amount of collagenous fibers around the renal tubules that was much increased in the shrunken glomerular tufts (arrows) (MTS., x 400).

Fig. (113). A photomicrograph of the medulla of the previous section, showing little amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (114). A photomicrograph of the cortex of the kidney of a 2nd pre irradiated ALA rat, showing the reincrease in the amount of collagenous fibers around the renal tubules and glomeruli (arrows) (MTS., x 400).

Fig. (115). A photomicrograph of the medulla of the previous section, showing the increase in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (116). A photomicrograph of the cortex of the kidney of a 3rd pre irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules and in the glomerular tufts (arrows) (MTS., x 400).

Fig. (117). A photomicrograph of the medulla of the previous section, showing the decreased amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (118). A photomicrograph of the cortex of the kidney of pre irradiated ALA rat left for one month after 3rd 2 Gy, showing increased amount of collagenous fibers around the renal tubules that was obviously seen in the glomerular tufts (arrows) (MTS., x 400).

Fig. (119). A photomicrograph of the medulla of the previous section, showing obviously increased in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).
Fig. (120). A photomicrograph of the cortex of the kidney of a normal control rat, showing the normal distribution of carbohydrate contents in renal tubules and glomerulus (arrows) (PAS., x 400).

Fig. (121). A photomicrograph of the cortex of the kidney of an ALA treated rat, showing moderate amount of carbohydrates in both basement membranes and brush borders of the renal tubules as well as in the glomerular tufts (arrows) (PAS., x 400).

Fig. (122). A photomicrograph of the cortex of the kidney of a 1st irradiated rat, showing that carbohydrate contents were reincreased in glomerulus and the basement membrane of renal tubules (arrows) (PAS., x 400).

Fig. (123). A photomicrograph of the cortex of the kidney of a 2nd irradiated rat, showing slight decrease of carbohydrate contents in both glomerulus and basement membranes of renal tubules (arrows) (PAS., x 400).

Fig. (124). A photomicrograph of the cortex of the kidney of a 3rd irradiated rat, showing obvious increase of carbohydrates at the glomerulus and basement membranes of renal tubules (arrows). Note the slightly decrease in the inner brush borders (PAS., x 400).

Fig. (125). A photomicrograph of the cortex of the kidney of an irradiated rat left for one month after 3rd 2 Gy, showing obviously increased amount of carbohydrates in most renal tubules as well as the glomerulus (arrows) (PAS., x 400).

Fig. (126). A photomicrograph of the cortex of the kidney of a 1st simultaneously irradiated ALA rat, showing obvious increase of carbohydrates at both glomerulus and most renal tubules (arrows) (PAS., x 400).

Fig. (127). A photomicrograph of the cortex of the kidney of a 2nd simultaneously irradiated ALA rat, showing the increase of carbohydrate contents in glomerulus and renal tubules (arrows) (PAS., x 400).
Fig. (128). A photomicrograph of the cortex of the kidney of a 3rd simultaneously irradiated ALA, showing the increase of carbohydrates in glomerulus and inner borders of renal tubules as well as basement membrane (arrows) (PAS., x 400).

Fig. (129). A photomicrograph of the cortex of the kidney of a simultaneously irradiated ALA rat left for one month after the 3rd 2 Gy, showing obviously increase of carbohydrates in the renal tubules (arrows). The high affinity to the stain in basement membranes and brush borders can be seen (PAS., x 400).

Fig. (130). A photomicrograph of the cortex of the kidney of a 1st pre irradiated ALA rat, showing the obvious increase of carbohydrate contents (arrows) (PAS., x 400).

Fig. (131). A photomicrograph of the cortex of the kidney of a 2nd pre irradiated ALA rat, showing the increased amount of carbohydrate contents (arrows) (PAS., x 400).

Fig. (132). A photomicrograph of the cortex of the kidney of a 3rd pre irradiated ALA rat, showing the obviously increased amount of carbohydrates (arrows) (PAS., x 400).

Fig. (133). A photomicrograph of the cortex of the kidney of pre irradiated ALA rat left for one month after 3rd 2 Gy, showing the increase of carbohydrate contents in glomerulus and most renal tubules (arrows) (PAS., x 400).

Fig. (134): Electron micrograph of a part of a normal glomerulus of albino rat’s kidney showing podocytes (P), foot process (arrows) and glomerular basement membrane. (X 2000).

Fig. (135): Electron micrograph of a part of the apical region of proximal convoluted tubule of albino rat’s kidney, showing normal cristae of mitochondria (M) and brush border (BB). Lipid droplets can be seen (L). (X 2600).

Fig. (136): Electron micrograph of a part of glomerular tuft of albino rat’s kidney irradiated with 2 Gy after 3 days from the exposure, showing complete degeneration of foot processes (arrows). (X 1300).
Fig. (137): Electron micrograph of a part of a proximal convoluted tubule of albino rat’s kidney irradiated with 2 Gy after 3 days from the exposure, showing ruptured brush border (BB) and many mitochondria with ruptured cristae (M). (X 1600).

Fig. (138): Electron micrograph of a part of a proximal convoluted tubule of albino rat’s kidney irradiated with 6 Gy (total) after 3 days from the exposure, showing ruptured endoplasmic reticulum (ER) and many mitochondria with ruptured cristae (M). (X 2000).

Fig. (139): Electron micrograph of a part of a glomerular tuft of albino rat’s kidney treated with ALA for 3 days post irradiation (2 Gy), showing 3 capillary (c) were observed contain RBCs. Note the reappearance of foot processes (arrow). (X 1300).

Fig. (140): Electron micrograph of a part of glomerular tuft of post-irradiated ALA rat’s kidney after 6 Gy, showing normal podocytes (P) with normal foot processes (arrow). (X 2000).

Fig. (141): Electron micrograph of a part of apical region of a proximal convoluted tubule of post-irradiated ALA rat’s kidney after 6 Gy exposure, showing many collapsed mitochondria (M) and some vacuolated areas in cytoplasm (arrows). (X 1300).

Fig. (142): Electron micrograph of a part of the apical region of a proximal convoluted tubule of pre-irradiated ALA rats kidney after 2 Gy, showing many elongated mitochondria (M) and nucleus (N) with heterochromatin restricted at the nuclear membrane. (X 1600).

Fig. (143): Electron micrograph of a part of a glomerular tuft of pre-irradiated ALA rat’s kidney after 6 Gy, showing epithelial cells lining Bowman’s capsule with basal nucleus, mesangial cell (m) and regenerated foot process (arrows). (X 660).

Fig. (144): Electron micrograph of a part of the apical region of a proximal convoluted tubule of pre-irradiated ALA rat’s kidney after 6 Gy, showing regenerated epithelial cells lining the proximal convoluted tubule rich with elongated mitochondria (M). (X 660).
Fig. (145). A photomicrograph of the cardiac muscle of a control rat, showing branching and anastomosing cardiac muscle fibers with central vesicular nucleus (thin arrows) and interstitial connective tissue spaces (thick arrows) (H&E x 400).

Fig. (146). A photomicrograph of the cardiac muscle of an ALA treated rat, showing branching and anastomosing cardiac muscle fibers with central vesicular nucleus (arrows) (H&E x 400).

Fig. (147). A photomicrograph of the cardiac muscle of a 1st irradiated rat, showing degenerated of cardiac muscle fibers losing striation (thin arrows) and widened connective tissue spaces infiltrated with many pyknotic nuclei (thick arrows) (H&E x 400).

Fig. (148). A photomicrograph of the cardiac muscle of a 2nd irradiated rat, showing infarction of the striated muscle and prominent changes in the cardiac muscle fibers including loss of striation (thin arrows) and appearance of many connective tissue spaces (thick arrows) (H&E x 400).

Fig. (149). A photomicrograph of the cardiac muscle of a 3rd irradiated rat, showing the marked infarction of cardiac muscle fibers (arrows) (H&E x 400).

Fig. (150). A photomicrograph of the cardiac muscle of an irradiated rat left for one month after 3rd 2 Gy, showing a slight improved picture, however infarct muscle fibers, pyknotic nuclei and widened connective tissue spaces were still apparent (arrows) (H&E x 400).

Fig. (151). A photomicrograph of the cardiac muscle of a 1st Simultaneously irradiated ALA rat, showing slight improvement of the cardiac muscle fibers striation, although infarction of muscle was still seen (arrows) (H&E x 400).

Fig. (152). A photomicrograph of the cardiac muscle of a 2nd Simultaneously irradiated ALA rat, showing the improvement of the cardiac muscle fibers architecture. Some pyknotic nuclei and many widened connective tissue spaces can be seen (arrows) (H&E x 400).

Fig. (153). A photomicrograph of the cardiac muscle of a 3rd Simultaneously irradiated ALA rat, showing the infarct cardiac muscle fibers with many blood cells in between the myocardial bundles (arrows) (H&E x 400).
Fig. (154). A photomicrograph of the cardiac muscle of a Simultaneously irradiated ALA rat left for one month after 3rd 2 Gy, showing slight recovery in cardiac muscle fibers architecture, although many pyknotic nuclei were still seen (arrows) (H&E x 400).

Fig. (155). A photomicrograph of the cardiac muscle of a 1st pre irradiated ALA rat, showing the slight improved structure of the cardiac muscle fibers (arrows) (H&E x 400).

Fig. (156). A photomicrograph of the cardiac muscle of a 2nd pre irradiated ALA rat, showing the slight improved picture of cardiac muscle fibers (arrows) (H&E x 400).

Fig. (157). A photomicrograph of the cardiac muscle of a 3rd pre irradiated ALA rat, showing the marked infarction of cardiac muscle fibers (arrows) (H&E x 400).

Fig. (158). A photomicrograph of the cardiac muscle of a pre irradiated ALA rat left for one month after 3rd 2 Gy, showing slight recovery in the architecture of cardiac muscle fibers (arrows) (H&E x 400).
ABSTRACT

Ionizing radiation interacts with living cells, causing a variety of biochemical changes depending on exposed and absorbed doses, duration of exposure, interval after exposure and susceptibility of tissues to ionizing radiation. So, it may increase the oxidative stress and damage of body organs. Alpha-lipoic acid (ALA—also known as thioctic acid) appears to be readily absorbed from an oral dose and converts easily to its reduced form, dihydrolipoic acid (DHLA), in many tissues of the body. ALA can neutralize free radicals in both fatty and watery regions of cells.

The present study has been designed to evaluate the possible efficiency of ALA as antioxidant and radio-protector against radiation induced oxidative stress in different organs (liver, kidney and heart) in rats through estimation of the activity of markers of serum liver, kidney and heart function, in addition to the histopathological differentiation of these organs by light and electron microscope.

Five equal groups were conducted for the study: control, ALA (30 mg/kg body wt), irradiated (each rat was exposed to 6 Gy as a fractionated dose of gamma (γ) radiation), irradiated plus ALA (each rat received ALA for 9 days simultaneously during exposure) and ALA plus irradiation plus ALA groups (each rat received ALA for a week pre-exposure plus 9 days during exposure). Radiation doses were fractionated dose levels of 2 Gy each 3 days to reach accumulative dose of 6 Gy. After 3 days of each exposure rats were sacrificed, except, those left for recovery test one month after last exposure.

The results revealed that whole body γ-irradiation of rats induces oxidative stress in liver, kidney and heart obviously manifested by significant elevation in alanine and aspartate transaminase (ALT & AST), alkaline phosphatase (ALP), urea, creatinine and creatine kinase (CK-MB). ALA treated-irradiated rats showed lower
significantly values indicating remarkable improvement in all measured parameters and histopathological alterations, compared to irradiated rats.

**In conclusion,** the results demonstrated that ALA may play a protective role against the destructive effects induced by $\gamma$-radiation and reduced the biochemical and histopathological events of radiation sickness.

Key words: Alpha lipoic acid, $\gamma$-rays, Rats.
INTRODUCTION

Natural radiation exists in all regions of the world, although its levels vary geographically. Individuals have been exposed to this environmental radiation since the beginning of evolution\(^1\). Most natural ionizing radiation results from radon 22 and uranium 226 decay\(^2,3\). It has been reported that chromosomal aberrations and specific cancers are well known macroscopic results of radiation exposure\(^4\).

Ionizing radiation collides with molecules in living cells generating clusters of free radicals, known as reactive oxygen species (ROS), including free radicals (H\(^+\): hydrogen ion, H\(^-\): hydrogen radical, H\(_2\)O\(_2\): hydrogen peroxide, OH\(^-\): hydroxyl radical). These free radicals randomly damage cellular constituents, including DNA and react with almost all structural and functional organic molecules, including proteins and lipids\(^5,6,7\). OH\(^-\) induces peroxidation of unsaturated membrane fatty acids, forming peroxyl (ROO\(^-\)) and alkoxyl (RO\(^-\)) radicals and resulting in a loss of cellular compartmentation that leads to metabolic disturbances\(^8\).

According to Tawfik et al.\(^9\), all organisms (i.e., bacteria, plants, or animals, including humans) are exposed each day to variable amount of radiation and 81% of the amount received from radiation comes from natural sources. Such percent was calculated as: 55% from radon; 8% from cosmic radiation; 8% from rocks and soil; and 10% from internal exposure to radiation from the radioactive materials in food and water consumed in the daily diet. The remaining 19% of the daily exposure dose may originate from man-made sources such as medical X ray exposure (11%), nuclear medicinal exposure (4%), consumer products (3%), and other sources (<1%). This last category includes occupational sources, nuclear fallout, the nuclear fuel cycle radioactive waste, hospital radioactive waste, radioactively contaminated sites and other miscellaneous sources. So, Human
beings are constantly exposed to ionizing radiations from different sources and it is well established that such radiations cause lesions in various mammalian tissues and organs. Studies, strongly, suggest that oxidative stress from ionizing radiation exposure can trigger a cascade of events, including altered immune function, cellular transformation, and tissue damage. It should be noted that the effect of natural ionizing radiation on living cells is dependent on the level of radiation exposure. During the previous decade, statistically significant evidence has indicated that whole body exposure of humans to low doses of ionizing radiation stimulates immune function, decreases total cancer mortality rates, and increases longevity. On the other hand, high dose radiation depresses immune function, increases the incidence of cancer, and induces higher mortality rates.

Antioxidants have proved to decrease the damage effects of ionizing radiation. Humans are endowed with antioxidant defense systems that scavenge and minimize the formation of free radicals. Alpha-lipoic acid (ALA), also known as thioctic acid, was discovered in 1951 as a molecule that assists in acyl-group transfer and as a coenzyme in the Krebs cycle. In the 1980s, the scientific community realized alpha-lipoic acid is a powerful antioxidant in both its reduced and oxidized forms. Several qualities distinguish alpha-lipoic acid from other antioxidants that can be synthesized by animals and humans. It neutralizes free radicals in both the fatty and watery regions of cells, in contrast to vitamin C (water soluble) and vitamin E (fat soluble).
AIM OF THE WORK

This work is an attempt to study the effect of whole body exposure to a fractionated dose of gamma radiation on some parameters as well as the histological status of albino rats, *Rattus norvegicus*. This study was also planned to examine the possible ameliorative effects of administration of ALA on the radiation-induced biochemical disorders in liver (alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP)), kidney (urea and creatinine) and heart (creatine kinase (CK-MB)) functions. In addition, light and transmission electron-microscopic studies were carried out on liver, kidney and heart samples of rats to assess the histological alteration induced after gamma radiation exposure as well as the ameliorative effects of ALA.
REVIEW OF LITERATURE

The extensive use of atomic energy now-a-days in various branches of national economy (science and technology, biology, physiology and medicine) has made radiation injury an urgent problem attracting the attention not only of specialists in a variety of clinical disciplines but also of a vast army of theoretical scientists \(^\text{17}\).

Radiation

Radiation is defined as energy in transit and comprises electromagnetic rays; such as X-rays or gamma rays and particulate radiation such as neutrons, alpha particles and heavily charged ions. Radiation affects people by depositing energy in body tissues. The extent of the damage depends upon the total amount of energy absorbed, the time period and dose rate of exposure and the particular organ(s) exposed \(^\text{18}\).

According to Yarmonenko \(^\text{18}\), radiation may be non-ionizing or ionizing according to energy emitted.

*Non-ionizing radiation* has enough energy to excite molecules and atoms causing them to vibrate faster, which is obvious in a microwave oven, where the radiation causes water molecules to vibrate faster creating heat.

*Ionizing radiation* has more energy than non-ionizing radiation; enough to cause chemical changes by breaking chemical bonds. This effect can cause damage to living tissues. The ionizing radiations of primary concern are alpha and beta particles, gamma and x rays.

Different types of ionizing radiations cause similar kinds of damage but all cells in the living body do not respond to radiations to the same degree. Apparent differences in radio-sensitivity of different cell populations were recognized early in the history of radiobiology and a law was formulated that "The radio-sensitivity of cells is directly proportional to their reproductive activity and inversely proportional to their degree of differentiation." \(^\text{17}\).
Ionizing radiation is defined as a specific form of radiation that possesses sufficient energy to remove electrons from the atoms in the tissue that they penetrate. This process is called ionization and it is the reason for the name "ionizing radiation." When this energy is received in appropriate quantities and over a sufficient period of time it can result in tissue damage \(^{19}\).

According to Harikumar and Kuttan, \(^{20}\), radiation is used therapeutically for investigating different types of diseases and treatment of various types of malignancies. The severe side effects of radiotherapy result from the damage of normal cells. Rapidly dividing cells of gastrointestinal tract and haematopoietic system are more prone to radiation induced damage.

Radiation is known as a producer of reactive oxygen species (ROS). When water which constitutes around 80% of the cell is exposed to ionizing radiation, decomposition occurs through which a variety of ROS, such as the superoxide radical \(O_2^\cdot\), the hydrogen peroxide \((H_2O_2)\) and the hydroxyl radical \((OH)\) are generated \(^{21}\).

\[
\begin{align*}
H_2O \xrightarrow{\text{Rad}} & \quad HOH + e^- \quad \text{(ionization leads to ion pair)} \\
H_2O + e^- & \rightarrow HOH^* \\
HOH & \rightarrow H + OH^* \\
HOH^* & \rightarrow H + OH^* \\
2O_2^* + 2H & \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2 \\
2H_2O_2 & \rightarrow O_2 + 2H_2O
\end{align*}
\]

The emitted electron reacts with other water molecule.

The presence of oxygen leads to the formation of powerful oxidizers as \(H_2O_2\) \& \(HO_2\).

These ROS attack cellular molecules like DNA, RNA, proteins and membranes causing their dysfunction and damage. ROS increases membrane lipid
peroxidation, which in turn can alter the integrity of membrane structure leading to inactivation of membrane bound enzymes, loss of permeability of the membrane and decrease in membrane fluidity \(^{22}\).

\[\text{RH} \rightarrow \text{R} + \text{H}\]

\[\text{RH} + \text{OH} \rightarrow \text{R} + \text{H}_2\text{O}\]

The organic radical (R) may give rise to permanent damage.

\[\text{R} + \text{O}_2 \rightarrow \text{RO}_2\]

The presence of \(\text{O}_2\) increases the damage by forming of peroxy radical (RO\(_2\)) followed by irreversible damage that can not be repaired.

Under physiological conditions, free radicals like ROS or reactive nitrogen species (RNS) perform useful function, such as cell differentiation and proliferation. They are also useful in inflammatory reactions and in signal transduction pathway-like intracellular messengers of the growth factors \(^{23}\). However, excess ROS / RNS production is induced by a wide variety of environmental factors. These include physical (radiation), chemical or infectious agents, and /or deficient ROS / RNS removal by antioxidant defenses including intracellular enzymes like glutathione peroxidase, superoxide dismutase (SOD), or low molecular-mass compounds (e.g. vitamin E and C). This may result in pathological stress to tissue and cells \(^{24}\).

Ionizing radiation causes defective hemopoiesis as a function of radiation dose, dose rate and radiation quality \(^{25}\). This is complicated by thrombocytopenia and concomitant hemorrhages besides effects on adaptive immune system resulting from apoptosis of lymphocytes and deficient lymphopoiesis \(^{26}\).

**Types of Ionizing Radiation**

According to Cember \(^{27}\), there are 5 types of ionizing radiation, known as:

**Alpha Radiation:** Alpha particles consist of two protons and two neutrons, and carry a positive charge. Alpha particles are barely able to penetrate skin and can be stopped completely by a sheet of paper.
**Beta Radiation:** Beta radiation consists of fast moving electrons ejected from the nucleus of an atom. More penetrating than alpha radiation, beta radiation is stopped by a book or human tissue.

**Gamma Radiation:** Gamma radiation is a very penetrating type of radiation. It is usually emitted immediately after the ejection of an alpha or beta particle from the nucleus of an atom. It can pass through the human body, but is almost completely absorbed by denser materials such as concrete or lead.

**X-rays:** X-rays are a form of radiation produced mainly by artificial means rather than by naturally occurring radioactive substances.

**Neutrons:** Less common, neutron radiation occurs when neutrons are ejected from the nucleus by nuclear fission and other processes. The nuclear chain reaction is an example of nuclear fission.

*Penetrating Powers of Alpha Particles, Beta Particles, Gamma and X-Rays*

(Fig. A)

**Chronic radiation exposure**

Exposure to ionizing radiation over an extended period of time is called chronic exposure. The natural background radiation is chronic exposure, but a normal level is difficult to determine due to variations. Geographic location and occupation often affect chronic exposure. Chronic exposure is continuous or intermittent exposure to low doses of radiation over a long period of time. With chronic
exposure, there is a delay between the exposure and the observed health effect. These effects can include cancer and other health outcomes such as benign tumors, cataracts, and potentially harmful genetic effects\textsuperscript{28}.

**Acute radiation exposure**

Acute radiation exposure is an exposure to ionizing radiation which occurs during a short period of time. Acute exposure is exposure to a large, single dose of radiation, or a series of moderate doses received during a short period of time. Large acute doses can result from accidental or emergency exposures or from specific medical procedures (radiation therapy). For approved medical exposures, the benefit of the procedure may outweigh the risk from exposure. In most cases, a large acute exposure to radiation causes both immediate and delayed effects. Delayed biological effects can include cataracts, temporary or permanent sterility, cancer, and harmful genetic effects. For humans and other mammals, acute exposure to the whole body, if large enough, can cause rapid development of radiation sickness, evidenced by gastrointestinal disorders, bacterial infections, hemorrhaging, anemia, loss of body fluids, and electrolyte imbalance. Extremely high dose of acute radiation exposure can result in death within a few hours, days, or weeks\textsuperscript{28}.

**Biological effect of radiation**

According to Weiss and Kumar,\textsuperscript{29} radiation can damage every tissue in the body. The fastest growing tissues are the most vulnerable, because radiation as much as triples its effects during the growth phase. Ionization of living tissue causes cell molecules to be broken apart. This interaction can kill the cell or cause them to reproduce abnormally. Damage to a cell can come from **direct action** or **indirect action** of the radiation.

**Direct action** occurs when the radiation interacts directly with cell's essential molecules. The radiation energy may damage cell components such as the cell walls or the deoxyribonucleic acid (DNA). When radiation interacts with a cell wall
or DNA, the cell either dies or becomes a different kind of cell, possibly even a cancerous one.

**Direct Effect**

Indirect action occurs when radiation interacts with water molecules, which are roughly 80% of cells composition. The energy absorbed by the water molecules can result in the formation of free radicals. Free radicals are molecules that are highly reactive due to the presence of unpaired electrons, which result when water molecules are split. Free radicals may form compounds, such as hydrogen peroxide, which may initiate harmful chemical reactions within the cells. As a result of these chemical changes, cells may undergo a variety of structural changes which lead to altered function or cell death.

**Indirect Effect**

Radiolytic Decomposition of Water in a Cell
Exposure of mammals to ionizing radiation leads to the development of complex, dose dependent series of changes including different aspects of immunity and injury to the lymphoid as well as haematopoietic systems which can cause septicemia and death. Damage to the highly sensitive bone marrow (major site of hematopoiesis) and secondary lymphoid organs such as spleen can result in increased susceptibility to infections, toxicity and even death. The development of new concerns comprised immune function and/or radiation induced genetic damage.

**Units of radiation dose**

According to the Canadian Nuclear Safety Commission (CNSC), the scientific unit of measurement for radiation dose, commonly referred to as effective dose, is the millisievert (mSv). Other radiation dose measurement units include rad, rem, Roentgen, Sievert, and Gray. Because different tissues and organs have varying sensitivity to radiation exposure, the actual radiation risk to different parts of the body from an x-ray procedure varies. The term effective dose is used when referring to the radiation risk averaged over the entire body. The effective dose accounts for the relative sensitivities of the different tissues exposed. More importantly, it allows for quantification of risk and comparison to more familiar sources of exposure that range from natural background radiation to radiographic medical procedures.

**Gray (Gy).** The SI unit of absorbed dose. One gray = 1 J/kg = 100 rad.

**Rad.** The unit of absorbed dose. One rad = 100 erg/g = 0.01 Gy.

**Sievert (Sv).** The SI unit of dose equivalent, equal to absorbed dose in gray multiplied by the quality factor. One Sv = 100 rem.

**Rem.** The conventional unit of dose equivalent. One rem = 0.01 Sv.
Effect of gamma radiation on some body organs

1- The liver

This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It lies below the diaphragm in the thoracic region of the abdomen. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. It also performs and regulates a wide variety of high-volume biochemical reactions requiring highly specialized tissues, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. It is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs between 1.4–1.6 kg, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body.

