HELWAN UNIVERSITY
FACULTY OF SCIENCE
CHEMISTRY DEPARTMENT

Role of Wheat Germ Oil in Radiation-Induced Oxidative Stress and Alteration in Energy Metabolism in Rats

Thesis

For the partial fulfillment of the requirements for the Master Degree in Biochemistry

Presented by

Shereen Mohamed Ebrahim Shedid

B.Sc. in Biochemistry 2002

Helwan University

Faculty of Science

Chemistry Department

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ABSTRACT

The liver is essential in keeping the body functioning properly while muscular strength is important in sport as well as in daily activities. Exposure to ionizing radiation is thought to increase oxidative stress and damage liver and muscle tissues. Wheat germ oil is a natural unrefined vegetable oil. It is an excellent source of vitamin E, octacosanol, linoleic and linolenic essential fatty acids, which may be beneficial in neutralizing the free oxygen radicals. This study was designed to investigate the efficacy of wheat germ oil, on radiation-induced oxidative damage in rat's liver and skeletal muscle. Wheat germ oil was supplemented orally via gavage to rats at a dose of 54 mg/ kg body weight for 14 successive days pre- and 7 days post-exposure to 5 Gy (single dose) of whole body γ- irradiation. Animals were sacrificed 7, 14 and 21 days post radiation exposure.

The results revealed that whole body γ-irradiation of rats induces oxidative stress in liver and skeletal muscles obvious by significant elevation in the levels of xanthine oxidase and thiobarbituric acid reactive substances (TBARS) associated with significant decreases in the content of reduced glutathione, as well as decreases in xanthine dehydrogenase, superoxide dismutase, catalase and glutathione peroxidase activities. Irradiated rats showed also significant decreases in creatine phosphokinase, glutamate dehydrogenase and glucose-6-phosphate dehydrogenase activities while lactate dehydrogenase were significantly increased. Total iron, total copper and total calcium levels significantly increased in the liver and skeletal muscles of irradiated rats group compared to control group. Wheat germ oil treated-irradiated rats

showed significantly less severe damage and remarkable improvement in all the measured parameters, compared to irradiated rats.

It could be concluded that wheat germ oil by attenuating radiation-induced oxidative stress might play a role in maintaining liver and skeletal muscle integrity.

1- INTRODUCTION

Ionizing radiation has proven to be a double-edged sword since its discovery by Dr Roentgen in 1895. Radiation is a potent mutagen and carcinogen; however, it is also used in the diagnosis and treatment of human diseases. All organisms (e.g., bacteria, plants, or animals, including humans) are exposed everyday to varying amounts of ionizing radiation that interact with molecules, cells, and tissues, causing reversible deviations from homeostatic equilibrium or irreversible damage. Many aspects of aging and many diseases are thought to stem from exogenous and endogenous deleterious agents acting on key components of cells within the body **(Anonymous, 1990).**

The lives of most organisms are dependent on breakdown of adenosine triphosphate (ATP) to produce energy which is used to power all body function. Generation of free radicals or reactive oxygen species (ROS) during metabolism or as a result of exposure to gamma radiation beyond the antioxidant capacity of a biological system gives rise to oxidative stress **(Mikulikova and Popes, 2001)**, which leads to a decrease in mitochondrial ATP synthesis, causing liver and muscle dysfunction and cell death **(Grace et al., 2006).** Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process **(Sian, 2003)**.

ROS are tightly controlled by antioxidant defense systems, including non-enzymatic radical scavengers as reduced glutathione and enzymes that can either directly detoxify ROS or indirectly regulate their levels. The major enzymatic antioxidants are superoxide dismutase, catalase, and the glutathione redox system **(Meister, 1992)**. Dietary antioxidants oppose the oxidative damage and lower risk of

degenerative disease **(Alasalvar et al., 2005)** and thus arises a necessity to extract these antioxidants from the plant matrices. These plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because the endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species **(Fridovich, 1998)**.

Wheat germ is high in fiber and a diet high in fiber can be useful in the regulation of bowel function (i.e. reducing constipation) and may be recommended for patients at risk for diabetes, colon and heart diseases **(Mahan and Stump, 2000)**. Wheat germ oil was claimed anti-inflammatory and described as a suitable natural antioxidant due to its high content of vitamin E **(Paranich et al., 2000)**. Wheat germ oil contains also octacosanol, a long chain fatty alcohol, reported to be helpful in cholesterol management **(Singh et al., 2006).** The oil was reported also to be a valuable source of essential fatty acids, including linoleic acid and linolenic acid**,** which insufficiency was observed to cause tiredness, dry skin, immune insufficiency, anorexia, digestion and cardiovascular disorders (**Mohamed et al., 2005)**.

AIM OF THE WORK

The present study was designed to evaluate and to elucidate the relationship between radiation-induced oxidative stress in liver and skeletal muscle tissues of albino rats and changes in some metabolic enzymatic activities after total body exposure to gamma radiation at 5 Gy applied as one shot dose and to evaluate the role of wheat germ oil against radiation-induced disturbances in energy metabolism.

This goal has been achieved by:

1- Determination of the radiation-induced oxidative stress in the liver and skeletal muscle by measuring the variations in the xanthine oxidoreductase system**,** reduced glutathione level and the activity of the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and changes in the level of thiobarbituric acid-reactive substances; marker of lipid peroxidation.

2- Determination of the radiation-induced changes in some metabolic enzymatic activities in the liver and skeletal muscle by measuring the changes in the activity of lactate dehydrogenase, creatine phosphokinase, glucose-6-phosphate dehydrogenase and glutamate dehydrogenase.