Biochemical changes of liver induced by gamma radiation

Liver function tests (LFTs or LFIs), which include liver enzymes, are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver. This testing is performed by a medical technologist on a patient's serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality (eg. albumin); some with cellular integrity (eg. transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to (1) detect the presence of liver disease, (2) distinguish among different types of liver disorders, (3) gauge the extent of known liver damage, and (4) follow the response to treatment.
**Alanine transaminase (ALT),** also called Serum Glutamic Pyruvate Transaminase (SGPT) or Alanine aminotransferase (ALAT) is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured. ALT rises dramatically in acute liver damage, such as viral hepatitis. Elevations are often measured in multiples of the upper limit of normal (ULN).
Normal Values 9 to 60 IU/L\(^\text{37}\).

**Aspartate transaminase (AST),** also called Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate Amino Transferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. Elevated AST levels are not specific for liver damage, and AST has also been used as a cardiac marker.
Normal Values 10 to 40 IU/L\(^\text{37}\).

**Alkaline phosphatase (ALP),** is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. ALP is also present in bone and placental tissue, so it is higher in growing children (as their bones are being remodelled) and elderly patients with Paget's disease.
Normal Values 30 to 120 IU/L\(^\text{37}\).

**Effect of whole body gamma radiation on liver aminotransferase activities (AST&ALT):**

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the plasma of rats following local liver irradiation with a single dose of 20 Gy were determined \(^\text{38}\). Plasma AST levels was increased between 2 h and 24 h after irradiation, while plasma ALT levels remained unchanged. In contrast, AST
and ALT levels, measured between 24 h and 16 days after radiation, did not show any differences between irradiated and control animals. Male Swiss Albino rats were subjected to daily fractionated dose delivered as 1 Gy to reach a total dose of 9 Gy of whole body gamma irradiation \textsuperscript{39}. The obtained results demonstrated significant increase in serum AST and ALT levels at 3, 6 and 9 Gy post-irradiation. Abdel-Gawad et al., \textsuperscript{40} exposed female rats to whole body gamma irradiation at a dose level of 7 Gy and found that gamma irradiation caused significant increase in serum levels of AST and ALT at time intervals 14, 24 and 34 days post irradiation. While, Ashry \textsuperscript{41} exhibited an insignificant decrease in AST level and significant increase in ALT level on 3rd and 7th days post irradiation at the same dose. Female rats were exposed to whole body gamma irradiation at a dose level of 7 Gy \textsuperscript{42}. Data revealed that gamma irradiation induced a significant increase in AST and ALT activity on 5th and 9th days post-irradiation.

When male albino rats were exposed to a single dose of 6.5 Gy whole body gamma irradiation, the results showed a highly significant increase in levels of serum aspartate aminotransferase and alanine aminotransferase during four weeks post-irradiation as reported by \textsuperscript{43}. Also, Todorov and Damianov, \textsuperscript{44} found that AST and ALT were rose strongly in the blood plasma of male rats over the first 24 hours following irradiation with a dose of 6.7 Gy. Albino rats were exposed to a single dose of ionizing radiation (6Gy) \textsuperscript{45}. They found that the changes in the levels of aspartate transaminase and alanine transaminase (AST and ALT) were dependent on radiation dose and time of examination. El-Gabry et al., \textsuperscript{46} exposed female rats to whole body gamma irradiation at a dose level of 1 & 6 Gy. The results indicated that exposure of rats to both doses (1 & 6 Gy) of gamma irradiation induced significant increase in plasma AST and ALT levels as a dose dependent manner after 2 hours, 2 days till 2 weeks post-
irradiation. Similar results were reported by Ramadan et al., \textsuperscript{47} after 1 and 6 days post irradiation at dose levels of 2 and 6 Gy in male rats.

When male Wistar rats were exposed to a single dose of 6 Gy whole body gamma irradiation, the results showed a highly significant increase in levels of plasma AST and ALT during three weeks post-irradiation \textsuperscript{48, 49, 50}. Male Swiss albino rats were subjected to fractionated dose delivered as 1.5 Gy every 2 days to reach a total dose of 6 Gy whole body gamma irradiation \textsuperscript{51}. Data revealed that gamma irradiation induced a significant increase in serum ALT level after 1st, 4th, 7th & 10th days post irradiation.

On the other hand, whole body exposure of rats to gamma radiation at a dose level of 3.5 Gy caused increases in the levels of AST and ALT \textsuperscript{52}. Such pronounced elevation was also reported by Jungowska et al., \textsuperscript{53} who exposed mice to a single whole body X-irradiation with a 9 Gy dose. The assays were performed in 6 h intervals during the first day and 24 h intervals from the 2nd until the 6th day after the exposure. The data recorded highly significant increase in the enzymatic activity of alanine transaminase and aspartate transaminase (ALT and AST) in irradiated animals. Interestingly, a single dose of 0.5 Gy whole body X-ray irradiation significantly increased AST and ALT activities in rats \textsuperscript{54}.

\textit{Effect of whole body gamma radiation on alkaline phosphatase activity (ALP)}:

Alkaline phosphatase are enzymes which catalyses the phosphoric acid splitted from monophosphoric esters. These enzymes seemed to be involved in the transport of phosphate across cell membranes of many organs. The Alkaline phosphatase level in normal serum of adults appears to be mainly derived from the liver with a small variable intestinal component \textsuperscript{55}.

Noaman et al., \textsuperscript{39} subjected male Swiss albino rats to fractionated dose delivered as 1 Gy daily to reach a total dose of 9 Gy whole body gamma irradiation. The
obtained results demonstrated that exposure of rats to whole body gamma irradiation induced significant increase in serum ALP level at 3, 6 and 9 Gy of radiation exposure.

Abdel-Gawad et al., 40 exposed female rats to whole body gamma irradiation at a dose level of 7 Gy. Data revealed that gamma irradiation caused significant decrease in serum level of ALP at time intervals 14, 24 and 34 days post-irradiation. But, Ashry and Hussein 42 used the same dose and found that gamma irradiation caused significant increase in serum level of ALP activity on 5th and 9th days post irradiation.

Exposure of male albino rats to a single dose of 6.5 Gy whole body gamma irradiation caused a highly significant decrease in ALP level during four weeks post-irradiation 43. While, Badr El-din 56 exposed female Swiss albino mice to whole body gamma irradiation at a dose level of 6.5 Gy and data revealed a significant elevation in plasma ALP level on day 1 and 7 post-irradiation.

Female albino rats were subjected to whole body gamma irradiation at dose levels of 1 Gy and 6 Gy 57. The authors reported that exposure of rats to both doses of gamma irradiation induced significant increase in ALP level after 2 hours, 2 days and 2 weeks post-irradiation as compared to controls.

Tawfik, 51 exposed male Swiss albino rats to fractionated dose of whole body gamma irradiation delivered as 1.5 Gy every 2 days to reach a total dose of 6 Gy. Data revealed that gamma irradiation induced a significant increase in serum ALP level after 1st, 4th, 7th & 10th days post irradiation.

Salovsky et al., 55 exposed male rats to a single external whole-body irradiation at a dose level of 4 Gy gamma rays. They indicated that irradiation caused elevation in serum alkaline phosphatase (ALP) activity.

**Histological changes induced by gamma radiation:**
Liver is the major detoxifying organ in mammalian body. Its high metabolic activity performs a vast number of functions. The liver parenchyma is composed of two types of cells, the hepatocytes (polygonal cells) and the kupffer's cells (reticulo-endothelial cells). These kupffer's cells remove the particular matter from the body by phagocytosis. Dawson et al. reported that radiation induced liver disease is a dose limiting complication of liver irradiation and the treatment options are limited resulting in liver failure and death may occur in sever cases. Furthermore, Koc et al. and Song et al. observed damage to the liver and blood system of adult Swiss albino mice. Geraci et al. observed that local irradiation of rat's liver (15 Gy) reduced liver size and caused minimal histological changes. A dose of 25 Gy showed significant histological abnormalities and liver injury. The authors recorded that hepatic vein lesions and cellular necrosis were the most prominent histological lesions in 25 Gy irradiated liver. Whole body exposure of rats to 7 Gy gamma irradiation delivered as single dose results in loss of normal hepatic architecture, extremely widened and dilated hepatic portal vein with rupture endothelial lining and clogged with lysing erythrocytes, hemorrhage, inflammatory cells and fibroblasts surrounding the portal vein and dilated sinusoids. The hepatocytes showed vacuolated cytoplasm, some cells were necrotic with ill defined cell membrane and pyknotic nuclei. Whole body exposure of rats at fractionated sub lethal dose level of 2 Gy up to accumulative dose of 6 Gy of gamma irradiation resulted in widening and dilated hepatic portal area, hemorrhage, inflammatory cells and fibroblasts surrounding the portal vein and vacuolated cytoplasm. Also, congested vessels and dilated sinusoids, necrotic cells and pyknotic nuclei were also observed.
Khamis et al., reported that administration of bone marrow to irradiated rats at a dose level of 6 Gy of gamma irradiation injury and/or to enhance recovery of enzyme activities, thus confirming its protective role on liver function. Recently liver regeneration was supported by bone marrow transplantation which has been proposed as an alternative source of functional liver cells.

Soliman et al., postulated that liver of rat irradiated at 5 Gy gamma irradiation one and three days post-irradiation showed destructed areas, infiltration with inflammatory cells in the portal area and a number of necrotic cells with pyknotic and karyolitic nuclei. Concerning liver sections stained with PAS showed depletion of PAS positive particles in some hepatocytes whereas others appeared nearly normal.

El-Missiry et al., reported that irradiated animals with 2 and 4 Gy of gamma rays, showed significantly increased oxidative stress markers, malondialdehyde level and protein carbonyl content of the liver. However a marked decrease in hepatic contents of DNA, RNA and glutathione that indicates the role of oxidative stress in radiation induced tissue damage.

Koshimoto et al., suggested that external doses of irradiation of more than 1 Gy impaired the normal transfer of the haematopoietic site from the blood islands of the yolk sac into the liver of rat fetus. The majority of hepatocytes appeared in the fetal liver of rats subjected to fractionated 2 Gy dose of gamma irradiation were devoid of nuclei and accompanied by cytoplasmic degeneration besides rupture of central vein and diminished hemopoiesis were also observed.

Similarly the effect of whole body gamma irradiation at doses 1, 2 and 3 Gy on rats was studied by Abdel-Gawad et al., and found that the liver showed the highest degree of histopathological changes, necrosis and degenerative changes of cytoplasm.
Kafafy et al., \textsuperscript{72} found that the most remarkable effect after gamma irradiation is the loss of the normal configuration of liver cell strands radiating around the central vein in many areas. There were nuclear pyknosis, lobular disarray, cell vacuolation, lymphatic infiltration around the portal areas and slight dilatation of sinusoids. Severe damage to hepatic parenchyma and focal necrosis were seen 10 days post irradiation. Male rats irradiated at a dose of 3 Gy gamma irradiation caused latent cytogenetic damage to the liver, which was expressed during the course of an induced proliferation of hepatocytes \textsuperscript{73}.

Liver sections of rats one week post gamma irradiation (6Gy) revealed different histopathological alterations. These alterations were manifested as dilatation and congestion in blood vessels and appearance of inflammatory cells. The hepatocytes showed vacuolated cytoplasm and often with nuclei. Blood sinusoids were dilated. While after two weeks post gamma irradiation liver sections exhibited an increase in the above mentioned effects. Blood vessels were congested, more dilated and infiltrated with inflammatory cells. Blood sinusoids showed also dilatation, hepatocytes revealed pyknotic nuclei \textsuperscript{74}.

2- The kidney

The kidneys are paired organs with several functions. They are an essential part of the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure. They serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium; the kidneys also are responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, renin, and erythropoietin \textsuperscript{75}.

The kidney has a bean-shaped structure; each kidney has concave and convex surfaces. The concave surface, the renal hilum, is the point at which the renal artery
enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia (of Gerota) and paranephric fat. The anterior (front) border of these tissues is the peritoneum, while the posterior (rear) border is the transversalis fascia. The superior border of the right kidney is adjacent to the liver; and the spleen, for the left border. Therefore, both move down on inspiration.

The kidney is approximately 11–14 cm in length, 6 cm wide and 3 cm thick. The substance, or parenchyma, of the kidney is divided into two major structures: superficial is the renal cortex and deep is the renal medulla. Grossly, these structures take the shape of 8 to 18 cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (of Malpighi). Between the renal pyramids are projections of cortex called renal columns (of Bertin). Nephrons, the urine-producing functional structures of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the renal corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct. The tip, or papilla, of each pyramid empties urine into a minor calyx, minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter.

**Biochemical changes induced by gamma radiation**

**Kidney Function:**

The kidneys' main job is to filter the blood and rid the body of excess water, salt, and waste products. The filtered waste products are concentrated into urine. Urine leaves the kidneys through long slender tubes called ureters that connect to the bladder. Urine flows down the ureters into the bladder where it is stored until urination.
Effect of whole body gamma irradiation on urea concentration:

Urea is known to be the major excretory product in plasma and urine as it is the end product of protein metabolism in mammals. In the degradation of all amino acids, ammonia is released. Ammonia, incorporated mainly into glutamine and alanine molecules, is then transported to the liver, where, it is detoxified by incorporation into urea, a metabolically inert molecule

The urea resulting from protein degradation is excreted by the kidney, where Van Rongen et al. irradiated left kidney of female rats with single doses of 2, 4, 10, 20, and 40 dose fractions of gamma irradiation. The data revealed that serum urea decreased continuously. Kidney function is affected by radiation exposure as reflected by the level of urea in plasma of rats. On the other hand, where both kidneys of male Wistar rats were irradiated with either a single dose of 10 Gy or 26 Gy of gamma irradiation at a rate of 2 Gy per fraction per day. The both dose induced significant increases in serum blood urea nitrogen level was observed at 16 weeks, and this was followed by an apparent improvement after 24 weeks.
Male Swiss albino rats were exposed to fractionated dose delivered as 1 Gy every other to reach a total dose of 9 Gy whole body gamma irradiation. The obtained results demonstrated that exposure of rats to whole body gamma irradiation delivered as 1 Gy every other day induced significant increase in the levels of urea at 3, 6 and 9 Gy post-irradiation.

Mahdy, revealed that whole body gamma irradiation of rats at a single dose of 7.5 Gy caused a significant decrease in the content of serum proteins accompanied by a significant increase of urea level as recorded 7, 10 and 14 days after irradiation. In contrast, exposure at 7.5 Gy in 3 equal successive doses at an interval of 72 hours decreased the levels of serum proteins and urea at all the experimental time periods.

When female albino rats were exposed to whole body gamma irradiation at a dose level of 7 Gy, the results revealed a gradual significant increase in plasma levels of urea and creatinine from the 1st day post irradiation up to 2 weeks in a dose dependent manner.

Exposure of female Swiss albino mice to whole body gamma irradiation at a dose level of 6.5 Gy caused a significant elevation in plasma creatinine and urea levels on 1st and 7th days post-irradiation.

Regarding the same aspect, Mohamed, showed that gamma irradiation at (6.5 Gy) induced significant increment in the concentration of serum urea throughout 20 days post-irradiation. However, Abou Safi et al., and Ramadan et al., found that exposure of male rats to whole body gamma irradiation (6 Gy) caused a significant decrease in the plasma urea levels after 1 hr and 3 hrs. Then a significant increase was recorded after 5 hrs post-irradiation.

When female albino rats were exposed to whole body gamma irradiation at a dose level of 1 Gy and 6 Gy, the results revealed a gradual significant increase in plasma levels of urea after 2 hours, 2 days till 2 weeks in a dose dependent manner.
Donnadieu et al.,\textsuperscript{88} exposed pigs to whole body gamma radiation at a dose level up to 6 Gy and they found that gamma radiation altered urea level as compared to control.

**Effect of whole body gamma irradiation on creatinine concentration:**

Creatinine is excreted in urine by glomerular filtration and minute amounts are present in the blood. Urinary output of creatinine may be taken as a sensitive parameter indicating the degree of impaired tissue metabolism due to radiation effect. Results conducted by Yildiz et al.,\textsuperscript{82} showed a significant increase in serum creatinine level when kidneys of male rats were irradiated with either 10 Gy single dose or 26 Gy at a rate of 2 Gy per day, and after 4 weeks of irradiation, glomerular and proximal tubular injury were observed.

Noaman et al.,\textsuperscript{39} exposed male Swiss albino rats to fractionated dose of gamma irradiation delivered as 1 Gy every day to reach a total dose of 9 Gy. The obtained results demonstrated that exposure of rats to whole body gamma irradiation delivered as 1 Gy every other day induced significant increase in the levels of creatinine at 3, 6 and 9 Gy post-irradiation. However, Hassan et al.,\textsuperscript{89} observed that the serum creatinine level was elevated when the rats were exposed to gamma irradiation at fractionated dose levels of 3 Gy to accumulative dose of 9 Gy on the 2nd hours, 1st and 7th days post exposure.

The kidney is relatively more resistant to ionizing radiation. On the other hand, exposure to gamma irradiation at a dose level of 6.5 Gy caused a significant decrease in creatinine clearance\textsuperscript{90,91}. In contrast, Abdel-Hamid et al.,\textsuperscript{92} revealed that the exposure of rats to gamma irradiation at a dose level of 6.5 Gy resulted in a significant increase in serum creatinine level.

El-Gabry et al.,\textsuperscript{57} exposed female albino rats to whole body gamma irradiation at a dose level of 1 Gy and 6 Gy, the results revealed a gradual significant increase in plasma levels of creatinine after 2 hours, 2 days till 2 weeks in a dose dependent
manner. However, Abou-Safi et al., 86 reported that insignificant decrease in creatinine plasma level was noticed after whole body gamma irradiation at dose level of 6 Gy.

In 1989 El-Kashef et al. 93 had revealed that whole body gamma irradiation (5.5 Gy) induced significant decrease in the level of creatinine in rats at the 3rd, 14th and 21st irradiation days.

**Histological changes induced by gamma irradiation.**

Geraci et al. 94 showed that kidney damage as measured by plasma urea nitrogen (PUN), EDTA, or hematocrit was detected after 10 Gy irradiation of rats. Radiation nephropathy has been well characterized by endothelial injury, mesangiolysis, and glomerular basement membrane expansion 95.

Abdel-Gawad et al., 71 noticed that the effect of whole body gamma irradiation at doses 1, 2 and 3 Gy in female rats showed changes that varied from mild tubular degeneration to renal necrosis.

Irradiation of kidney has been reported to cause progressive injury that results in fibrosis, renal failure and glomerular injury. Fibrin deposition is involved in glomerular and tubular radiation injury 96.

Renal irradiation leads to time-dependent reduction in renal function as manifested by increase in blood urea nitrogen. The histopathological changes reported due to radiation correspond with a decline in renal functions. Glomerular, tubular, and endothelial cell nuclear polymorphism and focal tubular cell injury, lysis, and karyorrhexis were observed as early as 10 weeks of irradiation. Progressive thinning of the cortex as a result of widespread tubulysis, collapsing of tubules, glomerular crowding, decrease in glomerular cellularity, interstitial inflammation, and an elevated juxtaglomerular cell count were recorded 97.

According to Eissa and Mostafa 98 the inspected kidney sections obtained from female rats irradiated daily with 1.5 Gy of gamma-rays for five days revealed
different histological lesions. These lesions included deformed and collapsed glomeruli, degenerated renal convoluted tubules and increased inflammatory cells.

3- The heart

The heart is a muscular organ that contracts rhythmically, pumping the blood through the circulatory system. It is also responsible for producing a hormone called atrial natriuretic factor. Its walls consist of three tunics: the internal, or endocardium; the middle, or myocardium; and the external, or pericardium. The fibrous central region of the heart, called, rather inappropriately, the fibrous skeleton, serves as the base of the valves as well as the site of origin and insertion of the cardiac muscle cells.

The endocardium is homologous with the intima of blood vessels. It consists of a single layer of squamous endothelial cells resting on a thin subendothelial layer of loose connective tissue that contains elastic and collagen fibers as well as some smooth muscle cells. Connecting the myocardium to the subendothelial layer is a layer of connective tissue (often called the subendocardial layer) that contains veins, nerves, and branches of the impulse-conducting system of the heart (Purkinje cells).

The myocardium is the thickest of the tunics of the heart and consists of cardiac muscle cells arranged in layers that surround the heart chambers in a complex spiral. A large number of these layers insert themselves into the fibrous cardiac skeleton. The arrangement of these muscle cells is extremely varied, so that in histological preparations of a small area, cells are seen to be oriented in many directions. The heart is covered externally by simple squamous epithelium (mesothelium) supported by a thin layer of connective tissue that constitutes the epicardium. A subepicardial layer of loose connective tissue contains veins, nerves, and nerve ganglia. The adipose tissue that generally surrounds the heart accumulates in this layer. The epicardium corresponds to the visceral layer of the
pericardium, the serous membrane in which the heart lies. Between the visceral layer (epicardium) and the parietal layer is a small amount of fluid that facilitates the heart's movements.

The cardiac fibrous skeleton is composed of dense connective tissue. Its principal components are the septum membranaceum, the trigona fibrosa, and the annuli fibrosi. These structures consist of dense connective tissue, with thick collagen fibers oriented in various directions. Certain regions contain nodules of fibrous cartilage.

The cardiac valves consist of a central core of dense fibrous connective tissue (containing both collagen and elastic fibers), lined on both sides by endothelial layers. The bases of the valves are attached to the annuli fibrosi of the fibrous skeleton.

**Biochemical changes induced by gamma radiation**

**Heart Function:**
The use of radiotherapy in the management of malignant tumors causes low or high radiation exposure doses to normal tissues and undesired side effects may occur in early and late period following irradiation. All mediastinal structures, lungs and heart can exposure to total therapeutic dose during radiation therapy for thoracal or mediastinal malignancies such as Hodgkin disease and breast cancer. While early deaths following irradiation are caused by primary malignancies among these patients, long term mortalities are resulted from radiation induced morphological and functional organ abnormalities. Cardiac changes are the most frequently seen. Significant cardiac anatomical changes occur particularly in pericardium, myocardium, valves and result in severe cardiac dysfunctions after 10 to 20 years following administration of radiation into mediastinal or thoracal areas. The precision of the development of radiation induced cardiovascular complications has increased due to achieving the complete cure of primary tumor and to the extended...
survival time of irradiated cases by using advanced radiotherapy and adjuvant chemotherapy modalities. Early detection of radiation-induced morphological changes leading to cardiac dysfunction offers the possibility for early intervention such as administration of cardiovascular drugs and/or cardiac surgery in order to reduce or delay severe irreversible late complications.
**Effect of whole body gamma irradiation on creatine kinase (CK) activity:**

Creatine phosphokinase (CPK) plays an important role in cellular energy metabolism in vertebrates \(^{101}\). It is specifically located at places of energy demand and energy production and plays an important role in the energetic of calcium homeostasis and mitochondrial membrane stability. Elevation of serum CPK is an indication of muscle damage \(^{102}\).

In 2009, Mansour and Abu El Nour, \(^{103}\) had revealed that whole body gamma irradiation (2 Gy) induced significant increase in serum CPK and LDH (lactate dehydrogenase) in rat heart. Also, the same results in mice were obtained by Kimura et al. \(^{104}\) and in rat by Abu Ghadeer et al. \(^{105}\), Malarkodi et al. \(^{106}\) and Saad et al. \(^{107}\).

In 2008, Tawfik \(^{51}\) had revealed that whole body gamma irradiation (5 Gy) induced significant increase in the level of creatine kinase in rat heart. In the same year, Fahim exposed male albino rats to fractionated doses of gamma radiation (1 Gy 3 times week for a period of 2 weeks to attain a cumulative dose of 6 Gy) and results revealed a significant increase in serum CPK and AST activities of heart.

**Histological changes induced by gamma radiation.**

The effect of radiation on the heart have been well described including acute and chronic pericarditis, myocardial fibrosis, accelerated arteriosclerosis of the coronary arteries. However, valvular dysfunction secondary to mediastinal irradiation has received less attention \(^{108}\).

Radiation injury to the heart includes not only constrictive pericarditis and myocardial fibrosis, but also appreciable valvular and coronary artery lesions. As patients with malignancies survive longer, the surgical relief of radiation-induced heart disease may become more prevalent \(^{109}\).
Examined the sections of the cardiac tissue of the animals irradiated by gamma radiation at dose 2 Gy showed degenerated of the cardiac muscle fibers with loss of striation. Also massive extended hemorrhagic areas were apparently seen.\textsuperscript{103}

**Destructive Changes Induced by Free Radicals**

Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radical oxidation plays important role in the radiation –induced cell and tissue damage. Numerous studies evidence a decrease in body antioxidant system activity and changes in nitric oxide (NO) levels during irradiation. It has been demonstrated that in the process of radiation damage, NO may play either radio- protective or radiotoxic role depending on body redox status.\textsuperscript{110}

Free radicals can be beneficial but harmful if uncontrolled. ROS affects cellular calcium metabolism. Uncontrolled rises in intracellular free calcium can result in cell injury or death. Excessive free radical formation can damage all cellular macromolecules including proteins, lipids and nucleic acids. Proteins perform numerous crucial functions in the cell, primary in the form of enzymes that mediate most biochemical reactions required for cellular functions. Proteins are made up of approximately 20 different building blocks called amino acids, which differ in their sensitivity to interactions with ROS. Alternatively, the ROS-induced oxidation of proteins can lead to changes in the proteins three-dimensional structure as well as to fragmentation, aggregation, or cross-linking of the proteins. The destructive effects on proteins may play a role in the causation of cataracts.\textsuperscript{111}

Lipids that contain phosphate groups (i.e., phospholipids) are essential components of the membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria. Consequently, damage to the phospholipids will
compromise the viability of the cells. The complete degradation (i.e., peroxidation) of lipids is a hallmark of oxidative damage. The poly unsaturated fatty acids present in the membranes phospholipids are particularly sensitive to attack by OH⁻ and other oxidants. In addition to damaging cells by destroying membranes, lipid peroxidation can result in the formation of reactive products that themselves can react with and damage proteins and DNA.