3- Determination of radiation-induced changes in total iron, total copper and total calcium levels in the liver and skeletal muscle.

4- Evaluation of the protective role of wheat germ oil against radiation hazards by comparing results obtained for irradiated rats with those obtained for wheat germ oil -treated irradiated rats.

2- REVIEW OF LITERATURE

All organisms (i.e., bacteria, plants, or animals, including humans) are exposed each day to some amount of radiation and 81% of the dose received from radiation comes from natural sources: 55% from radon; 8% from cosmic radiation; 8% from rocks and soil; and 10% from internal exposures to radiation from the radioactive materials in food and water consumed in the daily diet. The remaining 19% of the daily dose may originate from man-made sources: 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 **(NCRP 1987)**.

2.1. General Concept on 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 **(Yarmonenko, 1988)**. Radiation may be non-ionizing or ionizing according to energy.

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. Radio-waves, infrared and visible light are all examples of non ionizing radiation.

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 **(Yarmonenko, 1988)**.

Ionizing radiation triggers the formation of free radicals, which interact among themselves and with critical biological targets with the formation of a plethora of newer free radicals. It is generally believed that production of these free radicals is the main mechanism through which radiation induces biological damage at lower radiation doses **(Weiss and Kumar, 1988)**.

2.2. Biological Effect of Radiation

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 molecules in the cells 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.

1- Direct action occurs when the radiation interacts directly with a 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.

2- Indirect action occurs when radiation interacts with water molecules, which are roughly 80% of cells composition. The energy absorbed by the water molecule 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 (**Weiss and Kumar**, **1988)**.

Depending on the dose rate and dose of exposure, the effects of radiation range from nausea and vomiting to immune system compromise to death from either radiation-induced tissue damage or infection. Potential sequelae of radiation exposure include cataract formation, decreased fertility and fetal teratogenesis. Although it is believed that the effects of exposure to moderate doses (1–8 Gy) of γ-radiation result in hematopoietic dysfunction, the net injury is a result of a dynamic process involving cell killing, altered cell-to-cell communication, inflammatory responses, compensatory tissue hypertrophy and repair (**Stone et al., 2003**). Higher radiation doses compound these effects with gastrointestinal and neurovascular tissue damage (**Coleman et al., 2004**).

2.3. Energy Metabolism

The human body's main power comes from the mitochondrion which exists in every cell in the body. Energy metabolism consists of a series of chemical reactions that break down foodstuffs and thereby produce energy. The energy-rich chemical compound that provides virtually all the energy needed by the body is known as adenosine

triphosphate, or simply ATP. The mitochondria are the production centers for ATP**.** The energy released from the breakdown of ATP is used to power all body functions. The ATP is used for many cell functions including ***transport work,*** moving substances across cell membranes. It is also used for ***mechanical work***, supplying the energy needed for muscle contraction. It supplies energy not only to heart muscle (for blood circulation) and skeletal muscle (such as for gross body movement), but also to the chromosomes and flagella to enable them to carry out their many functions. A major role of ATP is in ***chemical work***, supplying the needed energy to synthesize the multi-thousands of types of macromolecules that the cell needs to exist (**Hickman et al., 1997)**.

2.4. Cellular Oxidants

Cellular oxidants, derivatives of oxygen, which are often called reactive oxygen species (ROS), are constantly produced in the cells. Among cellular ROS, the most aggressive entities are superoxides ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$). Superoxides, through the reaction catalyzed by superoxide dismutase (SOD) are transformed into the much less reactive hydrogen peroxide moiety (H_2O_2). However, when H_2O_2 interacts with ions of transition metals such as iron or copper, the most reactive ROS, hydroxyl radicals ($^{\bullet}OH$) are formed (Fenton reaction) (**Halliwell and Gutteridge, 2006**). (Fig.1) Besides ROS, cells also generate reactive nitrogen species (RNS) such as nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and peroxynitrite ($ONOO^{\bullet}$). Nitric oxide and nitrogen dioxide carry out a number of physiological functions but excessive $NO^{\bullet}$, $NO_2^{\bullet}$ and $ONOO^{\bullet}$ damage cell constituents **(Moncada et al., 1991)**. (Fig.1)

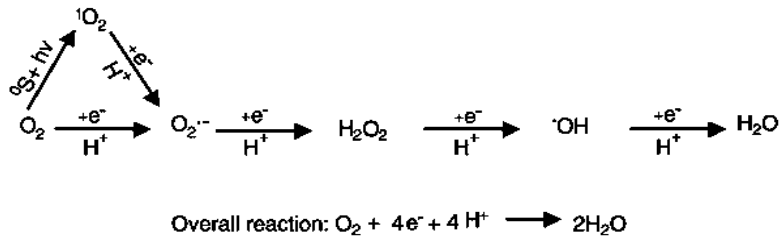

Fig.1: Pathways in the univalent reduction of oxygen to water leading to generation of various intermediate reactive oxygen species.

ROS play a crucial role in a few lifesaving biological mechanisms. Phagocytic cells protect the body from deadly microorganisms, killing them by producing an avalanche of ROS. When neutrophils and other phagocytic cells engulf bacteria, they greatly increase consumption of oxygen ("respiratory burst"), which is rapidly transformed to ROS that kill the dangerous intruders. Importantly, by a burst of ROS, phagocytes kill not only invading bacteria, but also cancer cells (**Halliwell and Gutteridge, 2006**).