DNA is the cell's genetic material and any permanent damage to the DNA can result in changes (i.e., mutations) in the proteins encoded in the DNA, which may lead to malfunctions or complete inactivation of the affected proteins. ROS are a major source of DNA damage, causing strand breaks and removal of nucleotides. Oxidative damage to cellular DNA can lead to mutations and may, therefore, play an important role in the initiation and progression of multistage carcinogenesis.

Severe oxidative stress with lipid peroxidation, protein oxidation, DNA damage and ATP depletion leads to cell death by necrosis, which is characterized by disruption of the cell membrane and cellular organelles.

**Antioxidants**

Antioxidant is a substance that prevents oxidation. In biological systems, antioxidants can work in several ways; they may reduce the energy of the free radical, stop free radical from being formed in the first place, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Interestingly, the body posse's defense mechanisms against free radical induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc. Non-enzymatic antioxidants are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, etc. All these
act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body’s natural antioxidant defenses or by supplementing with proven dietary antioxidants. This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area\textsuperscript{115}.

The intimate link between diet and oxidative stress is obvious, knowing that our body derives its main antioxidant defenses from essential nutrients mainly that they are necessarily obtained from food\textsuperscript{116}. Experimental studies have shown that most nutrients are biologically active in the body through synergistic effects, accordingly man and animals must be supplemented with a mixture of micronutrients\textsuperscript{117}. Furthermore, the vital importance of micronutrients for health and as reducing agents of oxidative stress may be through a direct action or act as component of antioxidant enzymes, like zinc in superoxide dismutase (SOD)\textsuperscript{118}.

**Alpha Lipoic Acid**

Alpha lipoic acid (ALA) is a natural molecule consisting of a five-membered cyclic disulphide and hydrocarbon tail ending with a carboxylic acid group. Hence, lipoic acid is a predominantly lipophilic molecule having an amphipathic character due to its carboxylic acid group attached to the ring structure. Lipoic acid is present in our diet mainly in animal foods such as meat and liver and at low or undetectable levels in plant foods such as potato. However, lipoic acid is also considered beneficial when used as a food supplement as its antioxidant function has been previously reported and several studies have revealed its protective effects in cases such as aging, diabetes mellitus and vascular and neurodegenerative diseases all in which free radicals are involved\textsuperscript{119, 120, 121}.

Studies are generally dealing with the biological consequences of lipoic acid administration in cases associated with oxidative stress or the differences between
the antioxidant activities of lipoic acid and its derivatives\textsuperscript{122, 123, 124}. There are also some studies on the spectroscopic and chromatographic analysis of the structural and quantitative aspects of lipoic acid and its derivatives in solution\textsuperscript{125}. However, structural information on the exact mode of interaction of lipoic acid with molecules of biological systems is lacking, despite the fact that investigation of the interactions of antioxidative drugs with biomolecules is important in understanding the mechanism of their action.

\textbf{Synonyms and trade names of ALA:}

1,2-Dithiolane-3-pentanoic acid- 1,2-Dithiolane-3-valeric acid; acetate-replacing factor; Biletan; 5-(1,2-dithiolan-3-yl)valeric acid; 6,8-dithiooctanoic acid; Heparlipon; lipoic acid; Liposan; Lipothion; protogen A; pyruvate oxidation factor; Thioctacid; thioctic acid; 6,8-thioctic acid; 6-thioctic acid; thioctidase; thiooctanoic acid; Tioctan\textsuperscript{126, 127, 128}.

\textbf{Biological sources of ALA}

Alpha lipoic acid is produced in the body, and found in food sources such as red meat (kidney, heart, liver), spinach, broccoli, and yeast extract\textsuperscript{129}. Red meat is among the rich sources of ALA\textsuperscript{130}. Naturally occurring lipoic acid is always covalently bound and not immediately available from dietary sources. Additionally, the amount of lipoic acid present is very low. For example: the purification of lipoic acid to determine its structure used an estimated 10 tons of liver residue, which yielded 30 mg of lipoic acid. As a result, all lipoic acid available as a supplement is chemically synthesized\textsuperscript{131}.

The amount of lipoic acid produced internally in the human body decreases naturally with age, which could set the stage for free radical induced damage. Although small amounts of lipoic acid are available in food sources, such as dark leafy greens like spinach and collards, broccoli, beef, and organ meats,
supplementation may be needed to achieve significant intake levels. In addition to, studies suggest that the most potent form of lipoic acid is R-dihydrolipoic acid.

**Chemical structure of ALA**

The chemical structure of lipoic acid is similar to that of biotin, and the authors suggest that lipoic acid can either displace biotin from its binding site at holocarboxylase synthetase, or compete with biotin for transport across the cell membrane.

**Molecular Formula of ALA:** $C_8H_{14}O_2S_2$

**Molecular Weight of ALA:** 206.33

**α-Lipoic Acid**

![Chemical Structure of α-Lipoic Acid](image)

**Dihydrolipoic Acid**

![Chemical Structure of Dihydrolipoic Acid](image)

**(Fig. B) structure of ALA and DHLA**

**Metabolism of lipoic acid:**

Lipoic Acid: In the mitochondria, "$\alpha$"-lipoic acid is covalently linked to a lysyl residue as a lipoamide in proteins. At the expense of NADPH, "$\alpha$"-lipoic acid is reduced to dihydrolipoic acid (DHLA) which reacts with oxidants such as superoxide and hydroxyl radicals. It also reduces oxidized vitamin C and glutathione, which in turn recycle ascorbic acid and vitamin E (Figure C). "$\alpha$"-Lipoic
acid also acts as a coenzyme in mitochondrial multienzyme complexes in the oxidative decarboxylation of "-keto acids such as pyruvate and "-ketoglutarate \(^{134, 135}\).

Food-derived "-lipoic acid originates from multienzyme complexes. Due to the lack of effective proteolytic enzymes, "-lipoic acid from the diet is absorbed as a lipoyllysine. In addition, "-lipoic acid can be produced by de novo biosynthesis, where cysteine appears to be the source of sulfur and octanoate serves as the intermediate precursor for the 8-carbon fatty acid. Only minor amounts of "-lipoic acid will enter the circulation from food or biosynthesis. The concentration of "-lipoic acid has been found to be highest in the liver \(^{136, 135}\).

Figure C. "-Lipoic acid: Antioxidant Regeneration \(^{135}\)

**Pharmacokinetics of ALA**

ALA appears to be readily absorbed from an oral dose and converts easily to its reduced form, dihydrolipoic acid (DHLA), in many tissues of the body \(^{133}\). The effects of ALA and DHLA are present both intra- and extracellularly. ALA contains an asymmetrical carbon and thus has two possible optical isomers. These are designated as R-lipoic acid (R-ALA) and S-lipoic acid (S-ALA). Naturally occurring ALA is in the R configuration, bound to a protein where it functions as
an essential cofactor for several mitochondrial enzyme complexes involved in energy production and the catabolism of alpha-keto acids and amino acids\textsuperscript{137}. Nutritional supplements of ALA are typically comprised either of R-ALA alone or a racemic mixture of R-ALA and S-ALA (RS-ALA). Human studies using oral racemic mixtures of ALA have observed plasma concentrations of R-ALA to be greater than that of S-ALA\textsuperscript{138,134}. Breithaupt-Grogler et al.\textsuperscript{134} found that, following oral administration of 50- or 600-mg racemic mixture of lipoic acid, maximum plasma concentrations (Cmax) of R-ALA were double that of S-ALA (following 50-mg dose: 135.45 ng/mL and 67.83 ng/mL, respectively; following 600-mg dose: 1,812.32 ng/mL and 978.20 ng/mL, respectively).
**Mechanisms of Action of ALA**

Alpha-lipoic acid is a potent antioxidant in both fat and water soluble mediums. Furthermore, its antioxidant activity extends to both its oxidized and reduced forms. DHLA is capable of directly regenerating ascorbic acid from dehydroascorbic acid and indirectly regenerating vitamin E \(^{139}\). Researchers have also found that ALA increases intracellular glutathione and coenzyme Q10 levels \(^{140}\). Alpha-lipoic acid appears capable of chelating certain metals. It forms stable complexes with copper, manganese, and zinc \(^{141}\). In animal studies, it has been found to protect against arsenic poisoning and in both animal and in vitro studies, ALA reduced cadmium-induced hepatotoxicity \(^{142}\). In an in vitro study, ALA chelated mercury from renal slices \(^{143}\).

It was also found that the antioxidant properties of lipoate originated from the studies of Rosenberg and Culik, \(^{144}\) who noticed that the administration of ALA prevented the symptoms of both vitamin C and E deficiency in guinea pigs and vitamin E deficiency in rats.

Mechanisms that may account for lipoic acid’s benefit in preventing diabetic complications include prevention of protein glycosylation \(^{145}\) and inhibition of the enzyme aldose reductase, the latter of which subsequently inhibits conversion of glucose and galactose to sorbitol. Accumulation of sorbitol has been implicated in the pathogenesis of various diabetic complications, including “sugar cataracts”, where sorbitol accumulates in the eye lens \(^{146, 147}\).

**Protective role of ALA**

The protective effects of ALA against free radical-mediated injury interested several groups in examining whether lipoic acid or dihydrolipoic acid protects haematopoietic tissues in mice from free radical damage induced by ionizing radiation. Recently, protective effects of ALA against radiation damage \(^{148}\). They determined the LD\(_{50}\) by the endogenous and exogenous spleen colony assay.
Interperitoneal administration of ALA at a nontoxic dose of 200 mg /kg body, 30 min. before irradiation, increased the LD$_{50}$ from 8.67 to 10.97 Gy in male mice. Dihydrolipoic acid also protects against UV radiation in mice skin $^{149}$. The table (1) below lists broad array of reactive oxygen species scavenged by lipoic acid and DHLA.

Table: R-α-lipoic acid /Dihydrolipoic Acid (DHLA)—The Most Versatile Antioxidant Pair

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Scavenged by Lipoic acid</th>
<th>Dihydrolipoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nitric oxide radical</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Superoxide radical</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypochlorous acid</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Peroxy radical</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Lipoic acid has long been associated with the treatment of liver conditions. The first human clinical studies using alpha- Lipoic acid (ALA) in the US were carried out by Bartter et al. $^{150}$ at the National Institute of Health in the 1980’s. They administered intravenous ALA to 79 people with acute and severe liver damage at various medical centers across the United States and 75 recovered full liver function. Drs. Bartter and Berkson were appointed by the FDA as principal investigators for this therapeutic agent as an investigational drug and Dr. Berkson went on to use it successfully for the treatment of chronic liver disease (viral hepatitis, autoimmune hepatitis, etc.) $^{151}$. Studies also support its role in metal chelation. Transition metals such as iron, copper, mercury, or cadmium can induce free radical damage in biological systems by catalyzing decomposition of hydroperoxides and generate highly toxic hydroxyl radicals. Lipoic acid and DHLA may exhibit antioxidant activity by metal chelating
which helps explain the usefulness of lipoic acid for detoxification in heavy metal poisoning \(^{153, 154, 155}\). Bioavailability of R- and S- lipoic acid has been studied extensively in humans using single dose administration \(^{156, 138, 157, 158, 134}\). After intravenous administration, there is no difference between R- and S-lipoic acid concentrations in plasma. However, after oral intake of the combined mixture, a 60% higher response is found for R-lipoic acid than for the S-form, which is highly significant \(^{157}\).

**Effectiveness of ALA in treating oxidative stress**

Hagen et al. \(^{120}\) investigated the effects of alpha lipoic acid supplementation on cellular and general metabolic activity, hepatocellular antioxidant status, oxidant production, and oxidative damage in rats. They used young and old rats on a diet with or without alpha lipoic acid (0.5% w/w) for two weeks. Alpha lipoic acid supplemented old rats showed a marked reversal in age associated oxygen consumption decline. The mitochondrial membrane potential in old rats increased by fifty percent with alpha lipoic acid supplementation, although it was still lower when compared to young untreated rats. Physiological data showed a doubling of ambulatory activity in supplemented old rats, which was not appreciably lower than the activity observed in untreated young rats. Levels of glutathione and ascorbate were substantially depressed in old untreated rats compared to young rats, but alpha lipoic acid supplementation restored both antioxidant levels these rats. To verify that the above findings translated to reduced oxidative stress, malondialdehyde levels, a lipid peroxidation indicator, was measured in all rat groups. Malondialdehyde formation was suppressed in old rats treated with alpha lipoic acid, compared the unsupplemented old rat group. Administration of alpha lipoic acid stimulates insulin-dependent and independent glucose uptake into cells and enhances non oxidative and oxidative glucose metabolism\(^{159}\).
Alpha-lipoic acid (ALA) and its reduced form dihydro-lipoic acid are present in all prokaryotic and eukaryotic cells. Lipoic acid was once considered a vitamin, but now it is commonly accepted that it can be synthesized de novo in human cells. It has long been known as a coenzyme of multi-enzymatic complexes catalyzing the decarboxylation of alpha keto acids\textsuperscript{160}. In addition, ALA is involved in the regulation of carbohydrate and lipid metabolism\textsuperscript{161}. Being easily absorbed from the gastrointestinal tract and able to cross the blood brain barrier without exhibiting any serious side effects along with the above mentioned features, ALA is considered a very promising drug\textsuperscript{161}.
MATERIALS AND METHODS

Materials:

Experimental animals:
A total number of 200 male albino rats (*Rattus norvegicus*) of about 120 - 160 g body weight were used in this study. They were purchased from the animal Breeding House of the National Research Center (NRC), Dokki, Cairo, Egypt. Animals were housed in standard metal cages and maintained in conditions of good ventilation, normal temperatures and humidity ranges and kept under observation for one week prior to experimentation. Rats were fed on standard pellets, containing all nutritive elements. Drinking water and food were provided *ad libitum* throughout the study.

Radiation facility:
Irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source of radiation was a Gamma Cell-40 (Cesium 137), which ensure a homogeneous distribution of irradiation. The dose rate was 0.61 Gy/minute during the experimental periods. Animals were whole body exposed to 6 Gy delivered as a fractionated dose (2 Gy each 3 days).

Drug administration:
Alpha lipoic acid (ALA) was obtained from Eva Company (Cairo- Egypt), dissolved in distilled water and administered orally by gavage after fasting overnight at doses 30 mg/kg body weight. The dose used in this study was selected on the basis of the report of a previous study done by Melhem et al.\textsuperscript{162}. 

\[162\]
Methods:

Experimental design:

120 rats were divided into five equal groups (each of 24 rats) as follows:

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Number of rats</th>
<th>Treatment method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>Rats of this group didn’t receive any treatment or expose to gamma irradiation.</td>
</tr>
<tr>
<td>ALA treated</td>
<td>24</td>
<td>Rats of this group received, daily, alpha lipoic acid (30 mg/kg body wt)*, for 16 successive days.</td>
</tr>
<tr>
<td>Irradiated</td>
<td>24</td>
<td>24 rats were exposed to whole body gamma irradiation. Radiation dose was fractionated at levels of 2 Gy, each 3 days to reach an accumulative dose of 6 Gy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 3 days of 1st exposure, 6 rats were sacrificed (2 Gy).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The other 18 rats were exposed to another 2 Gy from which 6 rats were sacrificed (4 Gy).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The other 12 rats were exposed after 3 days to the last exposure of 2 Gy and then 6 rats were sacrificed (6 Gy).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The remaining 6 rats were left for one month then sacrificed (for recovery test).</td>
</tr>
<tr>
<td>Simultaneously irradiated ALA</td>
<td>24</td>
<td>Rats of this group received, daily, alpha lipoic acid (30 mg/kg body wt)*, for 9 successive days simultaneously during exposure. Rats were divided exactly as the above irradiated control group.</td>
</tr>
<tr>
<td>Pre irradiated ALA</td>
<td>24</td>
<td>Rats of this group received, daily, alpha lipoic acid (30 mg/kg body wt)*, for 7 successive days before radiation exposure and 9 successive days, simultaneously, during exposure. Rats were divided exactly as the above</td>
</tr>
</tbody>
</table>
The dose used in this study was selected on the basis of the report of a previous study done by (Melhem et al., 2001). The experiment was repeated once, using 80 male rats (16 in each group), to confirm the obtained results. Rats of each group were sacrificed after 3, 6, 9 and 39 days of each treatment and blood was collected individually by heart puncture, in centrifuge tubes. Sera were separated and examined, freshly, for the assessment of liver and heart enzymes activities and kidney functions.

I-Biochemical studies:  

Assessment of aspartate and alanine transaminases (AST & ALT) activity.  

The activity of serum aspartate and alanine transaminases (AST & ALT) in liver was measured according to the method of Reitman and Frankel.

Principle:  
The method of ALT based on formation of the chromogenic dinitrophenylhydrazone of pyruvate and since dinitrophenylhydrazine reacts with α-ketoglutarate as well as pyruvate, high reagent blanks are obtained. The series of reactions involved in the assay system is as follows:  
The amino group is enzymatically transferred by ALT present in the specimen from alanine to the carbon atom of α-oxoglutarate yielding pyruvate and glutamate. Pyruvate formed is measured in its derivative form, 2,4-dinitrophenylhydrazone.

\[
\text{Alanine} + \alpha\text{-ketoglutarate} \rightarrow \text{Pyruvate} + \text{Glutamate} \]
On the other hand, the method of AST based on formation of the chromogenic dinitrophenylhydrazone of oxaloacetate and since dinitrophenylhydrazine reacts with α-ketoglutarate as well as oxaloacetate, high reagent blanks are obtained. The series of reactions involved in the assay system is as follows:

The amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of α-oxoglutarate yielding oxaloacetate and glutamate. Oxaloacetate formed is measured in its derivative form, 2,4-dinitrophenylhydrazone.

\[
\text{Aspartate} + \alpha\text{-oxoglutarate} \xrightarrow{\text{AST/GOT}} \text{oxaloacetate} + \text{glutamate}
\]

The activity was expressed as units/ml.

**Assessment of serum alkaline phosphatase (ALP) activity.**

The activity of serum alkaline phosphatase (ALP) in liver was measured according to the method of *Belfield and Goldberg*,\(^{164}\).

**Principle:**

\[
\text{Phenyl phosphate} \quad \xrightarrow{\text{ALP, pH=10}} \quad \text{phenol} + \text{phosphate}
\]

The liberated phenol is measured calorimetrically in the presence of 4-aminophenazone and potassium ferricyanide. The activity was expressed as IU/L.

**Assessment of serum urea:**

The activity of serum urea was measured according to the method of *Fawcett and Scott*,\(^{165}\).

**Principle:**

The method is based on the following reaction:

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2
\]
The ammonium ions formed are measured by the Berthelot reaction. The blue dye indophenol product reaction absorbs light between 530 nm and 560 nm proportional to initial urea concentration.

The activity was expressed as mg/dl.
**Assessment of serum creatinine.**

The activity of serum Creatinine was measured according to the method of Schirmeister et al.\textsuperscript{166}.

**Principle:**
Creatinine forms a colored complex with picrate in an alkaline medium. The activity was expressed as mg/dl.

**Assessment of serum creatine kinase (CK-MB) activity.**

The activity of serum creatine kinase (CK-MB) in heart was measured according to the method of Neumeier\textsuperscript{167} and Schumann,\textsuperscript{168}.

**Principle:**
CK-MB reagent contains an antibody inhibiting specifically CK-M subunits (i.e. 100% of CK-MM and 50% of CK-MB isozymes). The remaining activity, corresponding to CK-B fraction activity, is measured according to the IFCC reference method for measuring CK activity. CK-MB activity is then obtained by multiplying by 2 the remaining activity.

All kits were obtained from Biodiagnostic Company.

**II-Histological studies:**

**Light Microscopic studies**

At the end of each experimental period, rats were sacrificed and samples of liver, kidney and heart were taken rapidly from each rat, after dissection, and fixed in Bouin's (liver), Formal saline (kidney) and 10% formalin (heart) fixatives. All samples were dehydrated in ascending grades of ethanol, cleared in butanol and embedded in parablast. Sections of 5-6 μm thickness were obtained and stained with the following stains for:

**General histological studies**

1- Haematoxyline and Eosin for general histology of liver and kidney\textsuperscript{169}.

**Solutions and Reagents:**
1% Acid Alcohol Solution (for differentiation):
Hydrochloric acid -------------- 1 ml
70% ethanol ----------------- 100 ml
Mix well.

0.2% Ammonia Water Solution (Bluing):
Ammonium hydroxide (concentrated) ---- 2 ml
Distilled water ------------------- 1000 ml
Mix well.

Eosin Stock Solution:
Eosin Y -------------------------- 1 g
Distilled water ------------------ 100 ml
Mix to dissolve.

Hematoxylin Solution (Harris):
Potassium or ammonium (alum) ------- 100 g
Distilled water ------------------- 1000 ml
Heating to dissolve. Add 50 ml of 10% alcoholic hematoxylin solution and heat to boil for 1 minute. Remove from heat and slowly add 2.5 g of mercuric oxide (red). Heat to the solution and until it becomes dark purple color. Cool the solution in cold water bath and add 20 ml of glacial acetic acid (concentrated). Filter before use.

Procedure:
1. Deparaffinization of sections in 2 changes of xylene, 10 minutes each.
2. Re-hydration in 2 changes of absolute alcohol, 5 minutes each.
3. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.
4. Washing briefly in distilled water.
5. Staining in Harris hematoxylin solution for 8 minutes.
6. Washing in running tap water for 5 minutes.
7. Differentiation in 1% acid alcohol for 30 seconds.
8. Washing running tap water for 1 minute.
9. Bluing in 0.2% ammonia water or saturated lithium carbonate solution for 30 seconds to 1 minute.
10. Washing in running tap water for 5 minutes.
11. Rinsing in 95% alcohol, 10 dips.
12. Counterstaining in eosin-phloxine B solution (or eosin Y solution) for 30 seconds to 1 minute.
13. Dehydration through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.
14. Clearing in 2 changes of xylene, 5 minutes each.
15. Mounting with xylene based mounting medium.

Results:
Nuclei ---------------------------------------- blue
Cytoplasm ---------------------------------- pink to red

2- Mallory's Triple stain for collagen fibers\textsuperscript{170}.

Solutions and Reagents:

Mallory I
Acid fuchsin, C.I. 42685 ------------------ 1.0 gm
Distilled water --------------------------------100.0 ml
Phosphomolybdic acid
phosphomolybdic acid ----------------------- 1.0 gm
Distilled water ------------------------------- 100.0 ml

Mallory II
Aniline blue, WS, C.I. 42780 ------------------- 0.5 gm
Orange G, C.I. 16230 -------------------------- 2.0 gm
Distilled water ------------------------------- 100.0 ml.

Procedures:
1- Deparaffinization and hydration of slides to water; remove HgCl2. If Hg Cl2 is absent from fixative, moderate in saturated aqueous HgClo, plus glacial acetic acid: 10 minutes. %Wash, treat with Lugol's and 0.5% sodium thiosulfate, wash and rinse in distilled water.
2- Staining in Mallory I: 15 seconds.
3- Rinsing in distilled water: 10 or more seconds, to differentiate reds.
4- Treating with phosphomolybdic acid: 1-5 minutes.
5- Rinsing briefly in distilled water.
6- Staining in Mallory II: 2 minutes.
7- Rinsing in distilled water.
8- Differentiation of aniline blue in 90% ethyl alcohol.
9- Dehydration in absolute alcohol, clearing and mounting.

Results:
Nuclei—red
Muscle and some cytoplasmic elements—red to orange
Collagen—dark blue

Histochemical studies
1- Periodic acid Schiff’s reaction (PAS) for general carbohydrate of liver and kidney

Solutions and Reagents:

Periodic acid
Periodic acid (HIO4) -------------------------- 0.6 gm
Distilled water ------------------------------ 100.0 ml
Nitric acid, concentrated ---------------------- 0.3 ml
Schiff's reagent
Basic fuchsin, C.I. 42500 ------------------------------ 0.5-1.0 gm
Distilled water ------------------------------------------- 85.0 ml
Sodium metabisulfite, Na$_2$S$_2$O$_5$ ----------------------- 1.9 gm
N HCl ---------------------------------------------------- 15.0 ml
Sulfite solution
Sodium metabisulfite, Na$_2$S$_2$O$_5$ -------------- 0.5 gm
Distilled water ----------------------------------- 100.0 ml

**Procedures:**
1- Deparaffinization and hydration of slides to water.
2- Treating with periodic acid, aqueous: 5 minutes.
3- Washing in running water: 5 minutes.
4- Treating with Schiff's reagent: 10 minutes.
5- Transferring through sulfite solutions, 3 changes: 1.5-2 minutes in each.
6- Washing in running water: 5 minutes.
7- Dehydration, clearing, and mounting.