ROS are generated from many exogenous and endogenous sources in biological reactions.

2.4.1. Endogenous sources of ROS

ROS are constantly generated in living cells and are usually beneficial to the metabolic processes. Major sites of endogenous generation of ROS in the biological system are within the mitochondria, microsomes, endoplasmic reticulum, phagocytic cells, endothelial cells, and nuclei. Sources of free radicals are the mitochondrial electron transport chain, the enzymes such as xanthine oxidase, NADPH oxidase, lipoxygenase/cyclooxygenase and nitric oxide synthase (NOS) and auto-oxidation of various substances particularly catecholamines (Fig. 2).

Mitochondria

Among the various organelles in the cell, mitochondria are the major source of intracellular free radicals. Mitochondria, the intracellular powerhouses which produce the universal energy molecule, ATP, normally consume O_2 in this process and convert it to H_2O_2. Approximately 2-5% of O_2 used for aerobic metabolism in the mitochondria are converted to ROS such as $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$ due to incomplete reduction of the oxygen. It has been estimated that more than 20 billion molecules of oxidants per day are produced by each cell during normal metabolism **(Nishiyama et al., 1997)**.

Xanthine oxidase

Xanthine oxidase is a molybdenum and iron containing hydroxylating enzyme involved in the degradation of purine like nucleotides. This enzyme catalyzes the reaction of hypoxanthine to xanthine forming $O_2^{\bullet-}$ and of xanthine to uric acid forming H_2O_2 **(Leeuwenburgh and Heinecke, 2001)**.

Peroxisomes

Peroxisomes produce hydrogen peroxide as a by-product of the degradation of fatty acids and other molecules. In contrast to the mitochondria which oxidize fatty acids to produce ATP and water, peroxisomes oxidize fatty acids to produce heat and hydrogen peroxide (**Ames et al., 1993).**

Phagocyte cells

Other sources of ROS in the body are phagocyte cells, which help defend the body against invading microorganisms. Destroying of bacteria and virus infected cells by phagocyte cells releases a variety of ROS, including superoxide anions, nitric oxide and hydrogen peroxide.

ROS generated from this source play a central role in body's defense **(Ji, 1996)**.

Transition metals

Transition metals, such as iron and copper, play a major role in the generation of free radicals injury and the facilitation of lipid peroxidation. In myocytes, O_2 is converted to H_2O_2 in the presence of superoxide dismutase found in the mitochondrion and in the cytosol. H_2O_2, a non-radical, but a freely permeable and highly ROS, is converted to $^{\bullet}OH$ in the presence of copper or iron (the Fenton reaction). Alternatively H_2O_2 reacts with $O_2^{\bullet -}$ to form $^{\bullet}OH$ in the Haber-Weiss reaction. Glutathione peroxidase catalyzes the conversion of H_2O_2 to nonradical water and oxygen (Fig. 2). The Haber-Weiss reaction accelerates the non-enzymatic oxidation of molecules such as epinephrine and glutathione that generates $O_2^{\bullet -}$ and H_2O_2 and subsequently $^{\bullet}OH$ (**Halliwell and Gutteridge, 2006**).

Endogenous levels of ROS, which endanger our health, increase during chronic infection and inflammation, strenuous physical exercise, hyper-metabolic states seen in stress, trauma and sepsis and during exposure to exogenous sources.

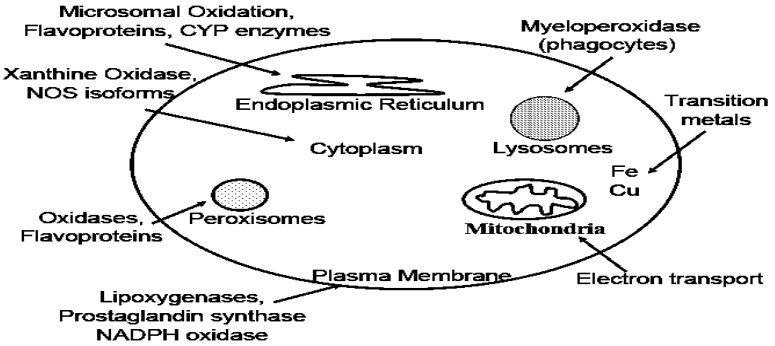

Fig.2: Endogenous sources of reactive oxygen species

2.4.2. Exogenous sources of ROS

Exogenous sources of ROS include the following: tobacco smoke, which has a broad spectrum of oxidant, ionizing radiation, which generates free radicals in exposed tissues, notably the highly reactive $^{\bullet}OH$ radical; UV light, which produces singlet oxygen ($^1O_2$) and $^{\bullet}OH$, ozone (O_3) and oxides of nitrogen in polluted air; industrial toxins such as carbon-tetrachloride; drugs such as phenobarbital, which is a known tumor promoter in liver; and charcoal-broiled foods, which form a variety of carcinogens, notably benzo(a)pyrene.

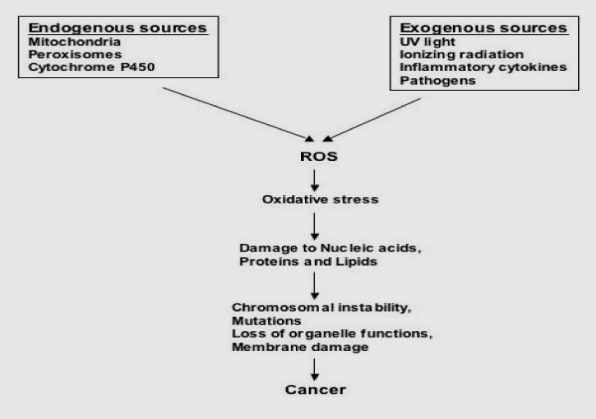

Fig. 3: Pathways illustrating the sources of reactive oxygen species and its role in the development of cancer.