**Results:**
Glycogen—Purplish-red.

**Transmission electron microscopic studies**
Liver and kidney specimens were quickly removed and cut into small pieces, immediately fixed in 2.5% glutaraldehyde diluted with 0.1M Phosphate Buffer Solution (PBS) (PH 7.3) at 4°C for 24 hours and post fixed in 1% Osmium tetroxide diluted in 0.1M PBS at 4°C for one hour.
After fixation, ultra-thin sections were prepared, viewed, examined and photographed with JEOL (JEM 100CX) Transmission electron microscope, at The
National Center for Radiation Research and Technology (NCRRT) in atomic energy authority.

**Statistical Analysis**

Data were analyzed using one way analysis of variance (ANOVA) followed by Fischer’s LSD test considering significant at $p \leq 0.05$ levels. The data were expressed as mean ± standard error (SEM). Statistical Package for the Social Sciences (SPSS inc.) was used for statistical analysis of the results.
RESULTS

Biochemical Results

A. Effects of gamma irradiation on liver enzymes:

1. Changes in serum AST:

Results represented in Table (2) and Fig. (D) showed a disturbance in the activity of liver AST lead to a significant elevation by 75.3 ± 2.3 and 76.1 ± 3 in irradiated rats at 2 Gy and 4Gy respectively. A significant decrease by 51.7 ± 3 at 6 Gy was recorded as compared to normal controls. Surprisingly, highly significant elevated values of AST were registered in rats which left for one month after 6 Gy. Treatment of irradiated rats with ALA pre and simultaneously-irradiation up to 4 Gy decreased the activity of liver AST with values near to control. While, only the pre-administration of ALA (7 days before the beginning of radiation) decreased the liver AST activity near to control and attaining intermediate values between normal controls and irradiated rats in rats left for one month. In contrast, simultaneous-administration of ALA showed no significant effect.
Table 2: Effect of alpha lipoic acid (30 mg/kg) on serum AST activities in γ-irradiated male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without treatment</th>
<th>AST (Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td>2 Gy</td>
</tr>
<tr>
<td>Control</td>
<td>65.6 ±2.3</td>
<td>-</td>
</tr>
<tr>
<td>ALA</td>
<td>-</td>
<td>70 ±3.9</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Irradiated</td>
<td>-</td>
<td>75.3 ±2.6</td>
</tr>
<tr>
<td>P</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Irradiated+ALA</td>
<td>-</td>
<td>66.8 ±2.4</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALA+irradiated+ALA</td>
<td>-</td>
<td>62.2 ±4</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P2</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means of 6 records ± standard error.

Statistical analysis was made by LSD at levels of P ≤ 0.05.

P          Difference from control at P ≤ 0.05.
P1         Difference from irradiated rat at P ≤ 0.05.
P2         Difference from irradiated plus ALA at P ≤ 0.05.
NS         Non-significant.
Fig. (D). Effect of ALA (30 mg/kg) and/or γ-radiation on the level of AST in serum liver.
2. Changes in serum ALT:

Table (3) and Fig. (E) demonstrate that the activity of liver ALT was significantly decrease by $19.5 \pm 1.6$, $18.2 \pm 1.2$ and $19.7 \pm 1.7$ in irradiated rats at 2 Gy, 4Gy and 6 Gy respectively as compared to normal controls. While, a highly significant elevation by $51.4 \pm 2.9$, in rats which left for one month after 6 Gy was reported. Also, treatment of irradiated rats with ALA pre- and simultaneously-irradiation up to 4 Gy improve the activity of liver ALT with values near to control one. While, only the pre-administration of ALA (7 days before the beginning of radiation) protected the liver ALT activity near to control and attaining intermediate values between normal controls and irradiated rats in those left for one month. On the other hand, simultaneous-administration of ALA showed no significant effect.
Table 3: Effect of alpha lipoic acid (30 mg/kg) on serum ALT activities in γ-irradiated male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without treatment</th>
<th>ALT (Units/ml)</th>
<th>2 Gy</th>
<th>4 Gy</th>
<th>6 Gy</th>
<th>After month of 6 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>27.2 ±1.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALA</td>
<td></td>
<td>-</td>
<td>26.9 ±2.7</td>
<td>28.8 ±1.8</td>
<td>28.4 ±1.9</td>
<td>31.5 ±0.97</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Irradiated</td>
<td>-</td>
<td>19.5 ±1.6</td>
<td>18.2 ±1.2</td>
<td>19.7 ±1.7</td>
<td>51.4 ±2.9</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Irradiated+ALA</td>
<td>-</td>
<td>23.6 ±1.3</td>
<td>22.3 ±1.1</td>
<td>20.8 ±1.7</td>
<td>44.2 ±3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALA+Irradiated+ALA</td>
<td>-</td>
<td>24.2 ±1.6</td>
<td>27 ±2.5</td>
<td>27.3 ±2.7</td>
<td>35.5 ±3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are expressed as means of 6 records ± standard error.

Statistical analysis was made by LSD at levels of P ≤ 0.05.

P Difference from control at P ≤ 0.05.

P1 Difference from irradiated rat at P ≤ 0.05.

P2 Difference from irradiated plus ALA at P ≤ 0.05.

NS Non-significant
Fig. (E). Effect of ALA (30 mg/kg) and/or $\gamma$-radiation on the level of ALT in serum liver.
3. Changes in serum ALP:

Results represented in Table (4) and Fig. (F) demonstrated non significant change in the activity of serum liver ALP at 2 Gy. While, a significant decrease by 102 ± 7 was observed at 4 Gy followed by a highly significant increase by 186.3 ± 3.9 and 213.3 ± 12 in irradiated rats at 6 Gy and after one month of 6 Gy, respectively was registered as compared to normal control ones. Administration of ALA pre and simultaneously-irradiation improved the values of liver ALP activity and only, the pre-administration of ALA preserved the values near to control ones at 6 Gy and achieved intermediate values between normal control and irradiated rats in those left for one month. But, ALP values after simultaneously-administration of ALA was not significantly affected.
Table 4: Effect of alpha lipoic acid (30 mg/kg) on serum ALP activities in γ-irradiated male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without treatment</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 Gy</td>
</tr>
<tr>
<td>Control</td>
<td>123.7 ±2.3</td>
<td>-</td>
</tr>
<tr>
<td>ALA</td>
<td>-</td>
<td>126.2 ±2.7</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Radiated</td>
<td>-</td>
<td>112 ±3.7</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Irradiated+ALA</td>
<td>-</td>
<td>118.5 ±7.7</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P₁</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALA+</td>
<td>-</td>
<td>119 ±2</td>
</tr>
<tr>
<td>Irradiated+ALA</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>P₁</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>P₂</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means of 6 records ± standard error.

Statistical analysis was made by LSD at levels of P ≤ 0.05.

P Difference from control at P ≤ 0.05.
P₁ Difference from irradiated rat at P ≤ 0.05.
P₂ Difference from irradiated plus ALA at P ≤ 0.05.
NS Non-significant
Fig. (F). Effect of ALA (30 mg/kg) and/or γ-radiation on the level of ALP in serum liver.
B. Effects of gamma irradiation on kidney functions:

1. Changes in urea concentration:

Results in Table (5) and Fig. (G) show that irradiation of animals at 2 Gy induced significant depressions by 21 ± 1.5 in urea concentration, while significant elevation was observed in rats exposed to accumulative dose of 4 Gy, 6 Gy and those left for one month after 6 Gy by 36 ± 5.7, 42.6 ± 1.3 and 55 ± 2.3 respectively as compared to normal controls. Rats received ALA after irradiation was not significantly affected at all, while rats received ALA pre-and simultaneously during irradiation recorded significant improvement except, those left for one month which attained intermediate values between normal controls and irradiated rats.
Table 5: Effect of alpha lipoic acid (30 mg/kg) on serum urea concentration in γ-irradiated male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without treatment</th>
<th>Urea (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 Gy</td>
<td>4 Gy</td>
<td>6 Gy</td>
<td>After month of 6 Gy</td>
</tr>
<tr>
<td>Control</td>
<td>26 ±1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALA</td>
<td>-</td>
<td>25.6 ±2</td>
<td>25.7 ± 2.4</td>
<td>26.8 ± 2.5</td>
<td>27.8 ± 1.7</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Irradiated</td>
<td>-</td>
<td>21 ±1.5</td>
<td>36 ± 5.7</td>
<td>42.6 ±1.3</td>
<td>55 ±2.3</td>
</tr>
<tr>
<td>P</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Irradiated+ ALA</td>
<td>-</td>
<td>19.7 ±1.2</td>
<td>33.3 ±1.9</td>
<td>34 ±2.6</td>
<td>46.5 ± 2</td>
</tr>
<tr>
<td>P</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P_1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALA+ irradiated+ ALA</td>
<td>-</td>
<td>22.5 ±1.4</td>
<td>30.8 ±1</td>
<td>31.4 ±1.4</td>
<td>43.3 ± 2</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P_1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>P_2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means of 6 records ± standard error.

Statistical analysis was made by LSD at levels of P ≤ 0.05.

P       Difference from control at P ≤ 0.05.
P_1     Difference from irradiated rat at P ≤ 0.05.
P_2     Difference from irradiated plus ALA at P ≤ 0.05.
NS      Non-significant
Fig. (G). Effect of ALA (30 mg/kg) and/or γ-radiation on the level of serum urea.
2. *Changes in creatinine concentration:*

Surprisingly, unlike urea, creatinine concentration was not significantly changed up to 6 Gy. But, rats left for one month after 6 Gy demonstrated highly significant increase as shown in Table (6) and Fig. (H).
Table 6: Effect of alpha lipoic acid (30 mg/kg) on serum creatinine concentration in γ-irradiated male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without treatment</th>
<th>Creatinine (mg/dl)</th>
<th>2 Gy</th>
<th>4 Gy</th>
<th>6 Gy</th>
<th>After month of 6 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.91 ±0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALA P</td>
<td>-</td>
<td>0.89 ±0.076</td>
<td>0.73 ±0.077</td>
<td>0.83 ±0.085</td>
<td>0.96 ±0.071</td>
<td>NS</td>
</tr>
<tr>
<td>Irradiated</td>
<td>-</td>
<td>0.97 ±0.039</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALA P</td>
<td>-</td>
<td>0.87 ±0.045</td>
<td>0.74 ±0.084</td>
<td>0.73 ±0.079</td>
<td>1.73 ±0.18</td>
<td>*</td>
</tr>
<tr>
<td>Irradiated+ALA P</td>
<td>-</td>
<td>0.87 ±0.037</td>
<td>0.68 ±0.083</td>
<td>0.76 ±0.077</td>
<td>1.2 ±0.063</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means of 6 records ± standard error.

Statistical analysis was made by LSD at levels of P ≤ 0.05.

- **P** Difference from control at P ≤ 0.05.
- **P1** Difference from irradiated rat at P ≤ 0.05.
- **P2** Difference from irradiated plus ALA at P ≤ 0.05.
- **NS** Non-Significant
Fig. (H). Effect of ALA (30 mg/kg) and/or γ-radiation on the level of serum creatinine.
C. Effects of gamma irradiation on heart CK-MB:

1. Changes in CK-MB activity:

Data presented in Table (7) and Fig. (I) illustrate significant increase in heart CK-MB by 626.3 ± 4 at 2 Gy followed by significant decrease by 555.2 ± 3.4 at 4 Gy and a highly significant increase by 728 ± 6.5 and 990.8 ± 5.9 at 6 Gy and those left for one month after 6 Gy respectively as compared to normal control ones. Administration of ALA simultaneously with irradiation did not induce any significant change at all. While, pre-and simultaneous treatment during radiation decreased values of CK-MB up to 4 Gy significantly was not significantly affected after the last exposure dose.
Table 7: Effect of alpha lipoic acid (30 mg/kg) on serum CK-MB activities in γ-irradiated male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Without treatment</th>
<th>CK-MB (U/L)</th>
<th>2 Gy</th>
<th>4 Gy</th>
<th>6 Gy</th>
<th>After month of 6 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>600±4.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALA</td>
<td>-</td>
<td>532.6 ±4</td>
<td>521.2 ±2.5</td>
<td>531.3 ± 6.1</td>
<td>518.2 ± 3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Irradiated</td>
<td>-</td>
<td>626.3 ±4</td>
<td>555.2 ±3.4</td>
<td>728 ± 6. 5</td>
<td>990. 8 ±5.9</td>
<td></td>
</tr>
<tr>
<td>Irradiated+ALA</td>
<td>-</td>
<td>617.8 ±4</td>
<td>566 ± 4.2</td>
<td>677.8 ±4</td>
<td>903.8 ± 8. 3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P1</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ALA+irradiated+ALA</td>
<td>-</td>
<td>604.4 ±7.2</td>
<td>600.7 ±7</td>
<td>662.3 ±5.4</td>
<td>839.3 ±26.7</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P1</td>
<td>*</td>
<td>*</td>
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<td>NS</td>
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<tr>
<td>P2</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are expressed as means of 6 records ± standard error.

Statistical analysis was made by LSD at levels of P ≤ 0.05.

P            Difference from control at P ≤ 0.05.
P1           Difference from irradiated rat at P ≤ 0.05.
P2           Difference from irradiated plus ALA at P ≤ 0.05.
NS           Non-significant.
Fig. (I). Effect of ALA (30 mg/kg) and/or γ-radiation on the level of serum CK-MB.
Histological and histochemical studies of the liver

I-Light microscopic studies

A. Haematoxyline and Eosin (H&E):

1. Control Group:

Investigations of normal liver sections, stained with Haematoxyline and Eosin (H&E) revealed that each hepatic lobule consists of a central vein lined with simple squamous epithelium surrounded by polygonal hepatic cells radially distributed in the form of strands containing quite clear blood sinusoids in-between (Fig. 1). The cytoplasm of the hepatocytes is eosinophilic and appeared homogenous containing many uniformly distributed coarse basophilic granules. The hepatocytes are normally uni- or binucleated. The nuclei are large, vesicular and contain one or more nucleoli. There are many kupffer's cells appeared between the hepatocytes as spindle shaped cells with deeply stained nuclei surrounded by attenuated cytoplasm. The hepatic portal tract is a combination of branches of the hepatic portal vein, hepatic artery and the bile duct (Fig. 2).

2. Alpha Lipoic Acid (ALA) Group:

A liver sections of rats treated with ALA, through the treatment period (16 days) and also after one month of the last dose, showed normal hepatic architecture with many binucleated well-defined hepatocytes and normal central vein (Fig. 3). Some hepatic portal areas showed damaged wall of portal vein and bile duct (Figs. 4).

3. Irradiated Group:

Whole body exposure of rats to 6 Gy gamma irradiation delivered as fractionated dose (2 Gy each 3 day intervals to reach accumulative dose of 6 Gy) showed
different histological changes and loss of normal hepatic architectures. Where, liver sections of rats after the 1st irradiated interval (1st 2 Gy) showed dilated sinusoids, most of hepatocytes lost their nuclei showing vacuolated cytoplasm and some others had pyknotic nuclei. Also, the chromatin of some nuclei was detected as clumps restricting at the nuclear membrane (Fig.5). Widened and dilated hepatic portal veins filled with amyloidial substances as well as erythrocytes were seen. Many pyknotic nuclei were also noticed surrounding the portal vein. Some cells of bile ducts wall were seen having pyknotic nuclei and others appeared with vacuolated ones (Fig. 6).

Liver sections of rats exposed to the 2nd irradiated interval (2nd 2 Gy) showed similar effects, in addition to the progressive wideness of hepatic sinusoids, as well as the detection of irregular central veins. Most of the hepatocytes lost their nuclei and appeared with homogenous non-vesiculated cytoplasm (Fig. 7). The hepatic portal vein became more widened, congested with hemolized blood and its wall was thickened invading with many fibroblasts. Furthermore, there was a remarkable proliferation in the cellular wall of a ruptured bile duct. In addition, many pyknotic nuclei were detected (Fig. 8).

On the other hand, after the 3rd irradiated interval (3rd 2 Gy), liver sections showed the presence of hemolized central vein, prominent cellular necrosis and increased number of pyknotic nuclei (Fig. 9). The hepatic portal veins became more dilated with ruptured wall and congested with blood. Ruptured bile ducts, necrotic cells and interstial hemorrhage were also observed. In general, the liver architecture was completely obscured (Fig. 10).

Sections of rats of recovery group (rats left for one month after 6 Gy) exhibited more acute effects than the above mentioned alterations. The hepatocytes were completely destroyed and were hardly distinguished from each others. Some
pyknotic nuclei were also seen (Fig. 11). The hepatic portal veins were much thickened and filled with hemolized blood. The wall of bile ducts appeared avoids of nuclei and surrounded by aggregation of pyknotic lymphocytes (Fig. 12).

4. Simultaneously Irradiated ALA Group:

Treatment of rats with ALA, after each intervals of irradiation exposure, showed different degrees of improvement in hepatocytes architecture. In the 1st simultaneously irradiated ALA rat (1st 2 Gy), liver sections of rats showed a marked decrease in size of the dilated hepatic sinusoids. Hepatocytes with one or two nuclei and normal amount of cytoplasm were prominently seen, except few hepatocytes appeared with vacuolated cytoplasm (Fig. 13). However, there was no detectable improvement at the portal area, where most hepatocytes appeared highly vacuolated losing their stranded appearance, in addition to the altered portal area (Fig. 14).

Liver sections of rats of the 2nd simultaneously irradiated ALA group exhibited less improved picture of hepatic architecture where many widened hepatic sinusoids and vesiculated hepatocytes were obviously seen. (Fig. 15). In addition, the impaired architecture of the portal area was still detected, except remarkable decrease in number of fibroblasts and pyknotic nuclei. A remarkable feature was seen surrounding the portal area, where many eosinophilic cells were observed (Fig. 16).

Liver sections of rats exposed to the 3rd simultaneously irradiated ALA group indicated little improved picture, where some hepatocytes were appeared nearly normal with one or two nuclei, but most of them had vacuolated cytoplasm (Fig. 17). The hepatic portal areas showed slight improvement, however the wall of
portal vessels were still thickened, ruptured and invaded with many fibroblasts (Fig. 18).

Liver sections of rats of the recovery rat, showed severe alterations in hepatic architecture around the central vein as well as at the portal area (Figs. 19 and 20).

5. Pre Irradiated ALA Group:

Prophylactic use of ALA showed a remarkable improvement in preserving hepatic architecture. In the 1st pre-irradiated ALA rat, liver sections of rats exhibited remarkable improved picture indicated by the observation of stranded hepatic cells with slightly widened hepatic sinusoids, kupffer's cells and normal central veins (Fig.21). The hepatic portal areas showed no improved picture, where destruction of the wall of portal vessels, many fibroblasts and some vacuolation in cytoplasm of the surrounding hepatocytes were still observed (Fig.22).

Liver sections of rats of the 2nd pre-irradiated ALA group exhibited the same picture as the previous section with slight decrease in size of the dilated hepatic sinusoids (Fig. 23). The hepatic portal area was slightly improved with obvious decrease in the number of fibroblasts but some spindle-shaped eosinophilic cells were detected (Fig. 24).

In rats of the 3rd pre-irradiated ALA group, liver sections exhibited little improvement of hepatic architecture, where many destructed and vacuolated hepatocytes were still seen (Fig. 25). The hepatic portal areas were still without remarkable improvement (Fig. 26).

Liver sections of rats left for one month after the 3rd 2 Gy exhibited slight improvement only around the central veins (Fig. 27). On the other hand, portal
areas seemed to be more resistible against the prophylactic action of ALA, showing the prominence of the previously detected destructive features (Fig. 28).
Fig. (1): A photomicrograph of a section of the liver of a control rat, showing normal hepatic architecture of hepatocytes (h), central vein (cv), hepatic sinusoids (hs) and kupffer’s cells (arrows) (H&E., x 400).

Fig. (2): A photomicrograph of a section of the liver of a control rat, showing normal hepatic architecture of portal area (portal vein (pv) and bile duct (bd)) (H&E., x 400).
Fig. (3): A photomicrograph of a section of the liver of ALA treated rat, showing central vein (cv), widened hepatic sinusoids (hs), kupffer’s cells (arrows) and some hepatocytes with vacuolated cytoplasm (h) (H&E., x 400).
Fig. (4): A photomicrograph of a section of the liver of ALA treated rat, showing the damaged wall of a portal vein (pv) and bile duct (bd). Note also the destructed hepatocytes (h) (H&E., x 400).
Fig. (5): A photomicrograph of a section of the liver of a 1st irradiated rat (1st 2 Gy), showing central vein (cv), dilated hepatic sinusoids (hs), hepatocytes with vacuolated cytoplasm (h) and some pyknotic nuclei (arrows) (H&E., x 400).

Fig. (6): A photomicrograph of a section of the liver of a 1st irradiated rat, showing dilated and congested hepatic portal vein (pv) filled with hemolized blood (thick arrow), some pyknotic nuclei (thin arrows) and many hepatocytes with vacuolated cytoplasm (h) can be also seen (H&E., x 400).
Fig. (7): A photomicrograph of a section of the liver of a 2<sup>nd</sup> irradiated rat (2<sup>nd</sup> 2 Gy), showing central vein (cv), hepatic strands, dilated hepatic sinusoids (hs) and some hepatocytes without nuclei (h) (H&E., x 400).
Fig. (8): A photomicrograph of a section of the liver of a $2^{nd}$ irradiated rat ($2^{nd}$ 2 Gy), showing bile ducts (bd), dilated portal vein (pv) congested with hemolized blood and inflammatory cells (arrows) (H&E., x 400).

Fig. (9): A photomicrograph of a section of the liver of a $3^{rd}$ irradiated rat ($3^{rd}$ 2 Gy), showing congested central vein (cv), many ill-defined hepatocytes (h) many of which with pyknotic nuclei (arrows) (H&E., x 400).
Fig. (10): A photomicrograph of a section of the liver of a 3rd irradiated rat (3rd 2 Gy), showing a widened portal vein (pv), pyknotic nuclei (thick arrows) and some fibroblasts (thin arrows) surrounding bile duct (bd) (H&E., x 400).
Fig. (11): A photomicrograph of a section of the liver of a rat, left for one month after the $3^{rd}$ 2 Gy exposure, showing irregular central vein (cv), hepatocytes with vacuolated cytoplasm (h) and pyknotic nuclei (arrows) (H&E., x 400).

Fig. (12): A photomicrograph of a section of the liver of a rat, left for month after the $3^{rd}$ 2 Gy, showing thickened wall of portal vein (pv) and bile duct (bd) as well as empty swollen hepatocytes (h) and inflammatory cells (arrows) surrounding the bile duct (H&E., x 400).
Fig. (13): A photomicrograph of a section of the liver of a 1st simultaneously irradiated ALA rat (1st 2Gy), showing hepatocytes (h) arranged in strands, central vein (cv), hepatic sinusoids (hs) and kupffer’s cells (arrows) (H&E., x 400).
Fig. (14): A photomicrograph of a section of the liver of a 1st simultaneously irradiated ALA rat (1st 2Gy), showing dilated portal vein (pv) and the destructed wall of bile duct (bd). Note, some hepatocytes lost their nuclei (h) (H&E., x 400).

Fig. (15): A photomicrograph of a section of the liver of a 2nd simultaneously irradiated ALA rat, showing hepatocytes with vesiculated cytoplasm (h), central vein (cv), kupffer’s cells (arrows) and dilated hepatic sinusoids (hs) (H&E., x 400).
Fig. (16): A photomicrograph of a section of the liver of a 2nd simultaneously irradiated ALA rat, showing hepatic portal vein (pv) with ruptured wall and bile duct (bd). Note some pyknotic nuclei (thin arrows) and many shrunken deeply eosinophilic stained cytoplasm (thick arrows) (H&E., x 400).
Fig. (17): A photomicrograph of a section of the liver of a 3rd simultaneously irradiated ALA rat, showing a central vein (cv) surrounded with few fibroblasts, kupffer’s cells (arrows), swollen hepatocytes with vacuolated cytoplasm (h) and narrow hepatic sinusoids (hs) (H&E., x 400).

Fig. (18) A photomicrograph of a section of the liver of a 3rd simultaneously irradiated ALA rat, showing the thickened wall of hepatic portal vein (pv) and bile duct (bd), both are infiltrated with pyknotic nuclei (arrows) and fibroblasts (highlight arrows) (H&E., x 400).
Fig. (19): A photomicrograph of a section of the liver of a rat, after one month of 3rd 2 Gy, showing ill-defined hepatocytes (h), central vein (cv), some pyknotic nuclei (thin arrows) and interstitial hemorrhage (thick arrow) (H&E., x 400).
Fig. (20): A photomicrograph in the portal area of the previous section, showing fibroblasts (arrows) surrounding both the hepatic portal vein (pv) and a damaged bile duct (bd) (H&E., x 400).

Fig. (21): A photomicrograph of a section of the liver of a 1st pre irradiated ALA rat, showing normal central vein (cv) and hepatic sinusoids (hs), kupffer’s cells (arrows) and the stranded pattern of hepatocytes (h) (H&E., x 400).
Fig. (22): A photomicrograph of a section of the liver of a 1st pre irradiated ALA rat, showing the destructed wall of hepatic portal vein (pv) and cells of bile duct wall (bd) losing their nuclei. Damaged hepatocytes were prominently seen (H&E., x 400).
Fig. (23): A photomicrograph of a section of the liver of a 2nd pre-irradiated ALA rat, showing central vein (cv), kupffer’s cells (arrows) and hepatocytes (h) some of which with vacuolated cytoplasm and hepatic sinusoids (hs) (H&E., x 400).

Fig. (24): A photomicrograph of a section of the liver of a 2nd pre-irradiated ALA rat, showing thickened wall of hepatic portal vein (pv) infiltrated with fibroblasts which were also observed surrounding both bile duct (bd) and portal vein (H&E., x 400).
Fig. (25): A photomicrograph of a section of the liver of a 3\textsuperscript{rd} pre-irradiated ALA rat, showing central vein (cv), hepatocytes (h) most of which without nuclei and slightly widened hepatic sinusoids (hs) (H&E., x 400).
Fig. (26): A photomicrograph of a section of the liver of a 3rd pre-irradiated ALA rat, showing portal vein (pv) and bile duct (bd) with hyaline wall infiltrated with many fibroblasts (arrows) (H&E., x 400).

Fig. (27): A photomicrograph of a section of the liver of a rat, left for one month after the 3rd 2 Gy, showing nearly normal central vein (cv), kupffer’s cells (arrows) and hepatocytes (h) with vesiculated cytoplasm. Slightly dilated hepatic sinusoids (hs) were also seen (H&E., x 400).
Fig. (28): A photomicrograph of a section of the liver of a rat, left for one month after the 3rd 2 Gy, showing the thickened hyaline wall of hepatic portal vein (pv), ill-defined hepatocytes (h) and large number of pyknotic nuclei (arrows) surrounding bile duct (bd) (H&E., x 400).

**B. Mallory's Tripple Stain (MTS):**

1. **Control Group:**

Sections stained with Mallory's Triple stain showed the appearance of collagenous fibers, in the liver section as blue condensed threads around the portal vessels and bile ducts. Also, they were seen as little amounts around the hepatocytes and hepatic sinusoids (Fig.29).

2. **Alpha Lipoic Acid (ALA) Group:**

The collagenous fibers appeared in small amount around the hepatic vein (Fig.30).

3. **Irradiated Group:**
The collagenous fibers of the 1\textsuperscript{st} irradiated rat (1\textsuperscript{st} 2 Gy) was obviously increased around the portal areas, also obvious congestion in the portal area was observed. (Fig. 31). The amount of loose condensed collagenous fibers, after the 2\textsuperscript{nd} dose of 2 Gy, was furtherly increased than the previous dose (Fig.32). While, these fibers, after the 3\textsuperscript{rd} dose of 2 Gy exposure, were highly increased around the portal areas, showing an obvious hyalinization, especially around the congested portal vessels (Fig.33). Concerning rats left for one month after the last dose (rats left for one month after 3\textsuperscript{rd} 2 Gy), the amount of such fibers was markedly decreased around the hepatic vein. Also, the interstitial hemorrhage was clear (Fig.34).

4. Simultaneously Irradiated ALA Group:

The collagenous fibers of liver section of rats of the 1\textsuperscript{st} simultaneously irradiated ALA rat (1\textsuperscript{st} 2 Gy) showed moderate increase of its amount around the portal area (Fig.35). However, collagenous fibers in sections of rats of the 2\textsuperscript{nd} simultaneously irradiated ALA rat (2\textsuperscript{nd} 2 Gy) showed destructive appearance of such fibers at the portal area (Fig. 36). In the 3\textsuperscript{rd} simultaneously irradiated ALA rat, (3\textsuperscript{rd} 2 Gy), the amount and pattern of distribution of the collagenous fibers was slightly increased around the portal areas (Fig. 37). Concerning rats left for one month after the last dose, the collagenous fibers were slightly increased around the portal area. Interstitial hemorrhage was also observed (Fig. 38).

5. Pre Irradiated ALA Group:

In the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} pre irradiated ALA rats, the amount of collagenous fibers was decreased at the portal areas (Figs.39, 40 and 41) respectively. The better existed picture was recorded in sections of rats of the 1\textsuperscript{st} pre irradiated ALA rat followed by these of the 3\textsuperscript{rd} and the 2\textsuperscript{nd} ones. While, liver section of rats of recovery group
appeared without any obvious improvement of collagenous fibers around the portal areas (Fig. 42).
Fig. (29): A photomicrograph of a section of the liver of a control rat, stained with Mallory's Tripple Stain (MTS), showing blue condensed threads of collagen fibers around hepatic portal vein and bile duct (arrows) (MTS., x 400).
Fig. (30): A photomicrograph of a section of the liver of ALA rat, stained with (MTS), showing small amount of collagenous fibers around the hepatic vein (arrows) (MTS., x 400).

Fig. (31): A photomicrograph of a section of the liver of a 1st irradiated rat, (1st 2 Gy) stained with (MTS), showing obvious increased amount of collagen fibers around hepatic portal area (arrows). (MTS., x 400).
Fig. (32): A photomicrograph of a section of the liver of a 2\textsuperscript{nd} irradiated rat, (2\textsuperscript{nd} 2 Gy) stained with (MTS), showing the concentration of loose collagen fibers around hepatic portal vein and bile ducts (arrows) (MTS., x 400).
Fig. (33): A photomicrograph of a section of the liver of a 3rd irradiated rat (3rd 2 Gy) stained with (MTS), showing the appearance of collagen fibers as dense hyaline ill-defined fibers (arrows) around the congested portal vein and bile duct (x400).

Fig. (34): A photomicrograph of a section of the liver of a rat left for one month after 3rd 2 Gy stained with (MTS), showing the moderate amount of collagen fibers around the hepatic portal vein and bile duct (arrows). Interstitial hemorrhage was also present (MTS., x 400).
Fig. (35): A photomicrograph of a section of the liver of a 1st simultaneously-irradiated ALA rat stained with (MTS), showing less amount of collagenous fibers (arrows) around the hepatic portal vein (pv) and bile duct (bd) as compared to the above mentioned rats (MTS., x 400).
Fig. (36): A photomicrograph of a section of the liver of a 2\textsuperscript{nd} simultaneously-irradiated ALA rat stained with (MTS), showing a slight amount of fragmented collagenous fibers around the hepatic portal vein and bile duct (arrows) (MTS., x 400).

Fig. (37): A photomicrograph of a section of the liver of a 3\textsuperscript{rd} simultaneously-irradiated ALA rat, stained with (MTS), showing a slightly increased amount of collagenous fibers (arrows) around the hepatic portal vein and bile duct (MTS., x 400).
Fig. (38): A photomicrograph of a section of the liver of a rat left for one month after 3rd 2 Gy stained with (MTS), showing the amount of collagenous fibers around the hepatic portal area (arrows). Interstitial hemorrhage was also observed (MTS., x 400).
Fig. (39): A photomicrograph of a section of the liver of a 1st pre-irradiated ALA rat stained with (MTS), showing little amount of collagenous fibers around the hepatic portal area (arrows) (MTS., x 400).

Fig. (40): A photomicrograph of a section of the liver of a 2nd pre-irradiated ALA rat stained with (MTS), showing scarce amount of collagenous fibers around the portal area (arrows) (MTS., x 400).
Fig. (41): A photomicrograph of a section of the liver of a 3rd pre-irradiated ALA rat stained with (MTS), showing the slight reincrease in the amount of collagenous fibers around the portal area (arrows). Note, the congested portal vein (MTS., x 400).
C. Periodic Acid Schiff’s Reagent (PAS):

1. Control Group:

Control liver sections, stained with PAS revealed that carbohydrates are not uniformly distributed within the cytoplasm of these cells, where they were found in the majority of the hepatocytes as coarse and fine pink granules. The nuclei showed negative affinity to the reaction. The deeper the stain, the more carbohydrate intensity (Fig. 43).

2. Alpha Lipoic Acid (ALA) Group:

The amount of carbohydrate contents was moderate, especially at the plasma membrane of hepatocytes (Fig. 44).

3. Irradiated Group:

The 1st irradiated rat liver sections showed that the amount of liver carbohydrates was decreased and mainly concentrated near the basement membrane of some hepatocytes (Fig. 45). In the 2nd irradiated rat liver sections, most destructed hepatocytes showed weak reaction (Fig. 46). The 3rd irradiated rat, showed obviously increased amount of carbohydrates concentrating at the basement membrane of damaged hepatocytes (Fig. 47). Concerning rats left for one month after 3rd 2 Gy, carbohydrates content were located at the cellular margin of some hepatocytes (Fig. 48).

4. Simultaneously Irradiated ALA Control Group:
The 1\textsuperscript{st} simultaneously irradiated ALA rat liver sections, showed moderate reincrease of carbohydrates content locating at the periphery of most hepatocytes (Fig. 49).

In the 2\textsuperscript{nd} simultaneously irradiated ALA rat liver sections, few hepatocytes showed weak affinity toward the stain (Fig. 50). The 3\textsuperscript{rd} simultaneously irradiated ALA rat, revealed obvious increase of the concentration of carbohydrate contents in most hepatocytes (Fig. 51). Such picture was still prominent in liver sections of rats of recovery group (Fig. 52).

5. \textit{Pre Irradiated ALA Group:}

Most of the hepatocytes revealed improved appearance of PAS positive reaction in the liver of 1\textsuperscript{st} pre irradiated ALA control rats (1\textsuperscript{st} 2 Gy) (Fig. 53). The 2\textsuperscript{nd} pre irradiated ALA rat showed a clear decrease in carbohydrates in most cells, except few hepatocytes were positively stained (Fig. 54). Whereas, in sections of the 3\textsuperscript{rd} pre irradiated ALA rat, the carbohydrates concentrated at the periphery of hepatocytes and the cytoplasm was negatively stained (Fig. 55). Concerning rats left for one month after 3\textsuperscript{rd} 2 Gy, showed approximately similar appearance as detected in the above mentioned sections (Fig. 56).
Fig. (43): A photomicrograph of a section of the liver of a control rat, stained with Periodic Acid Schiff (PAS), showing that carbohydrates appeared as coarse and fine pink granules in cytoplasm of hepatocytes (arrows) (PAS., x 400).
Fig. (44): A photomicrograph of a section of the liver of ALA rat, (30 mg/kg) stained with (PAS), showing moderate amount of carbohydrates content, especially at the plasma membrane of hepatocytes (arrows) (PAS, x 400).
Fig. (45): A photomicrograph of a section of the liver of 1st irradiated rat, (1st 2 Gy) stained with (PAS), showing that the amount of carbohydrates was mainly concentrated near the basement membrane of some hepatocytes (arrows) (PAS., x 400).

Fig. (46). A photomicrograph of a section of the liver of 2nd irradiated rat, (2nd 2 Gy) stained with (PAS), showing most hepatocytes having decreased amount of dispersed carbohydrates (arrows) (PAS., x 400).
Fig. (47): A photomicrograph of a section of the liver of 3rd irradiated rat, (3rd 2 Gy) stained with (PAS), showing that carbohydrates were obviously increased concentrating at the basement membrane of hepatocytes (arrows) (PAS., x 400).
Fig. (48): A photomicrograph of a section of the liver of rat after one month of 3rd 2 Gy stained with (PAS), showing that most hepatocytes were positively stained especially at the margin of cellular membrane as well as vesiculated cytoplasm (arrows) (PAS., x 400).

Fig. (49): A photomicrograph of a section of the liver of 1st simultaneously-irradiated ALA rat, (1st 2 Gy) stained with (PAS), showing marked reincrease in carbohydrates content of hepatocytes (arrows) (PAS., x 400).
Fig. (50): A photomicrograph of a section of the liver of 2\textsuperscript{nd} simultaneously-irradiated ALA rat, (2\textsuperscript{nd} 2 Gy) stained with (PAS) showing most of hepatocytes having weak affinity towards the stain. Note the stained nuclear membranes (arrows) (PAS., x 400).
Fig. (51): A photomicrograph of a section of the liver of 3rd simultaneously-irradiated ALA rat, stained with (PAS), showing the increased amount of carbohydrates concentrating at the margin of hepatocytes (arrows) (PAS., x 400).

Fig. (52): A photomicrograph of a section of the liver of 4th simultaneously-irradiated ALA rat, stained with (PAS), showing some hepatocytes with high carbohydrates content (arrows) and others with low content (pale colour) (PAS., x 400).
Fig. (53): A photomicrograph of a section of the liver of 1st pre-irradiated ALA rat, stained with (PAS), showing moderate increased carbohydrate contents at the cellular membrane of most hepatocytes (arrows) and others with low content (pale colour) (PAS., x 400).
Fig. (54): A photomicrograph of a section of the liver of 2\textsuperscript{nd} pre-irradiated ALA rat, stained with (PAS), showing clear decrease in carbohydrate contents of most hepatocytes (arrows) (PAS., x 400).

Fig. (55): A photomicrograph of a section of the liver of rat left for one month after 3\textsuperscript{rd} 2 Gy, stained with (PAS), showing obviously increased carbohydrate contents at the basement membrane of many hepatocytes (arrows) (PAS., x 400).
Fig. (56): A photomicrograph of a section of the liver of rat left for one month after 3rd 2 Gy, stained with (PAS), showing some hepatocytes with increased carbohydrate contents at the basement membrane (arrows) (PAS., x 400).

II-Ultrastructural studies

1. Control Group:

The ultrastructure of normal hepatocyte showed the detection of normal nucleus with euo-and-heterochromatin, mitochondria, rough endoplasmic reticulum and few droplets of lipid (Fig. 57).

2. Irradiated Group:

Whole body exposure of rats to 2 Gy gamma irradiation showed degeneration of cytoplasmic components of the hepatocytes and ruptured cristae of mitochondria. Dilated bile canal and detection of active lysosome as well as droplets of lipid were also seen (Fig. 58). On the other hand, after the 3rd irradiated interval (6 Gy), liver sections showed the appearance of dilated and congested blood sinusoid, swollen
spaces of degenerated mitochondria and fragmentation of rough endoplasmic reticulum (Fig. 59).

3. Simultaneously Irradiated ALA Group:

Treatment of rats with ALA, after each interval of irradiation exposure, showed different degrees of improvement in hepatocytes architecture. In the 1\textsuperscript{st} simultaneously irradiated ALA rat (1\textsuperscript{st} 2 Gy), liver sections of rats showed regenerated mitochondria, the detection of rough endoplasmic reticulum. Normal nucleus and bile canal were also observed (Fig. 60). Liver sections of rats of the 3\textsuperscript{rd} simultaneously irradiated ALA group indicated normal nucleus with euo and heterochromatin, endoplasmic reticulum, elongated mitochondria and bile canal. Active lysosome can be detected (Fig. 61).

4. Pre Irradiated ALA Group:

Prophylactic use of ALA showed a remarkable improvement in preserving hepatic architecture. In the 1\textsuperscript{st} pre-irradiated ALA rat, liver sections of rats exhibited remarkable improved picture indicated by regeneration of nucleus, rough endoplasmic nucleus and bile canal (Fig. 62). However hepatocytes of the 3\textsuperscript{rd} pre-irradiated ALA rats, exhibited active nucleus with two peripheral nucleolus and numerous electron dense mitochondria associated with the detection of many lipid droplets (Fig. 63).
Fig.(57): Electron micrograph of control rat liver cell showing normal nucleus (N), mitochondria (M) and rough endoplasmic reticulum (rER). Note the presence of few lipid droplets (L). (X 2600).

Fig.(58): Electron micrograph of hepatocyte of albino rat irradiated with 2 Gy after 3 days from the exposure showing degeneration in cytoplasmic components of the hepatocyte and active
lysosome (arrow) near the dilated bile canal (Bc). Also, rough endoplasmic reticulum (RER) was still seen (X 2600).

Fig.(59): Electron micrograph of section of a hepatocyte of albino rat irradiated with 6 Gy after 3 days from the exposure showing swollen spaces of degenerated mitochondria (SM) and dilated blood sinusoid (BS) in between 5 parts of hepatocytes and fragmentation of endoplasmic reticulum (arrow). (X 1600).
Fig.(60): Electron micrograph of a binucleated hepatocyte of albino rat treated with ALA for 3 days post irradiation (2 Gy), showing regeneration of mitochondria (M). The bile canal was observed (Bc). (X 2600).
Fig.(61): Electron micrograph of a hepatocyte in liver of albino rat treated with ALA for 3 days post irradiation (6 Gy), showing regenerated nucleus (N), rough endoplasmic reticulum (rER) and elongated mitochondria (M). The bile canal (Bc) and active lysosome (arrows) were observed. (X 2000).
Fig.(62): Electron micrograph of a binucleated hepatocyte of pre-irradiated ALA rat after 2 Gy, rich with mitochondria (M) and regenerated bile canals (Bc). Desmosomes can be also seen (arrow). (X 2000).

Fig.(63): Electron micrograph of a hepatocyte in liver of pre-irradiated ALA rat after 6 Gy, showing active nucleus (N) with peripheral nucleolus and numerous electron dense mitochondria (M). Lipid droplets were observed (L). (X 2000).
Histological and histochemical studies of the kidney

I- Light microscopic studies

A. Haematoxyline and Eosin (H&E):

1. Control Group:

Normal kidney consists of an outer cortex and inner medulla. The outer cortex contains the renal corpuscles which appear as large spherical structures and renal tubules (proximal and distal convoluted tubules). Each renal corpuscle is surrounded by thin capsule (Bowman's capsule) composed of simple squamous epithelial cells; this capsule encloses the urinary space and the capillary tuft or (glomerulus) which appears as large cellular mass consists of capillary endothelial cells, mesangial cells and outer podocytes which represent the visceral layer of Bowman's capsule. The proximal convoluted tubules have larger diameter than the distal tubules and have brush borders and narrow lumens, the walls of the proximal convoluted tubules consists of few cuboidal cells of large and spherical nuclei. The distal tubules have no brush borders their lumens are wider and more conspicuous than that of proximal convoluted tubules, the walls of the distal convoluted tubules are formed of large number of smaller cells than those of the proximal convoluted tubules (Fig. 64).
The medullary rays are composed of descending and ascending limbs of Henle's loop, and collecting tubules. The walls of the descending limbs are formed of simple squamous epithelium, while the ascending limbs are lined by low cuboidal cells. The collecting tubules consist of cuboidal epithelial cells with lumens as those of the distal tubules (Fig. 65).

2. **Alpha Lipoic Acid (ALA) Group:**

The kidney of male rats treated with ALA (30 mg/kg body wt) for 16 days showed slight histological changes (Fig. 66). On the other hand, the medullary region showed no changes and appeared almostly, like those of normal kidneys, except detection of few pyknotic nuclei (Fig. 67).

3. **Irradiated Group:**

Inspected kidney sections obtained from male rats irradiated with 2 Gy of gamma-rays (as 1st dose) revealed different histological lesions. These lesions included obvious shrunken in glomerulus, with great reduction in its cellularity and as a result, dilation in the urinary space occurred. Ruptured wall of Bowman's capsule, dilation in the lumen of renal tubules, pyknotic nuclei and large number of fibroblasts were also present (Fig. 68). Some renal tubules (ascending and descending loop of Henel) of the medulla showed vacuolated cytoplasm and some pyknotic nuclei, in addition to signs of interstitial hemorrhage were detected. Few areas of hyaline basophilic background were also observed (Figs. 69). After the 2nd dose of 2 Gy, progressive changes were reported manifested by the detection of pyknotic nuclei in cells of the destructed renal tubule. Some glomeruli appeared swollen with diffused and fragmented tufts congested with blood and others appeared damaged loosing, approximately, its cellularity (Fig. 70). Cells of the
renal tubules of the medulla showed vacuolated cytoplasm and pyknotic nuclei. In addition to the dilation of ascending and descending tubules, signs interstitial hemorrhage was also detected (Fig. 71).

After the 3rd dose of 2 Gy, the changes in the tissues were more obvious than those detected in the previous doses. Most of the cells of the renal tubules were destructed. The glomeruli showed an obvious degree of shrinkage with rupture and loose glomerular tufts. Pyknotic nuclei were prominently detected (Fig. 72). Cells of both ascending and descending loop of Henel of the medulla showed predominant pyknotic picture manifested by the detection of many pyknotic nuclei as well as other swollen ones. Also, marked interstitial congestion was seen surrounding large areas of hyaline basophilic background, including damaged Henle's loops were observed (Fig. 73). Rats left for one month after the 3rd 2 Gy of gamma radiation, revealed more progressive degenerated picture. The wall of Bowman's capsule became irregular and ruptured. Clear destructive alterations were also seen in the glomerular tufts and renal tubules (Fig. 74). Both types of renal tubules of the medulla were still abnormal, showing non-improved signs, where the pyknotic picture was prominent (Fig. 75).

**4. Simultaneously Irradiated ALA Group:**

Simultaneous treatment of rats with ALA and 1st 2 Gy of gamma radiation revealed slight improved picture. Fragmentation of the glomerular tufts was still seen in addition to the destructed cellularity of both proximal and distal tubules (Fig. 76). The medulla showed little improvement and some pyknotic nuclei were detected (Fig. 77). After the 2nd 2 Gy of gamma radiation, rats showed marked improvement in the cortex compared to those of the previous group (Fig. 78). However, cells of both types of renal tubules in the medulla showed some pyknotic nuclei and degenerated cytoplasm (Fig. 79).
After the 3rd 2 Gy, some cells of both proximal and distal tubules appeared normal. Many pyknotic nuclei associated with the detection of interstitial hemorrhage were obviously seen. Such hemorrhagic picture was also detected in the glomerular tuft of Bowman's capsule (Fig. 80). The renal tubules in the medulla were dilated, showing many pyknotic nuclei and degenerated cytoplasm (Fig. 81). Rats left for one month after the 3rd 2 Gy of gamma radiation "recovery test" showed limited improvement in both cortex and medulla as compared to that of the recovery test of the irradiated rat. Glomeruli were shrunken and others were swollen with fragmented tufts. Most cells of both types of tubules were destructed and few of these had vacuolated cytoplasm with pyknotic nuclei (Fig. 82). The renal tubules in the medulla showed the detection of many pyknotic nuclei and degenerated cytoplasm (Fig. 83).

5. Pre Irradiated ALA Group:

Kidney sections of rats of this group showed an obvious improvement compared to irradiated ones with the exception of the detection of some histological changes. After 1st 2 Gy, few nearly normal proximal and distal tubules were detected, but the glomerulus was congested with blood (Fig.84). The renal tubules of the medulla showed normal appearance, except the detection of few pyknotic nuclei and interstitial hemorrhage (Fig. 85). After the 2nd 2 Gy, many tubules were destructed and the glomerular tufts were more fragmented (Fig.86). Many pyknotic nuclei were seen in the medullary region (Fig. 87).

After the 3rd 2 Gy exposure, the pyknotic picture in renal tubules was almostly predominant and the interstitial hemorrhage in the vacuolated glomerular was still seen (Fig. 88). The medulla showed the presence of many pyknotic nuclei and
interstitial hemorrhage was still present (Fig. 89). Rats left for one month after the 3rd 2 Gy showed no marked improvement in few cortical renal tubules, but pyknotic nuclei, hemorrhage and vacuolated glomeruli were still seen (Fig. 90). The medulla showed the same destructive pictures in both types of tubules Many pyknotic nuclei were still seen (Fig. 91).
Fig. (64). A photomicrograph of a section in the cortex of the kidney of a normal control rat, illustrating the normal appearance of Bowman's capsule (thin arrows), urinary space (thick arrows) and glomerular tuft (g). Note the proximal convoluted tubules (pt) and distal convoluted tubules (dt) (H&E., x 400).

Fig. (65). A photomicrograph of a section in the medulla of the kidney of a normal control rat, illustrating the normal appearance of renal tubules. Note the ascending (al) and the descending (dl) loop of Henel (H&E., x 400).
Fig. (66). A photomicrograph of the cortex of the kidney of an ALA treated rat, showing the proximal convoluted tubules (pt), distal convoluted tubules (dt), Bowman's capsule (thin arrows), urinary space (thick arrows) and glomerular tuft (g) (H&E., x 400).
Fig. 67. A photomicrograph of the medulla of the kidney of an ALA treated rat, showing the medullary tubules (the ascending (al) and the descending (dl) loop of Henel) and few pyknotic nuclei can be also detected (arrows) (H&E., x 400).

Fig. (68). A photomicrograph of a section in the cortex of the kidney of a 1st irradiated rat (1st 2 Gy), showing ruptured wall of Bowman's capsule, obvious shrunken glomerular tuft (g) as well as the widened urinary space (thick arrows) and Cellular damaged of both distal (dt) and proximal (pt) tubules were also seen. Note also some fibroblasts (arrow head) and few pyknotic nuclei (thin arrows) (H&E., x 400).
Fig. (69). A photomicrograph of the medulla of the above mentioned section, showing pale cytoplasm in both ascending (al) and descending (dl) loop of Henel. Signs of interstitial hemorrhage (arrow head) and pyknotic nuclei (arrows) were detected (H&E., x 400).

Fig. (70). A photomicrograph of the cortex of the kidney of a 2\textsuperscript{nd} irradiated rat (2\textsuperscript{nd} 2 Gy), showing pale cytoplasm, some cells of renal tubule (pt & dt) lost their nuclei and others with
pyknotic ones (thin arrows). The glomeruli appeared swollen with diffused and fragmented tufts (g) and congested with blood. Interstitial hemorrhage (arrow heads) was also detected surrounding the Bowman's capsule (H&E., x 400).