The combination of oxidative damage by exogenously and endogenously produced free radicals has ominous consequences for body tissues. The oxidants induce alterations in the structures of tissues and in their functions which manifest as aging and chronic degenerative diseases like arthritis, atherosclerosis and cancer (Fig.3) **(Ames et al., 1993)**.

2.5. Damage Caused by Free Radicals

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.

1- Proteins perform numerous crucial functions in the cell, primarily 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 **(Bagchi and Puri, 1998)**.

2- 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 polyunsaturated fatty acids present in the membranes' phospholipids are particularly sensitive to attack by $^{\bullet}OH$ 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 (**Dhalla et al., 2000)**.

3- 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, removal of nucleotides, and a variety of

modifications of the organic bases of the 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 (**Gulam and Haseeb, 2006)**.

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 **(Kroemer et al., 1998)**.

2.6. Antioxidants

Biological organisms have evolved defense mechanisms against free radicals that are known collectively as antioxidants. An antioxidant is a substance that prevents oxidation. In biological systems antioxidants can work in various ways, including catalytic removal of free radicals, as scavengers of free radicals or in the form of proteins that minimize the availability of pro-oxidants such as metal ions **(Halliwell, 1996)**. Progress in understanding the deleterious effects of ROS on cell components and structures has led to the development of protective antioxidant supplements. The supplements are used to protect cell structures from oxidative damage, cancer and other ROS-dependent morbid conditions

2.7. Oxidative Stress

Oxidative stress is the inappropriate exposure to ROS and results from the imbalance between prooxidants and antioxidants leading to cell damage (damage of lipids, proteins, carbohydrates and nucleic acids) and tissue injury **(Sies, 1997)**; this imbalance may be due to an excess of pro-oxidant agents, a deficiency of antioxidant agents or both factors simultaneously (Fig. 4).

High oxidative stress is common in organs and tissues with high metabolic and energy demands, including skeletal and heart muscle, liver, lymphatic organs, and blood cells (**Eli et al., 2004)**. In liver excess of ROS can induce hepatocyte cell death by either apoptosis or necrosis leading to liver injury and loss of liver function.

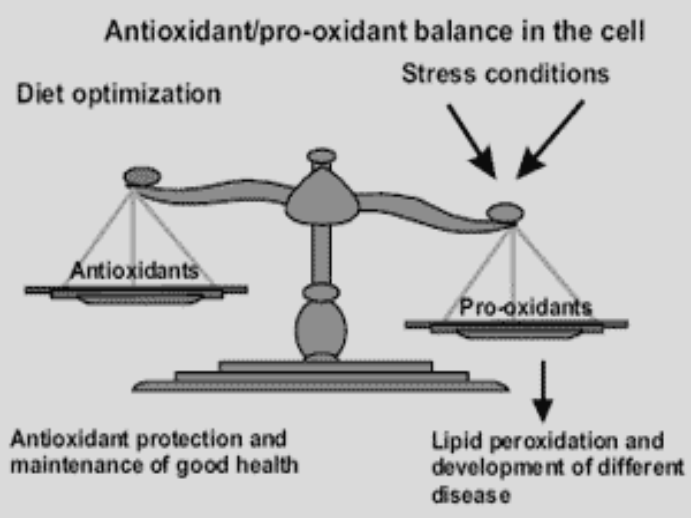

Fig. 4: Antioxidant/pro-oxidant balance in the cell

Contracting skeletal muscle generates an increased amount of reactive oxygen and nitrogen species **(Malcolm, 2005)**. Further studies have demonstrated that muscles release increased amounts of superoxide anion (**McArdle et al., 2001**) and nitric oxide (**Balon and Nadler, 1994**) into the extra-cellular fluid and that hydroxyl radicals are formed from hydrogen peroxide (H_2O_2) also released from the muscle cells (**McArdle et al., 2004**).

A large number of studies have reported that massive formation of RNS and/or ROS is closely associated with increased apoptotic processes in the myocytes of animals and humans. In such multinucleated cells, this may lead to atrophy and reduced muscle strength **(Reid et al., 1993)**.

2.8. Oxidative stress and radiation

Radiation injury to living cells is, to large extent, due to oxidative stress. Evidence suggests that a cell's oxidative state not only plays a role at the time of radiation exposure, but also has effects long after exposure. As the result of irradiation, cells can produce ROS for several minutes or even hours after being exposed. In addition to ROS production, cells are stimulated to increase their expression of antioxidants **(Spitz et al., 2004)**.

2.9. Physiological Defense against Oxidative Stress

To protect molecules against toxic free radicals and other ROS, cells have developed antioxidant defenses. Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. The antioxidative defense system is composed of methods to (i) transfer sensitive material to compartments better protected from the action of reactive species, (ii) complex transition metals, a potential source of electrons, thereby rendering them uncreative, (iii) inhibit vulnerable processes such as DNA replication, (iv) repair damaged molecules, (v) initiate apoptosis (vi) activate antioxidant enzymes and finally (vii) use a variety of direct free radical scavengers **(Halliwell and Gutteridge, 2006)**.

Antioxidants are substances that either directly or indirectly protect cells against the adverse effects of xenobiotics, toxicants, drugs, and carcinogens. Antioxidants can be endogenous (produced by the body) or exogenous (obtained through the diet).