Fig. (71). A photomicrograph in the medulla of the above mentioned section, showing cells of medullary tubules (al & dl) with degenerated cytoplasm, pyknotic nuclei (thin arrows), dilation of ascending (al) and descending (dl) loop of Henel. Also, signs of interstitial hemorrhage (thick arrows) was detected (H&E., x 400).
Fig. (72). A photomicrograph of the cortex of the kidney of a 3rd irradiated rat (3rd 2 Gy), showing damaged cellular wall of the proximal (pt) and distal (dt) tubules. The glomeruli showed obvious degree of shrinkage with rupture glomerular tufts (g) and dilated urinary spaces (thick arrows). Pyknotic nuclei were detected (thin arrows) (H&E., x 400).
Fig. (73). A photomicrograph of the medulla of the previous section, showing most cellular wall of both ascending (al) and descending (dl) loop of Henel with degenerated cytoplasm (thick arrows) and many pyknotic nuclei (thin arrows) (H&E., x 400).

Fig. (74). A photomicrograph of the cortex of a kidney of rat left for one month after 3rd 2 Gy, showing the degenerated renal tubules. Swollen and fragmented glomerular tufts (g) as well as widened urinary space (thick arrows) were also detected (H&E., x 400).
Fig. (75). A photomicrograph of the medulla of the above mentioned section, showing most cells of the ascending (al) and descending (dl) loop of Henel with degenerated cytoplasm (thick arrows), pyknotic nuclei (thin arrows) (H&E., x 400).
Fig. (76). A photomicrograph of the cortex of the kidney of a 1st simultaneously irradiated ALA rat, showing most proximal (pt) and distal (dt) tubules with destructed cellular wall and a glomerulus with fragmented tufts (g) (H&E., x 400).

Fig. (77). A photomicrograph of the medulla of the above section, showing many ascending (al) and descending (dl) loop of Henel having pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).
Fig. (78). A photomicrograph of the cortex of the kidney of a 2\textsuperscript{nd} simultaneously irradiated ALA rat, showing nearly normal renal tubules, glomerulus (g) and narrow urinary space (thick arrows). Both types of renal tubules (pt & dt) had narrow lumen filled with amyloid threads (H&E., x 400).
Fig. (79). A photomicrograph of the medulla of the above section, showing the ascending (al) and descending (dl) loop of Henel with some pyknotic nuclei (arrows) (H&E., x 400).

Fig. (80). A photomicrograph of the cortex of the kidney of a 3rd simultaneously irradiated ALA rat, showing shrunken and fragmented glomerular tufts (g) and widened urinary space (thick arrows). Some pyknotic nuclei in the damaged wall of renal tubules were also present (thin arrows) (H&E., x 400).
Fig. (81). A photomicrograph of the medulla of the above section, showing some cells in ascending (al) and descending (dl) loop of Henel with Pyknotic nuclei (arrows) (H&E., x 400).
Fig. (82). A photomicrograph of the cortex of the kidney of a simultaneously irradiated ALA rat left for one month after 3$^{\text{rd}}$ 2 Gy, showing the swollen glomerulus (g). Many pyknotic nuclei (thin arrows) and fibroblasts (arrow heads) were present (H&E., x 400).

Fig. (83). A photomicrograph of the medulla of the above section, showing the ascending (al) and descending (dl) loop of Henel with pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).
Fig. (84). A photomicrograph of the cortex of the kidney of a 1st pre irradiated ALA rat, showing few nearly normal renal tubules, fibroblasts (thin arrow) and interstitial hemorrhage (thick arrows). Glomerulus was also congested with blood (g) (H&E., x 400).
Fig. (85). A photomicrograph of the medulla of the above section, showing few ascending (al) and descending (dl) loop of Henel with pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).

Fig. (86). A photomicrograph of the cortex of the kidney of a 2nd pre irradiated ALA rat, showing damaged and ill-defined renal tubules. The glomerulus was slightly shrunken (g) (H&E., x 400).
Fig. (87). A photomicrograph of the medulla of the above section, showing both ascending (al) and descending (dl) loop of Henel with many pyknotic nuclei (thin arrows) and degenerated cytoplasm (thick arrows) (H&E., x 400).
Fig. (88). A photomicrograph of the cortex of the kidney of a 3rd pre irradiated ALA rat, showing the fragmented and vacuolated glomerulus (g). Some pyknotic nuclei (arrows) and destructed renal tubules (pt & dt) were detected (H&E., x 400).

Fig. (89). A photomicrograph of the medulla of the above section, showing both ascending (al) and descending (dl) loop of Henel with some pyknotic nuclei (thin arrows). Interstitial hemorrhage was detected (thick arrows) (H&E., x 400).
Fig. (90). A photomicrograph of the cortex of the kidney of a pre irradiated ALA rat left for one month after 3rd 2 Gy, showing obvious shrunken and vacuolated glomerulus (g). Damaged renal tubules with many pyknotic nuclei were observed (thin arrows) (H&E., x 400).
Fig. (91). A photomicrograph of the medulla of the above section, showing some widened ascending (al) and descending (dl) loop of Henel with many pyknotic nuclei (arrows) (H&E., x 400).

**B. Mallory's Triple Stain (MTS):**

1. **Control Group:**

Kidney sections stained with Mallory's Triple Stain revealed that a little amount of collagenous fibers were found around the renal tubules and a moderate amount, in the glomerular tuft. The blood corpuscles were stained with bright red colour (Fig. 92). Also, little amount of collagenous fibers were found around the ascending and descending tubules in the medulla (Fig. 93).

2. **Alpha Lipoic Acid (ALA) Group:**

The collagenous fibers showed little increase of those amounts around the renal tubules and in the glomerular tuft (Fig. 94). Such increase was also observed in an irregular manner around the renal tubules in the medulla (Fig. 95).

3. **Irradiated Group:**

In the 1st irradiated rat (2 Gy), the amount of collagenous fibers in both cortex and medulla were obviously increased around the damaged basement membranes of the renal tubules. A clear interstitial hemorrhage can be seen. Note, also the hemorrhage in the shrunken glomerular tufts (Figs. 96 & 97). The amount of collagenous fibers after the 2nd 2 Gy in both cortex and medulla were obviously decreased and the interstitial blood cells were still present (Figs. 98 & 99). While, the amount of collagenous fibers after the 3rd 2 Gy in cortex was rarely detected around the damaged renal tubules of the cortical region and Bowman's capsule (Fig. 100). The medullary tubules showed a slight reincrease in the amount of
collagenous fibers around some ascending (al) and descending (dl) loop of Henel. The interstitial blood cells were still present (Fig. 101). Concerning rats of the recovery group, the amount of collagenous fibers in both cortex and medulla was obviously reincreased around the renal tubules and glomerular tufts. Also, interstitial hemorrhage was still present. A marked appearance of amyloid substances was obviously recorded in the medullary region (Figs. 102 & 103).

4. Simultaneously Irradiated ALA Group:

In the 1\textsuperscript{st} simultaneously irradiated ALA rat (1\textsuperscript{st} 2 Gy), the amount of collagenous fibers in both cortex and medulla was moderately increased around the renal tubules and Bowman's capsules (Fig. 104). On the other hand, there was not any remarkable change in its amount around the ascending and descending tubules of the medulla (Fig. 105). The amount of collagenous fibers in the cortex of the 2\textsuperscript{nd} simultaneously irradiated ALA rat was increased in the glomerular tufts and around the renal tubules of both cortex and medulla (Figs. 106 & 107). In the 3\textsuperscript{rd} simultaneously irradiated ALA rat, the amount of collagenous fibers in both cortex and medulla was obviously increased around the renal tubules and altered glomerular tufts. Also, their amount was markedly increased around most of both ascending and descending tubules in the medulla. Interstitial blood cells still present (Figs. 108 & 109). Kidney sections of rats of the recovery rat showed a little amount of collagenous fibers around the cortical renal tubules (fig. 110). Obviously increased amount of collagenous fibers were seen around the ascending (al) and descending (dl) loop of Henel (Figs. 111).

5. Pre Irradiated ALA Group:

In the 1\textsuperscript{st} pre irradiated ALA rat (1\textsuperscript{st} 2 Gy) the amount of collagenous fibers in both cortex and medulla were obviously present around the renal tubules of the cortex as
well as around Bowman's capsule. Some of the medullary renal tubules appeared surrounded with little amount of collagen fibers (Figs. 112 & 113). The amount of collagenous fibers in cortex of the 2\textsuperscript{nd} pre irradiated ALA rat was slightly increased around the destructed renal tubules of cortex and medulla (Figs. 114 & 115).

In the 3\textsuperscript{rd} pre irradiated ALA rat, the amount of collagenous fibers in both cortex and medulla were obviously seen around some cortical renal tubules, but they were mainly in the glomerular tufts. Interstitial blood cells still present (Figs. 116 & 117). Kidney sections of rats of the recovery rat showed the pre irradiated ALA rat revealed that the amount of collagenous fibers in both cortex and medulla was markedly increased around the destructed renal tubules and glomerular tufts. The intensity of these fibers was markedly increased around most of ascending and descending tubules (Figs. 118 & 119).
Fig. (92). A photomicrograph of the cortex of the kidney of a normal control rat, showing the little amount of collagenous fibers around the renal tubules and the wall of Bowman's capsule (thin arrows). A moderate amount of these fibers was detected in the glomerular tuft (thick arrows) (MTS., x 400).
Fig. (93). A photomicrograph of the medulla of the previous section, showing little amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (94). A photomicrograph of the cortex of the kidney of an ALA treated rat, showing little increase in the amount of collagenous fibers around the renal tubules and glomerular tuft (arrows) (MTS., x 400).
Fig. (95). A photomicrograph of the medulla of the previous section, showing slight increase in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).
Fig. (96). A photomicrograph of the cortex of the kidney of a 1st irradiated rat (1st 2 Gy), showing the obvious increase in the amount of collagenous fibers around the renal tubules and inside glomerular tuft (arrows) (MTS., x 400).

Fig. (97). A photomicrograph of the medulla of the previous section, showing little increase in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).
Fig. (98). A photomicrograph of the cortex of the kidney of a 2nd irradiated rat (2nd 2 Gy), showing the obviously decreased amount of collagenous fibers around the renal tubules and inside the glomerular tuft (arrows) (MTS., x 400).
Fig. (99). A photomicrograph of the medulla of the previous section, showing rare amount of collagenous fibers around some ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (100). A photomicrograph of the cortex of the kidney of a 3rd irradiated rat (3rd 2 Gy), showing the marked decrease in the amount of collagenous fibers around the renal tubules and inside the glomerulus (arrows) (MTS., x 400).
Fig. (101). A photomicrograph of the medulla of the previous section, showing a slight reincrease in the amount of collagenous fibers around some ascending (al) and descending (dl) loop of Henel (arrows). Interstitial hemorrhage was prominently seen (MTS., x 400).
Fig. (102). A photomicrograph of the cortex of the kidney of an irradiated rat left for one month after 3rd 2 Gy, showing the increased amount of collagenous fibers around the renal tubules as well as glomerular tuft (arrows) (MTS., x 400).

Fig. (103). A photomicrograph of the medulla of the previous section, showing obvious increase in the amount of collagenous fibers around some ascending (al) and descending (dl) loop of Henel (thin arrows). Interstitial blood cells and amyloid substances (thick arrows) were seen (MTS., x 400).
Fig. (104). A photomicrograph of the cortex of the kidney of a 1<sup>st</sup> simultaneously irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules and the glomerular tufts (arrows) (MTS., x 400).
Fig. (105). A photomicrograph of the medulla of the previous section, showing moderate increased in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (106). A photomicrograph of the cortex of the kidney of a 2nd simultaneously irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules that was also seen in the glomerular tufts (arrows) (MTS., x 400).
Fig. (107). A photomicrograph of the medulla of the previous section, showing moderate increased in the amount of collagenous fibers around the ascending (al) and descending loop of Henel (dl) (arrows) (MTS., x 400).
Fig. (108). A photomicrograph of the cortex of the kidney of a 3rd simultaneously irradiated ALA rat, showing the obviously increased amount of collagenous fibers around the renal tubules and glomerular tufts (arrows) (MTS., x 400).

Fig. (109). A photomicrograph of the medulla of the previous section, showing the obviously increased amount of collagenous fibers around the ascending (al) and descending loop of Henel (dl) (arrows) (MTS., x 400).
Fig. (110). A photomicrograph of the cortex of the kidney of a simultaneously irradiated ALA rat left for one month after 3rd 2 Gy, showing the little amount of collagenous fibers around the renal tubules (arrows) (MTS., x 400).
Fig. (111). A photomicrograph of the medulla of the previous section, showing the obviously increased amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (112). A photomicrograph of the cortex of the kidney of a 1st pre irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules and few amounts in the glomerular tufts (arrows) (MTS., x 400).
Fig. (113). A photomicrograph of the medulla of the previous section, showing little amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).
Fig. (114). A photomicrograph of the cortex of the kidney of a 2nd pre irradiated ALA rat, showing the slight increase in the amount of collagenous fibers around the renal tubules and glomeruli (arrows) (MTS., x 400).

Fig. (115). A photomicrograph of the medulla of the previous section, showing the increase in the amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).
Fig. (116). A photomicrograph of the cortex of the kidney of a 3rd pre irradiated ALA rat, showing the amount of collagenous fibers around the renal tubules and in the glomerular tufts (arrows) (MTS., x 400).
Fig. (117). A photomicrograph of the medulla of the previous section, showing the decreased amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

Fig. (118). A photomicrograph of the cortex of the kidney of pre irradiated ALA rat left for one month after 3rd 2 Gy, showing increased amount of collagenous fibers around the renal tubules and glomerular tufts (arrows) (MTS., x 400).
Fig. (119). A photomicrograph of the medulla of the previous section, showing the obviously increased amount of collagenous fibers around the ascending (al) and descending (dl) loop of Henel (arrows) (MTS., x 400).

C. Periodic Acid Schiff’s Reagent:

1. Control Group:

Carbohydrates were demonstrated by Periodic Acid Schiff’s Reagent (PAS) and appeared as pink colour in the brush borders and basement membranes of the renal tubules of cortex. Also, the glomerular tufts were stained deeply than the brush borders of renal tubules. The cytoplasm of the tubules was stained faintly, while the nuclei, in general, appeared negatively stained (Fig. 120). The deeper the stain the increased carbohydrate contents.

2. Alpha Lipoic Acid (ALA) Group:
Carbohydrates were moderately increased in cortical renal tubules. The basement membranes and brush borders of the renal tubules as well as in the glomerular tufts showed higher affinity to the stain than their cytoplasm (Fig. 121).

3. **Irradiated Group:**

In the 1\textsuperscript{st} irradiated rat (1\textsuperscript{st} 2 Gy), the carbohydrate contents were slightly increased at the basement membrane of renal tubules, but they were slightly decreased in their brush borders (Fig. 122). In the 2\textsuperscript{nd} irradiated rat, carbohydrates were obviously decreased at the basement membranes of renal tubules and around the wall of Bowman's corpuscles (Fig. 123).

In the 3\textsuperscript{rd} irradiated rat, carbohydrates were obviously increased at basement membranes and brush borders of the cortical renal tubules, but they were slightly decreased in the brush borders of proximal tubules (Fig. 124). Concerning rats left for one month after the 3\textsuperscript{rd} 2 Gy, carbohydrates were obviously increased at the basement membranes and brush borders of the renal tubules as well as the glomerulus (Fig. 125).

4. **Simultaneously Irradiated ALA Group:**

In the 1\textsuperscript{st} simultaneously irradiated ALA rat (1\textsuperscript{st} 2 Gy), carbohydrates were obviously increased at the glomerulus and renal tubules (Fig. 126). In the 2\textsuperscript{nd} simultaneously irradiated ALA rat, an obvious increase of carbohydrate contents in the glomerulus and basement membranes of renal tubules was observed. A low affinity toward the stain was seen in the brush borders (Fig. 127).

The increase in carbohydrate contents in the 3\textsuperscript{rd} simultaneously irradiated ALA rat was greatly similar to those of the previously described rat (Fig. 128).
Carbohydrates were slightly increased at both basement membranes and brush borders of the proximal tubules in simultaneously irradiated ALA rat left for one month after the 3rd 2 Gy (Fig. 129).

5. Pre Irradiated ALA Group:

Carbohydrate contents were obviously increased in the renal tubules of the 1st pre irradiated ALA rat. The basement membranes and brush borders of the renal tubules showed higher affinity to the stain than their cytoplasm (Fig. 130). The increase in carbohydrate contents in the cortex of the 2nd and 3rd pre irradiated ALA rat was obviously seen at the basement membranes and brush borders of the renal tubules. The cytoplasm of most cells was negatively stained (Figs. 131 & 132). The amount of carbohydrates in the cortex of a pre irradiated ALA rat left for one month the 3rd 2 Gy was greatly similar to those of the last described rat (Fig. 133).
Fig. (120). A photomicrograph of the cortex of the kidney of a normal control rat, showing the normal distribution of carbohydrate contents in renal tubules and glomerulus (arrows) (PAS., x 400).
Fig. (121). A photomicrograph of the cortex of the kidney of an ALA treated rat, showing moderate amount of carbohydrates in both basement membranes and brush borders of the renal tubules as well as in the glomerular tufts (arrows) (PAS., x 400).

Fig. (122). A photomicrograph of the cortex of the kidney of a 1st irradiated rat, showing that carbohydrate contents were reincreased in glomerulus and the basement membrane of renal tubules (arrows) (PAS., x 400).
Fig. (123). A photomicrograph of the cortex of the kidney of a 2\textsuperscript{nd} irradiated rat, showing slight decrease of carbohydrate contents in both glomerulus and basement membranes of renal tubules (arrows) (PAS., x 400).
Fig. (124). A photomicrograph of the cortex of the kidney of a 3rd irradiated rat, showing obvious increase of carbohydrates in the glomerulus and basement membranes of renal tubules (arrows). Note the slightly decrease in the inner brush borders (PAS., x 400).

Fig. (125). A photomicrograph of the cortex of the kidney of an irradiated rat left for one month after 3rd 2 Gy, showing obviously increased amount of carbohydrates in most renal tubules as well as the glomerulus (arrows) (PAS., x 400).
Fig. (126). A photomicrograph of the cortex of the kidney of a 1st simultaneously irradiated ALA rat, showing an obvious increase of carbohydrates in both glomerulus and most renal tubules (arrows) (PAS., x 400).
Fig. (127). A photomicrograph of the cortex of the kidney of a 2\textsuperscript{nd} simultaneously irradiated ALA rat, showing the increase of carbohydrate contents in glomerulus and renal tubules (arrows) (PAS., x 400).

Fig. (128). A photomicrograph of the cortex of the kidney of a 3\textsuperscript{rd} simultaneously irradiated ALA, showing the increase of carbohydrates in glomerulus and inner borders of renal tubules as well as basement membrane (arrows) (PAS., x 400).
Fig. (129). A photomicrograph of the cortex of the kidney of a simultaneously irradiated ALA rat left for one month after the 3rd 2 Gy, showing the obvious increase of carbohydrates in the renal tubules (arrows). The high affinity to the stain in basement membranes and brush borders can be seen (PAS., x 400).
Fig. (130). A photomicrograph of the cortex of the kidney of a 1\textsuperscript{st} pre irradiated ALA rat, showing the obvious increase of carbohydrate contents (arrows) (PAS., x 400).

Fig. (131). A photomicrograph of the cortex of the kidney of a 2\textsuperscript{nd} pre irradiated ALA rat, showing the increased amount of carbohydrate contents (arrows) (PAS., x 400).
Fig. (132). A photomicrograph of the cortex of the kidney of a 3rd pre irradiated ALA rat, showing the obviously increased amount of carbohydrates (arrows) (PAS., x 400).
Fig. (133). A photomicrograph of the cortex of the kidney of pre irradiated ALA rat left for one month after 3\textsuperscript{rd} 2 Gy, showing the increase of carbohydrate contents in glomerulus and most renal tubules (arrows) (PAS., x 400).
II-Ultrastructural studies:

1. Control Group:

Electron micrograph of a part of a normal glomerulus showed normal glomerular basement membrane, podocyte and foot processes (Fig. 134). On the other hand, the apical region of proximal convoluted tubule exhibited the presence of nucleolus. Mitochondria having many cristae and brush border were also seen (Fig. 135).

2. Irradiated Group:

Inspected kidney sections obtained from male rats irradiated with 2 Gy of gamma-rays (as 1st dose) revealed complete degeneration of foot processes (Fig. 136). The cells of proximal convoluted tubule showed ruptured brush border and many mitochondria with ruptured cristae (Fig. 137). After the 3rd dose of 2 Gy, the changes in the tissues were more obvious than those detected in the previous doses. Most of the cells of the proximal convoluted tubules were destructed. Swollen mitochondria and vacuolated cytoplasm were also seen (Fig. 138).

3. Simultaneously Irradiated ALA Group:

The simultaneous irradiated ALA treated rats after 1st 2 Gy of gamma radiation, revealed the reappearance of glomerular foot processes (Fig. 139). After the 3rd 2 Gy, the glomerular tuft showed podocytes with normal foot processes (Fig. 140). The cells of proximal convoluted tubules showed many collapsed mitochondria and some vacuolated areas in cytoplasm (Fig. 141).

4. Pre Irradiated ALA Group:

Kidney sections of rats of the 1st pre-irradiated ALA group showed that the proximal convoluted tubule had many elongated mitochondria and nucleus with
heterochromatin restricted at the nuclear membrane (Fig. 142). After the 3rd 2 Gy exposure, the glomerular tuft of pre-irradiated ALA rats showed regenerated podocytes with normal foot processes. The epithelial cells lining Bowman’s capsule with normal basal nucleus (Fig. 143). The apical region of the proximal convoluted tubule revealed regenerated epithelial cells rich with elongated mitochondria (Fig. 144).
Fig. (134): Electron micrograph of a part of a normal glomerulus of albino rat’s kidney showing podocytes, foot process (arrows) and glomerular basement membrane. (X 2000).
Fig. (135): Electron micrograph of a part of the apical region of proximal convoluted tubule of albino rat’s kidney, showing normal cristae of mitochondria (M) and brush border (BB). Lipid droplets can be seen (L). (X 2600).

Fig. (136): Electron micrograph of a part of glomerular tuft of albino rat’s kidney irradiated with 2 Gy after 3 days from the exposure, showing complete degeneration of foot processes (arrows). (X 1300).
Fig. (137): Electron micrograph of a part of a proximal convoluted tubule of albino rat’s kidney irradiated with 2 Gy after 3 days from the exposure, showing ruptured brush border (BB) and many mitochondria with ruptured cristae (M). (X 1600).
Fig. (138): Electron micrograph of a part of a proximal convoluted tube of albino rat’s kidney irradiated with 6 Gy (total) after 3 days from the exposure, showing ruptured endoplasmic reticulum (ER) and many mitochondria with ruptured cristae (M). (X 2000).

Fig. (139): Electron micrograph of a part of a glomerular tuft of albino rat’s kidney treated with ALA for 3 days post irradiation (2 Gy), showing 3 capillary (c) were observed contain RBCs. Note the reappearance of foot processes (arrow). (X 1300).
Fig. (140): Electron micrograph of a part of glomerular tuft of post-irradiated ALA rat’s kidney after 6 Gy, showing normal podocytes with normal foot processes (arrow). (X 2000).
Fig. (141): Electron micrograph of a part of apical region of a proximal convoluted tubule of post-irradiated ALA rat’s kidney after 6 Gy exposure, showing many collapsed mitochondria (M) and some vacuolated areas in cytoplasm (arrows). (X 1300).

Fig. (142): Electron micrograph of a part of the apical region of a proximal convoluted tubule of pre-irradiated ALA rats kidney after 2 Gy, showing many elongated mitochondria (M) and nucleus (N) with heterochromatin restricted at the nuclear membrane. (X 1600).
Fig. (143): Electron micrograph of a part of a glomerular tuft of pre-irradiated ALA rats kidney after 6 Gy, showing epithelial cells lining Bowman’s capsule with basal nucleus, mesangial cell (m) and regenerated foot process (arrows). (X 660).
Fig. (144): Electron micrograph of a part of the apical region of a proximal convoluted tubule of pre-irradiated ALA rats kidney after 6 Gy, showing regenerated epithelial cells lining the proximal convoluted tubule rich with elongated mitochondria (M). (X 660).
Histological studies of the heart (Cardiac muscle)

A. Haematoxyline and Eosin (H&E):

1. Control Group:

Histological examinations of a section in heart of normal rats revealed that the cardiac muscle fibers appeared as short branching and anastomosing cylinders with moderately stained eosinophilic sarcoplasm and centrally located oval nuclei. Each bundle of cardiac muscle fibers is short and contains a single, centrally located nucleus. Binucleated (two nuclei) muscle fibers are also occasionally seen. Around these nuclei are the clear zones of non-fibrillar perinuclear sarcoplasm. Distinguishing and characteristic features of the cardiac muscles, are the intercalated disks. These disks are found at irregular intervals in the cardiac muscle; they represent the specialized junctional complexes between adjacent cardiac muscle fibers. The cardiac muscle has a vast blood supply, where numerous small blood vessels are found in the connective tissue that surrounds the muscle fibers. Capillaries are abundant in the delicate endomysium between individual muscle fibers (Fig. 145).

2. Alpha Lipoic Acid (ALA) Group:

The heart of male rats treated with ALA (30 mg/kg body wt) for 16 days showed branching and anastomosing cardiac muscle fibers with central vesicular nucleus. Few structural changes manifested by the detection of widened interstitial connective tissue spaces were detected (Fig. 146).
3. \textit{Irradiated Group:}

Examination of cardiac muscle sections of rats irradiated by gamma irradiation at a dose level of 2 Gy showed degenerated cardiac muscle fibers with loss of striation. Also, widened connective tissue spaces infiltrated with many pyknotic nuclei were observed (Fig. 147). Experimental rats exposed to another 2 Gy of gamma irradiation (as 2\textsuperscript{nd} dose), revealed progressive destruction in the cardiac muscle fibers, deformation of their striation and appearance of widened interstitial space (Fig. 148).