Antioxidant defenses can be classified into three categories enzymatic, non-enzymatic and dietary (nutritional) antioxidants.

1. Enzymatic antioxidant defenses that specifically metabolize ROS precursors, such as catalase, superoxide dismutase and glutathione peroxidase.

2. Non-enzymatic systems (intrinsic molecules such as glutathione, albumin, bilirubin and uric acid).

3. Dietary (nutritional) antioxidants are low molecular weight compounds ingested in the diet such as vitamin C, vitamin E, the carotenoids, flavonoids, other plant phenolics and wine phenolics.

Antioxidants can also be manufactured synthetically. These belong to the class of synthetic antioxidants. The main disadvantage with these antioxidants is their side effect when taken *in vivo* **(Chen et al., 1992)**. Most of the natural antioxidants are found to have higher antioxidant activity when compared with that of the synthetic ones. Several arguments suggest that the antioxidant components of fruits and vegetables contribute in the defense effect. Epidemiological studies and intervention trials on prevention of diseases such as cancer and cardiovascular disease in people have shown the positive effects of taking antioxidant supplements **(Ames et al., 1993; Rimm et al., 1993)**.

2.9.1. Enzymatic antioxidant defenses

Superoxide dismutase (SOD) is a metalloenzyme, meaning that in addition to amino acids, it contains metal ions. Superoxide dismutase helps the body use zinc, copper, and manganese. SOD plays a different role in keeping cells healthy and protects the cells' cytoplasm, and their mitochondria from free radical damage. Abnormalities in the copper- and zinc-dependent superoxide dismutase gene may cause deterioration

of motor nerve cells in the brain and spinal cord **(Cluskey and Ramsden, 2001)**.

The superoxide radical ($O_2^{-\bullet}$) is formed in the mitochondria as a byproduct of electron transport or by ionizing radiation (**Tortora et al., 1995)**. It is very dangerous to the cell because they steal electrons from neighboring molecules, starting a cascade of electron stealing, the end result being damaging of the cell. The superoxide dismutase enzyme is the first step that cells use to stop this process. SOD converts superoxide radicals to a less toxic ROS, hydrogen peroxide (H_2O_2), as follows:

$$\mathbf{2\ O_2^{-\bullet} + 2H^+ \text{ --------SOD-------}\rightarrow H_2O_2 + O_2}$$

The hydrogen peroxide molecule, which is still a danger to cells, is then further processed to nontoxic by-products.

Catalase, an iron-containing enzyme is primary found in peroxisomes except in cells like erythrocytes that do not contain these organelles. In an aqueous environment, the enzyme **catalase** degrades the hydrogen peroxide as follows:

$$\mathbf{2\ H_2O_2 \text{ -------Catalase-----}\rightarrow 2H_2O + O_2}$$

The oxygen gas created in this reaction, accounts for the bubbles seen when hydrogen peroxide is poured on a skin injury. Many bacteria do not have catalase, and the cells are destroyed, which is why hydrogen peroxide is effective against bacteria.

Glutathione peroxidase is a cytoplasmic and mitochondrial enzyme that is important for detoxifying H_2O_2 in most cells. The body uses glutathione peroxidase to degrade the H_2O_2 as follows:

$$\mathbf{H_2O_2 + 2H^+ \text{ ----glutathione peroxidase --}\rightarrow 2H_2O}$$

Glutathione peroxidase system consists of several components including the enzymes glutathione peroxidase and glutathione reductase and the cofactors glutathione and NADPH (Fig. 5). Lipid membranes are very susceptible to damage by free radicals, especially peroxide radicals. Glutathione peroxidase prevents destruction of cell membranes by removing several classes of these lipid peroxides. Increasing fruit and vegetable intake can improve production of GSH-Px **(Dragsted et al., 2004)**.

2.9.2. Non-enzymatic defense

The non-enzymatic antioxidant defense system includes compounds of intrinsic antioxidant properties, such as glutathione (GSH), β-carotene, and vitamin A. Vitamin E is lipophilic, and its major role is to block the chain reaction of lipid peroxidation **(Sies et al., 1992)**. Pre-treatment with vitamin E has been shown in rabbits to prevent lung injury caused by hyperoxia **(Jacobson et al., 1990)**. Ascorbic acid (vitamin C) and α-tocopherol cooperate in cellular defense against ROS. There is a balance between both the activities and intracellular levels of these antioxidants that are essential for the survival of organisms and their health.

Glutathione is the base material for several other key antioxidant enzyme systems including glutathione-peroxidase, glutathione-reductase, and glutathione-transferase. Reduced glutathione (GSH) protects cellular constituents from the damaging effects of peroxides formed in metabolism and through other ROS reactions.

Decreased tissue GSH levels are associated with cell damage, depressed immunity and the progression of aging, and may increase the risk of cancer development. In addition to being essential in the

glutathione redox cycle, glutathione can scavenge ROS non-enzymatically **(Freeman and Crapo, 1981)**, as well as detoxify xenobiotics via the glutathione S-transferase reaction. Glutathione protects thiol groups of proteins from oxidation and maintains vitamin-C levels **(Meister, 1992)**.

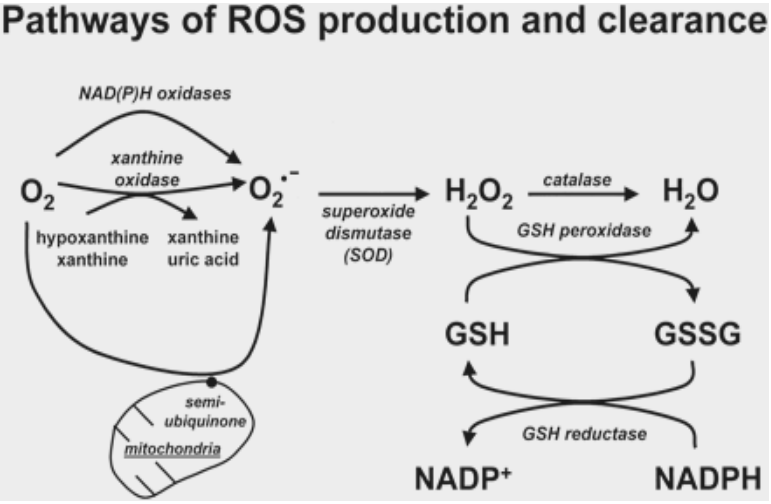

Fig. 5. Pathways of reactive oxygen species (ROS) production and clearance.GSH, glutathione; GSSG, glutathione disulfide.

2.9.3. Dietary (nutritional) antioxidants

Dietary (nutritional) antioxidants are low molecular weight compounds ingested in the diet such as vitamin C, vitamin E, the carotenoids, flavonoids and other plant phenolics. Many epidemiological studies have shown a link between diet and/or nutrition and the development of disease. Studies have shown that a high consumption of fruits and vegetables have been associated with a low risk of cancer, cardiovascular disease, and other chronic diseases.

2.10. Effect of Gamma Irradiation

The **liver** is the largest internal organ in the human body, and is an organ present in vertebrates and some other animals. It 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 and detoxification.

Skeletal muscle may be subjected to a greater level of oxidative stress during exercise than liver and heart due to increased ROS production. Therefore, the muscle needs greater antioxidant protection against potential oxidative damage occurring during and/or after stress **(Ji, 1995)**.

Among the sub-cellular organelles, mitochondria form some of the key components of cell killing induced by oxidative stress or radiation **(Slyshenkov et al., 1996)**. During oxidative stress, the flow of oxygen through muscle cells is greatly increased at high levels of oxygen uptake. At the same time, the rate of adenosine triphosphate (ATP) utilization exceeds the rate of ATP generation that can lead to free radical generation and has been implicated in fatigue, muscle soreness, myofibril disruption, and impairment of immune function (**Yu, 1994)**.

Gamma radiation alters the metabolism of the most common type of cell found in the liver, the hepatocyte. Under normal circumstances the blood supplies enough oxygen to the liver, but if hepatocytes use up more oxygen, oxygen deficits (i.e., hypoxia) can develop in some liver areas. Hypoxia, in turn, may impede the liver cells' ability to produce an energy–rich molecule ATP, which is generated during the breakdown of nutrients and supplies energy needed for numerous biochemical reactions. Sufficiently high levels of ATP are essential to the survival of all cells; reduced ATP levels in the liver are one factor contributing to liver cell death and may contribute to development of liver cirrhosis **(Carol et al., 2003)**.

2.10.1. Antioxidants status

The results of extensive studies showed that exposure to ionizing radiation results in significant alterations of antioxidant enzymes which depends upon the dose of radiation exposure, the time of biochemical analysis and the tissue analyzed. Radiation exposure results in changes in antioxidant enzyme levels: most consistently reported is elevation in SOD **(Weiss and Landauer, 2000)**. Irradiation caused increased lipid peroxide and decreased GSH levels in the intestine. Intestinal superoxide dismutase and glutathione peroxidase activities were increased, but glutathione transferase activity decreased following irradiation (**Umit et al., 2000**).

Lipid peroxidation is believed to be an important cause of destruction and damage to cell membranes and has been shown to be a contributing factor to the development of oxygen radicals-mediated tissue damage. Commonly measured parameter of lipid damage after ionizing radiation exposure is thiobarbituric acid reactive substances (TBARS) **(Samuni et al., 1997)**. **Palanivel et al., (1998)** reported that radiation represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipid peroxidation, or by indirect evidence of reduced antioxidant reserve, like SOD and CAT, in animal models.

Saada et al., (1999) reported that whole body gamma irradiation of rats with a fractionated dose of 8 Gy (4 x 2 Gy weekly) produces significant increase of blood GSH content, GSH-Px and GSH-reductase activities 1hr and 1day post-irradiation followed by a significant decrease on the 3rd day. **(Saada et al., 2003)** found that gamma

irradiation of rats with 7 Gy induces significant decrease in liver and kidney SOD and CAT activities 3, 7, 10 days post-irradiation. On the contrary lung SOD and CAT activities were increased. TBARS levels showed significant increases in all the tissues.

Koc et al., (2003) reported that total body irradiation with a single dose of 6 Gy resulted in a significant increase in the liver TBARS levels and a decrease of SOD and GSH-Px activities. **Kalpana and Menon, (2004)** reported that in irradiated rats the activities of SOD, CAT and GSH-Px were decreased in the blood and liver tissues. **Said, (2004)** showed that 5 Gy whole body gamma irradiation results in significant decreases of brain SOD and GSH-Px activities associated with an increase in TBARS content 1, 7 and 14 days post-irradiation. Also, **Said et al., (2004)** showed that whole body gamma irradiation of rats with 6 Gy (single dose) resulted in significant increase in cardiac TBARS along with reduction in cardiac SOD, CAT and GSH-Px activities 1, 2 and 4 weeks following radiation exposure.