After exposure of rats to another 2 Gy of gamma irradiation (as 3\textsuperscript{rd} dose), revealed that the cardiac muscle fibers were badly affected showing marked infarction and hyalinization of cardiac muscle bundles most of which lacking nuclei (Fig. 149). On the other hand, rats left for one month after a 3\textsuperscript{rd} 2Gy, demonstrated a slight improved picture. However infarct muscle fibers, pyknotic nuclei and widened connective tissue spaces were still apparent (Fig. 150).

4. \textit{Simultaneously Irradiated ALA Group:}

After 1\textsuperscript{st}, 2\textsuperscript{nd} ad 3\textsuperscript{rd} 2 Gy of gamma irradiation, the cardiac muscle fibers of irradiated rats showed slight improvement, where many widened interstitial space was still seen. In addition to the detection of prominent infarct muscle bundles in varying degrees of degeneration. The degree of degeneration ranged from loss of striation to complete necrosis and fragmentation of fibers (Figs. 151, 152 and 153). Cardiac muscles of rats of the recovery group showed weak improved picture, where the pyknotic picture was still seen together the abnormal structure of muscle fibers (Fig. 154).

5. \textit{Pre Irradiated ALA Group:}
Cardiac muscle sections of rats exposed to the 1\textsuperscript{st} 2 Gy of gamma irradiation showed the slight improved structure (Fig. 155). After the 2\textsuperscript{nd} 2 Gy, the cardiac muscle fibers showed an obvious improvement nearly close to control architecture, except some pyknotic nuclei as well as few infarct muscle bundles were observed (Fig. 156).

However, After the 3\textsuperscript{rd} 2 Gy, the architecture of the cardiac muscle fibers showed less improved picture compared to that detected after the last mentioned dose (Fig. 157). Cardiac muscles of rats of the recovery rat, demonstrated slight recovery in picture, but still not as that of normal ones (Fig. 158).
Fig. (145). A photomicrograph of the cardiac muscles of a control rat, showing branching and anastomosing cardiac muscle fibers with central vesicular nucleus (thin arrows) and interstitial connective tissue spaces (thick arrows) (H&E x 400).
Fig. (146). A photomicrograph of the cardiac muscles of an ALA treated rat, showing branching and anastomosing cardiac muscle fibers with central vesicular nucleus (arrows) (H&E x 400).
Fig. (147). A photomicrograph of the cardiac muscles of a 1st irradiated rat, showing degenerated of cardiac muscle fibers losing striation (thin arrows) and widened connective tissue spaces infiltrated with many pyknotic nuclei (thick arrows) (H&E x 400).

Fig. (148). A photomicrograph of the cardiac muscles of a 2nd irradiated rat, showing infarction of these muscle and prominent changes in the cardiac muscle fibers including loss of striation (thin arrows) and appearance of many connective tissue spaces (thick arrows) (H&E x 400).
Fig. (149). A photomicrograph of the cardiac muscles of a 3rd irradiated rat, showing the marked infarction of cardiac muscle fibers (arrows) (H&E x400).
Fig. (150). A photomicrograph of the cardiac muscles of an irradiated rat left for one month after 3rd 2 Gy, showing a slight improved picture, however infarct muscle fibers, pyknotic nuclei and widened connective tissue spaces were still apparent (arrows) (H&E x 400).
Fig. (151). A photomicrograph of the cardiac muscles of a 1st simultaneously irradiated ALA rat, showing slight improvement of the cardiac muscle fibers striation, although infarction of muscle was still seen (arrows) (H&E x 400).
Fig. (152). A photomicrograph of the cardiac muscles of a 2nd simultaneously irradiated ALA rat, showing the improvement of the cardiac muscle fibers architecture. Some pyknotic nuclei and many widened connective tissue spaces can be seen (arrows) (H&E x 400).

Fig. (153). A photomicrograph of the cardiac muscles of a 3rd simultaneously irradiated ALA rat, showing the infarct cardiac muscle fibers with many blood cells in between the myocardial bundles (arrows) (H&E x 400).
Fig. (154). A photomicrograph of the cardiac muscles of a simultaneously irradiated ALA rat left for one month after 3rd 2 Gy, showing slight recovery in cardiac muscle fibers architecture, although many pyknotic nuclei were still seen (arrows) (H&E x 400).
Fig. (155). A photomicrograph of the cardiac muscles of a 1\textsuperscript{st} pre irradiated ALA rat, showing the slight improved structure of the cardiac muscle fibers (arrows) (H&E x 400).

Fig. (156). A photomicrograph of the cardiac muscles of a 2\textsuperscript{nd} pre irradiated ALA rat, showing the slight improved picture of cardiac muscle fibers (arrows) (H&E x 400).
Fig. (157). A photomicrograph of the cardiac muscles of a 3rd pre irradiated ALA rat, showing the marked infarction of cardiac muscle fibers (arrows) (H&E x 400).

Fig. (158). A photomicrograph of the cardiac muscles of a pre irradiated ALA rat left for one month after 3rd 2 Gy, showing slight recovery in the architecture of cardiac muscle fibers(arrows) (H&E x 400).
DISCUSSION

Ionizing radiation produces harmful effects on the organisms and due to wide spread use of radiation in diagnosis therapy and industry, so pharmacological intervention could be most potent strategy to protect human or ameliorates the deleterious effect of ionizing radiation $^{172, 173}$. 

Experimental studies on animals have shown that exposure to ionizing radiation induces oxidative stress in different tissues $^{174, 175, 176}$ as also observed in the present study. The interaction of ionizing radiation with the biological system results in the generation of ROS $^{177, 178}$. ROS significantly affects the cellular membrane and induces peroxidation of the lipids, thereby producing damaging effects to the cells $^{22}$.

Most of radiation-induced damages to organisms are thought to originate from active oxygen species produced from water and oxygen in living organisms. These active oxygen species react with various intracellular components including DNA, protein, lipids, carbohydrates, then induced genetic damage and abnormal cell function. One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides in tissues. Oxidant-induced injury is believed to be mediated by reactive oxygen species and is associated with acute-phase response, which involves increased synthesis of a number of stress proteins and mediated through the synthesis of chemkines $^{179}$.

Human and animal bodies are endowed with endogenous antioxidant defense system (as ALA) that scavenge and minimize the formation of ROS. However, these systems are not always fully operative, therefore, diet derived antioxidants become particularly important in diminishing cumulative oxidative damage $^{180, 181}$. When there is an imbalance between free radicals generated and the protective
mechanisms that remove them, the excessive radical production (oxidative stress) can be damaged. Lipid peroxidation of biological membranes contributes significantly to the development of radiation induced cell injury, because these cellular elements play a decisive role in the functional organization of the cell \textsuperscript{182}. The oxidative stress free radical-formation was greatly augmented during ionizing-radiation exposure \textsuperscript{183}. Coping with irradiation stressing conditions required a complex adjustment of the physiological and biochemical metabolic pathways to ensure survival by minimizing intracellular damage. It was likely that animal had particular antioxidants generally decrease the level of oxidation in such systems by transferring hydrogen atoms to the free radical structure \textsuperscript{184}.

Tissue injury is mediated by excessive free radical production, which is encountered in many human disease, environmental stresses and radiation exposure. Humans are endowed with antioxidant defense systems that scavenge and minimize the formation of reactive oxygen species. However, in overload oxidation these systems are always fully operative. Therefore, diet derived antioxidant became particularly essential in diminishing cumulative oxygen damage \textsuperscript{185}.

**The liver**

**Biochemical changes:**

According to Fawcett, \textsuperscript{186} liver is an exocrine gland, secreting bile through a system of bile ducts into the duodenum and act as endocrine gland synthesizing a variety of substances released directly into the blood stream. Liver received any toxic substance from the general circulation or intestine and is capable of degrading them by oxidation or hydroxylation or detoxifying them by conjugation, the product of their degradation are excreted in the bile.

The results obtained in the present study, showed that whole body gamma irradiation; delivered as 2 Gy each 3 days up to a total dose of 6 Gy, except those
left for one month as recovery test, induced several biochemical and histopathological changes in liver, kidney and heart. Concerning the activity of serum liver AST, ALT and ALP, significant changes were recorded fluctuated between decreased and increased levels up to 6 Gy and recorded highly significant increase after month of 6 Gy “recovery test”. This fluctuation may be attributed to the decrease in synthesis of those enzymes induced by gamma irradiation, but accumulation of radiation doses induced highly significant elevation of serum AST, ALT and ALP activities as a consequences of rat irradiation (irreversible reaction) as also detected by Farag et al. They supposed that irradiation causes drastic dysfunction of liver cells which may lead to increase serum aminotransferase levels. They ascribed the elevated serum enzymes to the cellular membrane-gamma ray interaction leading to increment hepatic cell membranes permeability. Furthermore, the increased serum enzymatic activities may also be referred to the damage of liver parenchymal cells (a hypoxia state) and extrahepatic tissues caused by irradiation, followed by a release of intracellular enzymes into the circulation. In addition, Kafafy, Ramadan et al. and Nada explained that the changes in the enzymatic activities after irradiation may be due either to the release of enzymes from radiosensitive tissues or to changes in its synthesis. They added that such changes may be also related to the extensive breakdown of liver parenchyma as had been observed, histologically, in liver samples of irradiated rats in the present study.

It has been well documented that both AST and ALT are considered among the most sensitive markers of hepatocellular injury. The registered increase in serum AST and ALT activities in irradiated rats is in accordance with the findings of Abd El-Salam et al. The last authors attributed the increase in ALT activity in irradiated rats to extensive breakdown of liver parenchyma with subsequent enzyme release, or to increase in permeability of the cell membrane that could
enhance the movement of enzymes from their sites of production. One of the proposed mechanisms in this model is considered to be initiated by the accumulation of free radicals which causes consecutive lipid peroxidation of the cell membranes and endoplasmic reticulum. The peroxidative products caused the cell membrane to become leaky with the consequent release these enzymes into the blood. This suggestion was supported by the work of *Masayuki et al.* who reported that lipid peroxidation is recognized to be a major factor in the liver injury model.

*El-Khafif et al.* and *Omran and Abu-Zied,* suggested that the increase of serum ALT is particular to drastic physiological effects on liver caused either by direct interaction of cellular membranes with gamma-rays or indirectly through an action of free radicals produced by these reactions.

Moreover, the results of the present study showed that whole body gamma irradiation significantly increased ALP activity in the serum as also reported. It is well known that ALP plays an important role in maintaining the cell membrane permeability. Radiation exposure caused damage to the cell membrane that augmented the ALP activity. The increases in ALP activity also might be due to the changes in the amino acid resides and catalytic activity of ALP.

**Histological changes**

The recorded biochemical changes of liver were associated with histological damage that might result from an increase in the process of lipid peroxidation and a decrease in the activity of antioxidant enzymes of the body with the consequent damage of cellular bio-membranes.

In a study done by *Saada et al.*, it was suggested that histological damage induced in the liver of irradiated rats was associated with an increase in the content of lipid peroxides and a decrease in the activity of the antioxidant enzymes SOD and catalase. In addition, exposure to radiation causes injury to blood vessels.
provoking anoxia of tissues with degeneration and necrosis of hepatic parenchyma. Also, cytoplasmic changes including swelling, vacuolation and alteration in the various components of the plasma membrane were seen.

In the present study, sections of the liver of irradiated rats showed different histological changes and loss of normal hepatic architecture. Where, the radiation effects began with dilated central vein with an obvious congestion, dilated hepatic sinusoids, loss of hepatocyte nuclei, vacuolated and degenerated cytoplasm and dilated hepatic portal vein. All the previous histopathological changes were progressively obvious at high dose (6 Gy) and additional inflammatory cells (fibroblasts and lymphocytes) aggregated around the blood vessels and in the sinusoids. Furthermore, followed by progressive widening of hepatic sinusoids, hemolized central vein and prominent cellular necrosis.

This was expected but surprisingly, rats left for one month after the last dose of gamma radiation (6 Gy) as a recovery test, exhibited highly significant hypotrophied hepatocytes, ill defined cell membrane and pyknotic degenerated nuclei. Blood vessels were dilated and clogged with erythrocytes, particularly the tributaries of the hepatic portal vein in which the endothelial lining was ruptured. The hepatocytes were completely destroyed. The same results were reported by Yarmonenko, (1998), Zavodnik et al. (2003) and Guryev, (2005). The ultrastructural studies of liver of the present study indicated that gamma radiation induced cytoplasmic degeneration of hepatocytes, ruptured cristae of mitochondria, dilated bile canal, blood sinusoid, degenerated mitochondria and fragmented rough endoplasmic reticulum. Degeneration and necrosis of the hepatic cells following exposure to radiation could be explained according to the suggestion of Ritter who reported that liver cell necrosis may be either due to progressive degenerative action of intracellular enzymes of the injured cells or to a metabolic disturbance and inhibition of synthesis needed of DNA and hence protein synthesis for the
growth and maturation of the liver. It is well established that most of the physiological activities in the animal body are disturbed after exposure to ionizing radiation. These disturbances are either due to the direct harmful effect of irradiation on the biological system or indirect effect of free radicals liberated in the body after irradiation \textsuperscript{206}. Also, Geraci and Mariano \textsuperscript{207} suggested that radiation induced alterations of a non-parenchymal cell population in liver might have been responsible for fibrosis which led to hepatocytes loss.

According to \textit{Levier et al.} \textsuperscript{208}, the pyknotic and darkly stained nuclei may be as a result of coiling and shortening of chromosomes which became inactive especially in protein synthesis. Trigueiro \textit{et al.} \textsuperscript{209}, revealed that the dilated hepatic sinusoids and central vein occurred as a result of the shrunken in some hepatocytes.

The congestion of the blood sinusoids and central veins may be due to the dilation of peripheral blood vessels induced by irradiation. This dilation lead to the decrease in the velocity of blood through the sinusoids and central vein, so blood draining decreased and so crowdedness of erythrocytes is prominent \textsuperscript{210}.

The present results revealed that the amount of collagen fibers was obliviously increased by increasing radiation dose up to 6 Gy, where they appeared as thin hyaline rims around blood sinusoids and hepatic strands. These fibers were highly increased around the portal areas, showing an obvious hyalinization, especially around the congested portal vessels. Also, it is well known that chronic active hepatitis which results from exposure to gamma irradiation followed by fibrosis of liver tissue. The increase in the fibers may be also attributed to condensation of the stroma following destruction of liver cells \textsuperscript{211}.

\textbf{Histochemical changes:}

Liver cells take up glucose from the blood and polymerize it to form glycogen, the storage form of carbohydrate in animals. As the need arise, glycogen is broken down to glucose again in a process catalyzed by the enzyme phosphorylase, this
enzyme is activated by hormones (epinephrine and glucagon) which act upon the liver and release glucose into the blood \cite{186}.

The present results concerning carbohydrate contents (glycogen) in liver of rats exposed to gamma-irradiation revealed that most hepatocytes showed depletion in carbohydrate contents and few hepatocytes appeared nearly normal. The decrease of glycogen contents in liver induced hyperglycemia. These observation are in agreement with those reported by Ahlersova et al. \cite{212}, El Gharib et al. \cite{213} and Abd El Maguid et al. \cite{214} who found that radiation induced hyperglycemia.

So, the decrease in glycogen content in liver of irradiated rats of the present work induced hyperglycemia in these animals. This may be explained in two ways: it may be due to direct effects of irradiation on liver and an stimulation in hepatic rate of glucose production because of the abnormalities in liver function which induced as a result of hepatitis \cite{215}. Secondly, the release of corticosteroid hormone (glucocorticoid) disregulate carbohydrate metabolism and decrease the cellular synthesis of glycogen in the liver tissue.

General speaking, the inhibition of protein in the liver attributed to the retarded protein biosynthesis may be due to the possible decrease in DNA biosynthesis as a result of coiling and shortening in the chromosomes, in the highly pyknotic nuclei as revealed in these results \cite{216}. Finally the inhibition in protein may be also due to the inhibition in the respiratory rate which caused systemic hypoxia so the limits of energy production decrease the rate of protein synthesis.

The control distribution of ATPase revealed that the plasma membrane forming the wall of hepatic canaliculi contain the enzyme ATPase, so it has been suggested that bile secretion is an energy-dependent process and hence the enzyme is localized well around the portal spaces rather than other areas. The histological results also, revealed a great degeneration in the liver cells which decreased the ability of liver
cells to bile secretion and a corresponding decrease in ATPase activity in bile canaliculi.
The kidney

Biochemical changes

The present data showed that irradiation exposure increased serum urea and creatinine. It is known that radiation causes an increase in glutamate dehydrogenase enzyme levels, which might increase carbamoyl phosphate synthetase activity, leading to an increase in urea concentration.  

The current data revealed a significant elevation in serum urea and the increase was in direct proportional to the radiation dose. On the other hand, serum creatinine levels showed unexpected results, where it was still constant up to 6 Gy exposures and exhibited highly significant increase after one month of 6 Gy, monitoring to nephropathy induction. Such results have been reported by several authors. Nephropathy is characterized by a slow and progressive reduction of renal function associated with concomitant glomerulosclerosis and tubulo-interstitial fibrosis leads to renal failure. It is hypothesized that the renal response to irradiation involves modulation of several kidney cell phenotypes, mediated by autocrine and/or paracrine interactions with a variety of cytokines and biological mediators. However, observed that the serum creatinine level was elevated when the rats were exposed to gamma irradiation at fractionated dose levels of 3 Gy to accumulative dose of 9 Gy on the 2nd hour, 1st and 7th days post exposure. They suggested that the increased serum creatinine level is an evidence of marked impairment of kidney function and accumulative effect of fractionated gamma exposure significantly alters renal function. They concluded that fractionated exposure to gamma radiation effectively altered the glomerular filtration rate (GFR) in rat and elevation in serum creatinine level may be attributed to the back leakage of the filtered creatinine, which may occur through damaged tubular
epithelium along the concentration gradient established by salt and water reabsorption.

Mahdy, 83 revealed that whole body gamma irradiation of rats at 7.5 Gy (single dose), caused a significant decrease in the content of serum proteins accompanied by a significant increase of urea level as recorded 7, 10 and 14 days after irradiation. The authors suggested that the elevation in urea level in the serum may be due either to an increased oxidative deamination of amino acids in the liver, resulting in excess urea formation. On the other hand, the decreased level of serum urea at 7.5 Gy (fractionated dose) may be due to the increased protein catabolism. The resulting amino acids undergo transamination rather than oxidative deamination which seems to be suppressed in favour to transamination to enhance gluconeogenesis. They also, suggested that the decrease in urea content may be attributed to the damage occurred in the liver cells of irradiated animals as has been also detected in the studied liver sections of the present study.

Histological changes
The present study showed that gamma rays (6 Gy) induced different histopathological lesions in the kidney of male rats. These lesions were represented mainly by collapsed glomeruli, degenerated renal convoluted tubules and the increase in the inflammatory cells as has been reported by Eissa and Mostafa, 98. Previous histological studies on the effects of gamma irradiation on the kidney structures of experimental animals have been documented. Some authors emphasized the presence of glomerular damage under the effect of irradiation 94. Kidney is one of the organs that show high sensitivity toward gamma-radiation 219, 220. In the present study damage in glomerular tuft was noticed with widening in urinary space of Bowman's capsule, and finally its rupture in the late stages as a result of gamma irradiation. This result was in agreement with the findings of Stephens et al. 221 and Abu-Nour et al. 222, who indicated that, the structural
changes have led to the concept that glomeruli appeared to be very radiosensitive because after the clinically relevant dose of 6 Gy in 3 fractions essentially all glomeruli were altered in the irradiated kidneys as compared to controls. The lobulation and shrinkage in some glomerular tufts with rupture in Bowman's capsule in the present study may be due to whole body gamma irradiation in rats. Moreover, sections of kidney showed shrunken or swollen glomeruli infiltrated with proliferated mesangial cells and matrix. In others, the glomerular tufts became fenestrated as a result of dilated glomerular capillaries. Curran \textsuperscript{211} described such changes as glomerulonephritis which induced as a result of inflammatory changes. Capell and Anderson \textsuperscript{223}, divided glomerulonephritis into several types; the 1\textsuperscript{st} named diffuse proliferative glomerulonephritis. They stated that the glomeruli, in this type, are diffused, enlarged and increased in cellularity resulting narrow capsular spaces. Bowman’s capsule appeared normal or proliferated as recorded in the present study. The 2\textsuperscript{nd} fenestrated tuft which characterized by dilated blood capillaries with no increase in cellularity. The 3\textsuperscript{rd} type of glomerulonephritis in which the proliferated mesangial cells exhibited lobular feature. The same investigators stated that further renal changes represented by hypertension which develops as a consequence of various types of glomerulonephritis were shown in glomerular tufts where, they became collapsed and shrunken. This may explain the detection of shrunken glomeruli which appeared in many figures in the present work. On the other hand, Capell and Anderson \textsuperscript{223} attributed the changes in the renal tubules in both cortex and medulla as a secondary symptom to the glomerular lesion. Clear rudimentary glomerular structures (glomeruloid) which appeared as ovoid deeply basophilic nuclei. In addition, an acute tubular injury represented by degeneration in the cytoplasm and pyknosis of the nuclei of the renal tubules were seen. Furthermore, in the high doses of gamma irradiation, an extracellular fibrillar
eosinophilic material (amyloid deposition) was clearly seen. Curran, postulated that such deposition usually produces nephritic syndrome. Also, the interstitial congestion detected in both cortex and medulla and also in the glomerular tufts may attributed to the dilation in the peripheral blood vessels which induced by gamma irradiation as suggested by Katzung.

In addition, there was a marked degeneration in the epithelial cells of renal tubules. Robbins et al. mentioned that the renal irradiation resulted in a progressive decline in glomerular filtration rate (GFR), alterations in glomerular and tubular cell proliferation and morphology within 2-4 weeks of irradiation. Irradiation can also induce complete glomerular capillary obstruction due to thrombus formation. The earliest histological damage due to radiation exposure which was observed in the present work was noticed in glomerular capillary endothelial cells. In agreement with the present results the experimental data for mice, pigs and primates as well as data from clinical studies suggested that glomerular damage develops before tubular damage and that glomerular damage is responsible for renal failure.

According to Capell and Anderson, the glomerulonephritis was accompanied by many physiological disturbances; the 1st was the disturbance in glomerular filtration rate which reduced as a result of narrowing of glomerular capillary lumina attributed to the increase in number and size of glomerular cells, hence the renal plasma flow has been reduced and the fraction of plasma filtrated by glomeruli was also reduced. As a result of inhibition in ATPase in the brush borders of the proximal tubules and inner borders of distal convoluted tubules. It can be expected disturbance in reabsorption of different electrolytes because this activity depends on the energy which was supplied in the form of ATP. This may agree with the finding of Capell and Anderson, who stated that electrolytes disturbance represented mainly in disturbance of selective reabsorption of proximal tubules. He
also stated that the selective tubular reabsorption depends on the concentration of solutes in the lumen of tubules. Also, the hypertension that develops as a consequence of glomerulonephritis cause a disturbance in aldosterone hormone which secreted by adrenal cortex. This hormone is responsible for the blood pressure regulation as mentioned by Wheater et al. 226. So, a disturbance in the reabsorption of Na\(^+\) and selection of H\(^+\) and K\(^+\) by distal tubules which stimulated by aldosterone may be happen. On the other hand, this disturbance in reabsorption of Na\(^+\) may be also lead to the inhibition in ATPase.

Furthermore, the emerged results showed severe hemorrhagic areas scattered in-between degenerated renal tissue, due to gamma irradiation in rats. The detection of destructed cells lining the proximal and distal tubules was similar to that observed by Soranson and Denekamp 227, Jaenke et al. 95 and Abu-Nour 228. They concluded that the tubular cells are among the most important target cells for radiation injury and the endothelial cell injury represents the primary site of radiation damage in the kidney.

The collagen fibers were increased around the renal tubules and in the glomerular tufts with an obvious increase in the mesangial matrix. Also, an increase in the interstitial collagenous fibers in both cortex and medulla was observed. These results were discussed by Curran, 211 who stated that in glomerulonephritis and chronic renal failure, there was a marked periglomerular and interstitial fibrosis.

Conclusively, the changes of the kidney as a result of irradiation indicated various types of glomerulonephritis and consequently reduction in glomerular filtration rate which finally lead to renal failure 217.

**Histochemical changes**

The results obtained in the present study indicated that there was an increase in the amount of carbohydrates content in both cortex and medulla of the kidney which became highly significant in the high doses of irradiation. This increase in
carbohydrates may be attributed to the release of corticosteroids induced by irradiation, which disregulate carbohydrate metabolism and increase glycogen synthesis in the tissues. On the other hand, radiation caused disturbance in the selective reabsorption of the proximal tubules to glucose molecules increasing its flow in urine. Furthermore, radiation induced a clear inhibition in protein contents in kidney (cortex and medulla) this may be due to the release of glucocorticoid which breakdown protein in the tissue. In addition and according to Curran, who found that nephritic syndrome led to (proteinuria) which may explain a great loss of protein molecules in the urine due to disturbance in reabsorption activity, leading to inhibition in protein contents in the tissue.