Said et al., (2005) reported that whole body gamma irradiation of rats with 6 Gy produces an increase in liver and plasma TBARS and a decrease in GSH content 1 and 2 weeks post-irradiation**. Haldun et al., (2006)** found that Lipid peroxidation level was increased and the SOD, CAT and GSH-PX activities were decreased in the brain of irradiated rats.

Said and Azab, (2006) reported that 7 Gy (single dose) of whole body gamma irradiation induced significant elevation in TBARS levels with decrease in reduced glutathione content in heart tissues. Furthermore, gamma radiation at different doses (1, 2 and 4Gy) was found to significantly increase TBARS levels whereas the level of GSH

and the activities of SOD, CAT and GSH-Px in lymphocytes were significantly decreased. The maximum damage to lymphocytes was observed at 4Gy irradiation (**Srinivasan et al., 2006)**.

El-Missiry et al., (2007) reported that in irradiated rats subjected to two doses of 2 and 4Gy from Cesium-137 source, the TBARS, protein carbonyl contents and CAT activity were significantly increased in the liver, 5 days after irradiation. **Prabhakar et al., (2007)** reported that there is a depletion of glutathione transferase, SOD and CAT activities in the liver of irradiated mice. Irradiation with 5 Gy significantly increased the MDA level as an end product of lipid peroxidation. Irradiation also significantly decreased SOD activity and increased GSH-Px activity, 10 days after irradiation, indicating the generation of oxidative stress and an early protective response to oxidative damage **(Ibrahim et al., 2007)**.

2.10.2. Enzymes activities

Glutamate dehydrogenase

Glutamate dehydrogenase (GDH) is an enzyme, present in mitochondria of eukaryotes, as are some of the other enzymes required for urea synthesis, that converts glutamate to α-ketoglutarate, and vice versa. Under caloric restriction and low blood glucose, glutamate dehydrogenase activity is raised to increase the amount of α-Ketoglutarate that is produced. The product α-Ketoglutarate can be used to provide energy by being used in the citric acid cycle to ultimately produce ATP.

Zyrianova and Lavrova, (1998) found a significant decrease in the activities of glutamate dehydrogenase and aspartate aminotransferase in the liver of rats 1, 7 and 15 days after gamma whole

body gamma irradiation. **Saada and Azab, (2001)** showed that whole body exposure of rats to 7 Gy gamma irradiation results in a significant decrease in the activity of glutamate dehydrogenase in mitochondria of the liver after radiation exposure and this was accompanied by an increase in its activity in the cytosol.

Radiation-induced cellular dysfunction has been attributed, at least in part; to impairment of mitochondrial function as this organelle is both a major source of oxidants and a target for their damaging effects, which can result in a reduction of energy production, thereby compromising cell function. Radiation-induce significant decrease in glutamate dehydrogenase activity in rat liver due to glycation damage (**Hamelin et al., 2007)**.

Lactate dehydrogenase

Lactate dehydrogenase (LDH) is responsible for metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in the activity of LDH enzyme leads to biochemical impairment and lesions of the tissue and cellular function **(Khan et al., 2001)**.

Elevated LDH levels can be caused by a number of conditions, including heart failure, hypothyroidism, anemia, and lung or liver injury. **Franken et al., (2000)** reported that plasma LDH level was increased between 2 h and 24 h after local heart irradiation with a single dose of 20 Gy while cardiac enzyme (LDH) were reduced.

Sanchez et al., (2002) found a release of enzyme activity of LDH to blood plasma in mice exposed to oxidative stress. **Azab et al., (2003)** found also that whole body gamma irradiation of rats with a shot dose

of 6 Gy produces remarkable increases of serum LDH activity 1, 7, and 14 days post exposure.

Said and Azab (2006) reported that 7 Gy (single dose) of whole body gamma irradiation produced a significant rise in serum creatine phosphokinase and lactic dehydrogenase activities on the 7^{th} day post-irradiation **Said and Hanafy, (2006)** showed that rats exposed to 1 Gy whole body gamma irradiation exhibited a significant increase LDH activity in the serum.

Creatine phosphokinase

Creatine phosphokinase (CPK) plays an important role in cellular energy metabolism in vertebrates (**Saksa et al., 1996**). It is specifically located at places of energy demand and energy production and plays an important role in the energetic of calcium (Ca^{2+}) homeostasis and mitochondrial membrane stability. The muscle cells contain creatine phosphate and this used to restore ATP levels under the very short-term high-intensity conditions. Creatine phosphokinase mobilize a phosphate group from the creatine phosphate and this is quickly transferred to ADP to form ATP. So the muscle cell turns ATP into ADP and the phosphagen quickly turns ADP back into ATP. Elevation of serum CPK is an indication of muscle damage **(Holger et al., 2005).**

Franken et al., (2000) reported that plasma CPK level was increased between 2 h and 24 h after local heart irradiation with a single dose of 20 Gy while cardiac enzyme CPK was reduced. **Sánchez et al., (2002)** found a release of enzyme activity of CPK, to blood plasma in mice exposed to oxidative stress. **Sridharan and Shyamaladevi, (2002)** observed that exposure of rats to gamma-rays (3.5 Gy) caused increases in lipid peroxides and the excessive production of free radicals

and lipid peroxides might have caused the leakage of cytosolic enzymes LDH.