The control renal tubules rich in ATPase and centrally localized in proximal and distal convoluted tubules since their cells are essentially responsible for Na\(^+\) reabsorption and active transport and secretion of H\(^+\) and K\(^+\) in a mechanism which is called sodium pump, while the other renal substructures are essentially responsible for water reabsorption and waste excretion. Also, the plasma membrane of the microvilli which contains a variety of transport protein and enzyme involved in selective reabsorption of solutes from the glomerular filtrate depend on the energy which was supplied in the form of ATP by mitochondria which crowded in the cytoplasm of proximal convoluted tubules.

All the histological results on kidney revealed that radiation caused a glomerulonephritis and finally renal failure as discussed before. These syndromes affected the reabsorption mechanism of the renal tubules and as a result an inhibition in the activity of ATPase was occurred.

**The heart**

**Biochemical changes**

Radiation is associated with a decrease in antioxidants and an increase in oxidant free radicals resulting in increased oxidative stress which is followed by
development of a variety of subcellular changes in the myocardium, typical of radiation induced cardiac injury \(^{229, 230}\). Supplementation with antioxidants could prevent harmful radiation from reaching the heart tissue, counteracting radiation-induced free-radical damage \(^{231}\).

Creatine kinase (CK), also known as Creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme that catalyses the conversation of creatine to phosphocreatine. In tissues that consume ATP rapidly, especially muscle, phosphocreatine serves as an energy reservoir for the rapid regeneration of ATP.

In the present study, gamma irradiation caused a significant elevation in the activities of CK -MB in the serum of irradiated rats in agreement with Tawfik, \(^{51}\) and Fahim \(^{232}\), they indicated the severity of radiation-induced necrotic damage of the myocardial membrane and the release of these enzymes from damaged heart tissue into the blood stream. Similar results were recorded by Sridharan and Shyamaladevi \(^{52}\), who explained that the excessive production of free radicals and lipid peroxides might have caused the leakage of cytosolic enzymes such as aminotransaminases (AST and ALT), LDH, creatine kinase(CK) and phosphatases. Also, it could induce lipid peroxidation of cell membrane structure by oxygen derived free radicals leading to ionic leakage through cellular membranes and excessive calcium influx with ensuring cellular dysfunction and death from calcium overload \(^{233}\). These results agree with those of Ramadan et al. \(^{234}\). The high serum level of CK-MB may be attributed to the alterations in dynamic permeability of membranes induced by ionizing radiation, allowing leakage of biological active material out of the injured cell \(^{232}\). Moreover, the increase in CK-MB activity after irradiation may be related to the muscular injury \(^{235}\). In contrast, Gissel, \(^{236}\) indicated that the decrease of CPK activity may be caused by increasing membrane permeability due to secondary or delayed onset damage as a result of increasing
Ca²⁺ leakage into the muscle, and thus increased activity and further reductions in membrane integrity (i.e. increased permeability).

In the present study, generation of free radicals in the myocardium during radiation might have exceeded the ability of free radicals scavenging enzymes to dismutate the radicals, resulting in myocyte lesions and reduction of scavengers. In addition, oxidative stress stimulates apoptosis in cardiomyocytes as supposed by Childs et al. 237.
Histological changes

These results suggest that the low dose of ionizing radiation has distinct different histological changes in rat's heart. These changes varied from hypertrophy of the cardiac muscle fibers and the disruption of the striation appeared. In addition, massive extended hemorrhagic area found in this study which was prominent. In consistence with the present findings, Zheng et al.\textsuperscript{238} reported that the exposure to low dose of gamma radiation simulated weightlessness and caused more serious damage on myocardial cells. Weiss and Landauer\textsuperscript{14} referred the mechanism by which radiation causes damage to tissue (as has been observed in infarct cardiac muscles of the present study) or any other material to ionization of atoms in the material. Ionizing radiation absorbed by tissue has enough energy to remove electrons from the atoms that make up molecules of the tissue. When the electron that was shared by the two atoms to form a molecular bond is dislodged by ionizing radiation, the bond is broken and thus, the molecule falls apart.

Role of ALA

The role of ALA as a natural antioxidant against radiation revealed significant biochemical and histological changes in liver, kidney and heart. The results obtained, in the present study, indicated that the pre-irradiated ALA rats recorded highly significant improvement in most accumulative doses up to 6 Gy and insignificant improvement in rats of the recovery group. On other hand, the simultaneously irradiated ALA rats, revealed less significant improvement which may reach up to 4 Gy. The decreased levels of these enzymes as well as the improved histological picture were observed after administration of ALA which indicates that the release of these enzymes had been inhibited. Using ALA led to the formation of normal spherical nuclei, mitochondria with normal cristae and prevented (pyknotic nuclei) apoptosis features. Additionally, many cells undergoing mitotic division were seen in ultrastructural investigations. This could
be explained on the basis that exposure to radiation increase mitotic delay. Hence, the recorded significant improvement revealed that LA/DHLA are considered as an ideal therapeutic antioxidant because they are naturally existing, low molecular weight compounds with very powerful antioxidant effective in both aqueous and lipid domains. Their effects include free radical quenching, metal chelation and regeneration of other antioxidant such as ascorbic acid, vitamin E and glutathione. It has been found that exogenously supplied α-lipoic acid has antioxidant properties and is effective in preventing or lessening damage caused by reactive oxygen species (ROS). Because of its antioxidant properties, α-lipoic acid has been tested as a possible therapeutic agent in many common diseases like diabetes. This raises the possibility of using α-lipoic acid which can help against oxidative damage in other cases such as exposure to ionizing radiation.

According to Bonomi et al. as a potent antioxidant, ALA not only scavenges free radicals, but also raises the intracellular level of antioxidants by recycling them, and chelates heavy metals to prevent free radical generation. ALA’s antioxidant role involves protecting cells from damage by preventing the destruction of lipids in cell membranes. Unlike other antioxidants, ALA is soluble in both water and fat. Because of these unique antioxidant functions, ALA is known as the “universal antioxidant” and the “antioxidant of antioxidants.”

In the body, ALA is converted (reduced) to DHLA, or dihydrolipoic acid. Together, these two forms of LA make up a "redox couple," which means that each form can chemically change into the other and back again. DHLA also functions as an antioxidant and is an essential component in the interaction between vitamin C, E, and glutathione. Studies showed that the addition of lipoic acid to liver tissue results in increased vitamin C levels. It has been found that DHLA is responsible for regenerating vitamin C, which in turn regenerates vitamin E. DHLA also converts glutathione from its oxidized form back into its free radical
scavenging reduced form \(^{244}\). The LA/DHLA pair is vital for prevention of "oxidative stress," which occurs when the balance is tripped in favor of oxidation in cells. DHLA helps preserve antioxidants in both the watery cell interior and the fatty structure of cell membranes. By regenerating vitamin C, E, and glutathione in tissue, LA/DHLA helps reestablish the antioxidant/oxidant balance in the body.\(^{120}\)

Antioxidants such as lipoic acid prevent tissue damage by neutralizing free radicals and reducing oxidative stress.\(^{245, 246}\) According to \textit{Zhang et al.}\(^ {247}\), ALA supplements may offer several different mechanisms to enhance cardiovascular health in addition to its antioxidant and glucose metabolism functions. ALA also appears to reset and normalize metabolic processes in a variety of other ways, including helping to support healthy arterial function, helping to maintain healthy weight as part of a healthy diet, and supporting healthy lipid metabolism.

Lipoic acid and its reduced form, DHLA, appear in tissues in free form indicating that lipoic acid is activated metabolically to DHLA in vivo. DHLA is a strong reductant that can regenerate oxidized antioxidants. Specifically, when antioxidants such as ascorbate (vitamin C), glutathione, coenzyme Q10 and vitamin E scavenge radicals, they become radicals themselves. DHLA can directly and indirectly recycle these substances and “reactivate” them.\(^{248, 152, 249}\). From this came the term “antioxidant network”. For example, when vitamin E scavenges a peroxyl radical, a vitamin E radical is formed. The vitamin E radical may be reduced at the lipid/water interface by several antioxidants, such as ascorbate, ubiquinol, and reduced glutathione (GSH). DHLA is able to reduce all these antioxidants and thus regenerate them and therefore take a central position in the antioxidant network. Remarkably, lipoic acid has both water-soluble and membrane-soluble characteristics, enabling it to reduce oxidized antioxidants at the lipid/water interface. The predominant form that interacts with reactive oxygen species is DHLA, but the oxidized form of lipoic acid can also inactivate free radicals. Lipoic
acid increases ATP synthase activity, maintains critical thiol groups in a reduced state, and allows mitochondrial protein carriers to function more effectively, all which may lead to enhanced metabolism. A radioprotective effect of ALA was observed by Korkina et al., Following the Chernobyl accident. Children living in contaminated areas (15-40 Ci/km2) were given either 400 mg daily of ALA or the same dose with 200 mg daily of alpha tocopherol for 4 weeks. The spontaneous leukocyte chemiluminescence, as assessed by the luminol test, returned to normal levels following 4 weeks of both treatments. In addition, only the high dosed ALA group resulted in a significant decrease in the erythrocyte content of glutathione. It was also mentioned that ALA treatment allowed the normalization of kidney and liver functions.

In the present study, it is noteworthy to say that the ameliorative effects of ALA were mainly best registered in rats of the 5th group (preirradiated ALA group), where ALA was firstly administrated before, during and after radiation exposure. Hence, ALA acts as a protective agent due to its action in:

1- Preventing or lowering the damage caused by ROS liberated after radiation exposure.

2- Preventing free radical generation.

3- Protecting cells from damage by preventing the destruction of lipids in cell membranes.

In summary, in order to protect normal tissues from potential radiation damage, it would be important to identify biological or chemical agents which, when given before radiation exposure, could protect all normal tissues. In addition, extensive radiobiological research yielded numerous agents such as ALA which, when given before radiation exposure, protected animals against radiation injury, allowing the normalization of kidney and liver as has been observed in the present study.
On the other hand, we have to consider that the liver is the primary organ, where ALA is metabolized. This can raise the question of the efficiency of the chemical form (redox state, enantiomer and dose), the use of vectors (as cyclodextrins) for better internalization of ALA in cells, and should encourage other clinical trials in disorders where oxidative stress, especially in liver diseases, plays a major pathogenic role.

**Recommendation**

It appears that ALA, alone or together with vitamin E, is an effective treatment for radiation exposure, lessening indices of oxidative damage and normalizing organ function. The interesting antioxidant properties of ALA and its interaction with other important antioxidants like vitamin E, ascorbate and glutathione will provide a fertile field for continued research.
SUMMARY

Exposure to doses of ionizing radiation is associated with physiopathological and histopathological changes. These changes differ in their severity according to the radiosensitivity and responses of individual organs and tissue. The human diet contains an array of natural antioxidants that may contribute the endogenous antioxidant defense system. Among the most common dietary sources of natural antioxidants, alpha lipoic acid. The present study was designed to investigate the ameliorative effect of alpha lipoic acid (ALA) on the induced oxidative stress in liver, kidney and heart after gamma radiation exposure.

Male albino rats weighting 120-160 g were used for the different investigations carried out in this work. ALA was administrated to animals by gavage at doses of 30 mg/kg body weight. The animals were randomly divided into 5 groups, each of 24 rats. The experimental period was extended to 46 days. The groups of animals were categorized as follows: **control group**, **ALA group**: rats were received ALA 30 mg/kg orally by gavages daily; **irradiated group**: rats were submitted to 2 Gy every 3 days to reach accumulative dose of 6 Gy; **irradiated + ALA group**: rats were received ALA simultaneously during exposure and **ALA + irradiation +ALA group**: rats were received ALA for one week before exposure then exposed to radiation and received ALA simultaneously during exposure.

**Radiation effects on liver enzymes and tissues**

The result revealed that exposure to ionizing gamma irradiation of rats with 6 Gy (fractionated dose) induced significant increase in the levels of AST, ALT and ALP which was concomitant with severe histopathological hepatic changes manifested by dilated sinusoids, most of hepatocytes lost their nuclei, showing vacuolated cytoplasm and some others had pyknotic nuclei. The presence of hemolized central vein, prominent cellular necrosis and increased number of pyknotic nuclei were
also seen. Ruptured bile ducts and interstitial hemorrhage were also observed. In general, the liver architecture was completely obscured.

**Radiation effects on kidney functions and tissues**

The results obtained in the present study showed that whole body gamma irradiation of rats with 6 Gy (as fractionated doses) provoke oxidative stress in the kidney functions and tissues. Significant increases in urea and creatinine concentration were recorded. Different histological lesions were detected. These lesions included obvious shrunken in glomeruli, with great reduction in its cellularity and as a result, dilation in the urinary space occurred. Ruptured wall of Bowman's capsule, dilation in the lumen of renal tubules, pyknotic nuclei and large number of fibroblasts were also reported. Some renal tubules (ascending and descending loop of Henel) of the medulla showed vacuolated cytoplasm and some pyknotic nuclei, in addition to signs of interstitial hemorrhage were detected. Few areas of hyaline basophilic background were also observed. Progressive changes were reported manifested by the detection of pyknotic nuclei in cells of the destructed renal tubule. Some glomeruli appeared swollen with diffused and fragmented tufts congested with blood and others appeared damaged loosing, approximately, its cellularity. Cells of the renal tubules of the medulla showed vacuolated cytoplasm and pyknotic nuclei. In addition to the dilation of ascending and descending tubules, signs of interstitial hemorrhage were also detected.

**Radiation effects on heart enzymes and tissues**

The results revealed that whole body gamma irradiation of rats with 6Gy induced significant increases in the activity of creatine kinase (CK-MB). These biochemical changes were accompanied by histopathological changes manifested by cardiac muscle infarction, hemorrhage, dilated interstitial connective tissue spaces, pyknotic nuclei and loss of striation.

**Protective role of ALA**
The results obtained in the present study, demonstrated that administration of alpha lipoic acid for 9 days simultaneously during exposure to gamma irradiation at 6 Gy (fractionated dose) has significantly minimized the severity of biochemical and histological changes. ALA protection against oxidative stress is mediated by decreasing radiation disorders.

On the other hand, rats received 30 mg/kg body weight ALA for 7 successive days before and 9 days simultaneously and after whole body exposure to 6 Gy of ionizing gamma radiations (pre irradiated ALA group) showed an increased resistance to oxidative damage.

The results showed that ALA enhances antioxidant defenses and recorded significant amelioration of biochemical values as well as variable improved histological picture when compared to their corresponding values and pictures in irradiated rats.

**In conclusion**, oxidative stress refers to the cytotoxic consequence of oxygen free radicals: superoxide anions, hydroxyl radicals and hydrogen peroxide, which are generated as by-products of normal and abnormal metabolic processes induced by irradiation may lead to DNA damage and mutagenesis, protein and carbohydrate oxidation and metabolic disorders. According to the results obtained it could be concluded that ALA might attenuates radiation induced oxidative organ injury and improve their enzymes activity. ALA also maintains the integrity of cell membranes. So, LA/DHLA which is present in our diet mainly in animal sources such as meat and liver may help reestablish the antioxidant/oxidant balance in the body.
REFERENCES


الملخص العرabi

كثرت في الأونة الأخيرة العوامل التي تودى إلى زيادة الجهد التأكسدي للجسم ومنها كثرة الإشعاعات الذرية وزيادة التلوث البيئي والضغط النفسي وتناول الطعام غير الصحي والتدخين. ونتيجة زيادة عمليات الأكسدة في الجسم تنتج ذرات الأكسجين الحر غير المستقرة التي تنتج بدورها الشوارد الحرة (وهي جزيئات غير مستقرة)، كما أن الشوارد الحرة تنتج ثانويا عن عملية انتاج الطاقة في الخلية، ولأن الميتوكوندريا هي المركز النشط لانتاج الطاقة لكل الخلية فإنها تنمو بعدم من الشوارد الحرة التي تسبب تلف خلايا الميتوكوندريا ذاتها فيضعضع انتاجها للطاقة مما يؤدي إلى تلف الأنسجة والخلايا في الجسم وحدود كثير من الأمراض مثل الجلطات والشيخوخة المبكرة وبعض الأمراض العصبية والسرطان.

خلق الله تعالى في أجسادنا بعض الأساليب الدفاعية التي تقوم هذه الشوارد الحرة وتحميمنا من اختارها حيث يقوم الجسم يوميا بتثبيض الأنسجة والخلايا التالفة. ولكن إذا كانت سرعة تكوين الشوارد الحرة أكبر من طاقة الجسم الدفاعية فإن الجسم لا يستطيع التخلص منها وتحدث أضرار جسيمة للخلايا مما يؤثر سلبا على وظائفها وتظهر عليه علامات المرض.

لذلك فإن الحاجة إلى تحديد مواد لها القدرة على تقليل هذه الأضرار يمثل ضرورة كبيرة.

ويعتبر حمض الفا ليبوبك "بكلا شكله الموكسد والمختزل" من أقوى مضادات الأكسدة حيث أنه يستطيع معادلة الشوارد الحرة في كلا من الوسط المائي والدهني. يوجد حمض الفا ليبوبك طبيعيًا في الجسم ولكن تقل نسبته مع تقدم العمر وتعتبر اللحوم الحمراء من المصادر الغنية بحمض الفا ليبوبك.

لذلك تهدف هذه الدراسة إلى تقييم دور الوقائي المحتمل لحمض الفا ليبوبك في تحسين الأضرار التي يحدثها التعرض للإشعاع الجامى وذلك من خلال دراسة التغيرات البيوكيميائية والهستولوجية التي تحدث في نشاط بعض الأنزيمات مثل الأدينوامينوترينفسيريز - اسبرتيت، الأمينوبيرانونيسفريز - الصوفيات الخفية، كركبات كينيز والبولينيا والكربناتين وذلك في خلايا الكبد والقلب وخلايا الجرذان البيضاء بعد تعرض جسمها الكلي لجرعة إشعاعية قدرها 6 جرائز مقسمة بمعدل 2 جرائز كل ثلاثة أيام. وقد تم إعطاء الفا ليبوبك عن طريق الفم في الجرذان
يوميا لمدة 16 يوم متتالية بجرعة قدرها (30 ملجرام/كيلو جرام من وزن الجسم). تم تقسيم الجرذان البيضاء وزن (120-160 جرام) إلى خمس مجموعات متساوية:

المجموعة الأولى: تركت كمجموعة ضابطة حيث وضعت في نفس الظروف البيئية وظروف التغذية كباقي المجموعات.

المجموعة الثانية: تم إعطائها بحمض الفا ليبيوك عن طريق الفم بجرعة فعالة بدرجة يومية قدرها 30ملي جرام اكيلوجرام لمدة 6 أيام.

المجموعة الثالثة: تم تعرضها لأشعة جاما. كانت جرعة الإشعاع المستخدمة في هذه الدراسة 6 جراى مقسمة بـ 2 جراي كل ثلاثة أيام.

المجموعة الرابعة: تم تعرضها لأشعة جاما ثم إعطائها حمض الفا ليبيوك.

المجموعة الخامسة: تم إعطائها حمض الفا ليبيوك ثم تعرضها لأشعة جاما ثم إعطائها حمض الفا ليبيوك.

وقد تم ترك عدد 6 جرذان من كل مجموعة لمدة 30 يوم بدون أي معاملات لمتابعه ما قد يحدث في خلايا الانسجة الكبدية والقلبية والكلوية والدم من استشفاء وتعافيها. بعد توقفها عن حقن العقار لهذه المدة.

الجرذان الضابطة والتجريبية كان يجرى ذبحها وتشريحها للحصول على عينات صغيرة من الكبد والقلب والكلية. حيث أعدهت هذه العينات لأغراض الفحص الهيستولوجي والهيستوثولوجي والهستوكيميائي حيث تم تحضير قطعات ذات سمك 5-6 ميكرومتر مصغرة بالهيماتوكسيلين والأولوسين والملورى للفحص الهيستولوجي والهيستوثولوجي كما اتبعت الطريق الهستوكيميائي المتخصص للتوضيح: المكونات الأساسية مثل المواد الكروبيدراية كما تم سحب عينات من الدم في أوقات محددة ومعاملتها بالطرق المعروفة.

ويمكن تلخيص النتائج التي تم التوصل إليها كما يلي:
أولاً: تأثير تعرض الجسم الكلى لأشعة جاما على:

1. الإنزيمات الكبدية: أظهرت نتائج هذه الدراسة أن تعرض جسم الكلى لأشعة جاما أدى إلى زيادة معيَّنة في مستوى الإنزيمات الكبدية والمتعلقة بتغيرات في الخلايا الكبدية.

الوظائف الكلوية: هذه الدراسة تأكّدت من أن تأثير تعرض الجسم الكلى لأشعة جاما في جرعة قدرها (6 جرعة) مقسمة بمعدل 2 جرعة إلى أربعة جرات أدى إلى زيادة معيَّنة في مستوى الكرياتينين والكوليسترول، حيث حصلت زيادة مصحوبة بتغيير شكل الخلايا وفقدان صفاتها.

3. الانزيمات القلبية: نتائج هذه الدراسة أظهرت أن تعرض جسم الكلى لأشعة جاما في جرعة قدرها (6 جرعة) سماحية بمعدل 2 جرعة تأدي إلى زيادة معيَّنة في مستوى الكرياتينين والكوليسترول، بحيث صاحبته تغييرات في الخلايا القلبية وزيادة درجة التهابيّة.

ثانياً: دور حمض الفا ليبويك في تقليل الأضرار الإشعاعية:

توضح نتائج هذه الدراسة أن حقن الجرذان من حمض الفا ليبويك لمدة أربع Wochen قبل وعاء أ يصل في خلايا كبد وقلب وكلية الجرذان المعرضة للإشعاع، حيث أدى إعطاء الجرذان حمض الفا ليبويك إلى تقليل مستوى الإنزيمات الكبدية والقلبية وخلايا الطوارم النسيجيّة للأعضاء المذكورة أكثر من تلك التي تناولت حمض الفا ليبويك بعد الإشعاع.
من نتائج هذه الدراسة يمكن أن ينتج أن حمض الفا ليبود كأحد مضادات الأكسدة الطبيعية يحافظ على الوظائف الحيوية للجهاز العصبي والقلب والكبد، وثورة الشوارد الحرة حماة الناتجة من تعريض الجرذان للأشعة السينية.
المستخلص العربي

تهدف هذه الدراسة إلى تقييم الدور الوقائي المحتمل لحمض الفا ليبوية في تحسين الأضرار التي يحدثها التعرض للإشعاع الجامى وذلك من خلال دراسة التغيرات البيوكيميائية التي تحدث في نشاط الإنزيمات الكبدية (امينوترانسفيريز، أسبرتيت أمينوترانسفيريز، الفوسفاتيز القلوى) والإنزيمات الكلبية (كرياتين كينيز) والوظائف الكلوية (البولينا، الكرياتينين) للجرذان بعد تعرض جسمها الكلى لجرعة إشعاعية قدرها 6 جرائ مقسمة بمعدل 2 جرائ كل ثلاثة أيام (يتم قياس التغيرات البيوكيميائية بعد كل 2 جرائ) وتم ترك مجموعة لمدة شهر بعد آخر تعرض للإشعاع. وكذلك تم دراسة التغيرات الهستوبيولوجية في الأعضاء المذكورة باستخدام الصبغات المختلفة. يتعتبر حمض الفا ليبوية من أقوى مضادات الأكسدة حيث إنه يستطيع معادلة الشوارد الحرة في كلا من الوسط الدهني وال-reader.

أوضحت النتائج أن التعرض الكلي لأشعة جاما أحدث تغيرات بيوكيميائية واضحاً في كلا من الإنزيمات الكبدية (الاثنين أمينوترانسفيريز، أسبرتيت أمينوترانسفيريز، الفوسفاتيز القلوى) والإنزيمات الكلبية (كرياتين كينيز) والوظائف الكلوية (البولينا، الكرياتينين) وكان مصحوبة بتغيرا هستولوجي واضحا في تركيب الخلايا. ولقد اظهرت المعاملات الإحصائية أن مقدار التحسن في المجموعة المعالجة بحمض الفا ليبوية قبل الإشعاع أعلى معنوي في القياسات البيوكيميائية والفحوصات الهستولوجية من المجموعة التي تناولت حمض الفا ليبوية بعد الإشعاع مقارنة بالنتائج المأخوذة من المجموعة التي تعرضت للإشعاع دون تلقي علاج. ومن هنا نخلص أن حمض الفا ليبوية كمضاد للأكسدة يحقق فاعليته في المجموعات المعالجة أولاً قبل التعرض للإشعاع.
الدور الوقائي لحمض ألفا ليبوك ضد التأثيرات الضارة التي تحدثها أشعة جاما

السادة المشرفين

أ.د. وفاء محمد صادق زهران
أستاذ الأنسجة والمناعة النسيجية
كلية العلوم
جامعة المنيا

أ.د. سعاد عبد السلام عبد القادر
أستاذ البيوكيمياء الفسيولوجية
المركز القومي لبحوث وتقنية الأشعاع
هيئة الطاقة الذرية

أ.د. محمود هاني أيوب
أستاذ الفسيولوجى
كلية الطب
جامعة عين شمس
الدور الوقائي لحمض الفا ليبويك ضد التأثيرات الضارة التي تحدثها أشعة

جامعة مينيا

رسالة مقدمة من

خالد نادي محمد عبد العظيم

بكالوريوس علوم بيولوجى قسم علم الحيوان
جامعة المنيا

للحصول على درجة الماجستير في العلوم
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