Azab and Nada, (2004) reported that exposure of rats to 7 Gy (acute dose) gamma irradiation induces significant decrease in the activities of mitochondrial CPK of brain and heart. In addition, **Said and Azab, (2006)** reported that 7 Gy (single dose) of whole body gamma irradiation produced a significant rise in creatine phosphokinase, and lactic dehydrogenase activities on the 7^{th} day post-irradiation.

Glucose -6- phosphate dehydrogenase

Glucose -6- phosphate dehydrogenase (G6PD) is the first enzyme in the pentose phosphate pathway and is widely distributed in nature, being found in almost all animal tissues and microorganisms. It is present in large amounts in blood cells, smaller amount in liver, kidney, heart and skeletal muscle and in only trace amount in serum. G6PD plays a key role in protection against oxidative stress especially in erythrocytes **(Beutler, 1978)**.

Savitskii et al., (1985) noticed that a single total-body exposure of rats to gamma rays in an absolutely lethal dose caused significant changes in the activity of G6PD in the brain, liver, myocardium and skeletal muscles. **Abady et al., (2003)** revealed significant increase G6PD in blood and spleen of rats, one hour after exposure to fractionated doses of whole body gamma irradiation up to cumulative dose of 6 Gy. This increase was followed by significant decrease on the 7^{th} day post radiation exposure.

Babu et al., (2008) observed that oxidative stress induced lipid peroxidation and decrease catalase, glutathione reductase, glucose-6-phosphate dehydrogenase enzyme activities and glutathione levels.

Xanthine oxidoreductase (XOR) system

Xanthine oxidoreductase (XOR) system is one of the major sources of free radicals in biological systems which consist of two enzymes; xanthine dehydrogenase (XDH) and xanthine oxidase (XO).The xanthine oxidoreductase system is exists mainly as a dehydrogenase form (XDH); only the oxidase form of the enzyme is associated with the generation of large amounts of superoxide and hydrogen peroxide. XO has been associated with ischemic reperfusion injury, radiation injury and acute viral infection **(Galley et al., 1996)**.

Xanthine oxidase is highly expressed in liver and intestine, while its expression and activity are low or non-detectable in human lung (**Linder et al., 1999)**. The percentage of oxidase activity seems to be correlated with tissue damage and consequent liver impairment (**Fiorenzo et al., 2002)**. Free radicals produced by XO are involved in the development and complications of many pathological conditions including respiratory distress syndromes, aging, cancer and diabetes as well as in injuries to neurons **(Atlante et al., 1997)**.

Ionizing radiations cause cell damage and death; the xanthine oxidoreductase system may contribute to the detrimental effects in irradiated systems. The mode and magnitude of change in the specific activities of XO and XDH were found to depend on radiation dose. At doses above 3 Gy, the specific activity of XO increased rapidly and continued to increase with increasing dose. However, the specific activity of XDH was decreased. These findings are suggestive of an inverse relationship between the activity of XO and XDH. The ratio of the activity of XDH to that of XO decreased with radiation dose.

However, the total activity (XDH + XO) remained constant at all doses. These results indicate that XDH may be converted into XO **(Srivastava et al., 2002)**.

Kale, (2003) found that XO may catalyze the generation of free radicals and potentiate radiation effects in the post-irradiation period. Free radical generating systems could be activated in the radiolytically damaged cell and in turn contribute to the cause and complications of late effects and their persistence in post-irradiation period.

Said and Azab, (2006) reported that 7 Gy (single dose) of whole body gamma irradiation induced significant elevation in xanthine oxidase activity. **Celik and Erdogan, (2008)** showed that oxidative stress and inflammation increase the activity of xanthine oxidase in diabetic rat brain.

Devrim et al., (2008) reported that increased xanthine oxidase activity is one of the leading factors for the oxidant stress in the late phase of sepsis. **Gomez-Cabrera et al., (2008)** found that sever oxidative stress causes oxidation of glutathione.

2.10.3. Minerals

In nutrition, the term “minerals” refers to chemical elements that are necessary for proper functioning of the body. There are two kinds of minerals: **macro minerals** and **trace minerals**. Macro means "large" in Greek (and the body needs **larger** amounts of macro minerals than trace minerals). The macro mineral group is made up of calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur. A trace of something means that there is only a little of it. So even though the body needs trace minerals, it needs just a tiny bit of each one. Trace minerals

includes iron, manganese, copper, iodine, zinc, cobalt, fluoride, and selenium**.**

Calcium is necessary for healthy bones and teeth, for clotting of the blood for the functioning of nerve tissue and muscles, for enzymatic processes, and for controlling the movement of fluids through cell walls. It also acts to balance the amounts of other minerals and promotes better use of iron by the body.

Copper is essential (with iron) for the formation of hemoglobin in red blood cells; and it is important for protein and enzyme formation, as well as for the nervous and reproductive systems, bones, hair, and pigmentation. Excess copper consumption may lead to liver damage.

Iron is essential to form the oxygen-carrying hemoglobin in red blood cells and it is also involved in muscle functioning and in enzyme reactions for producing energy.

Noaman and El-Tahawy, (2003) observed that exposure of rats to whole body γ-irradiation at 6.5 Gy provoked a deleterious effect presented by significant decrease in serum iron and copper concentrations while non-significant changes in the zinc, manganese and calcium concentrations.

Osman et al., (2003) reported that whole body gamma radiation of rats (4 Gy) induced a significant increase in iron level in serum, spleen and liver 1, 2 and 4 weeks post radiation exposure. Significant elevation of copper level in serum and spleen but significant reduction in copper in the liver was recorded on the 2^{nd} week post irradiation.

Mansour et al., (2006) noticed that exposure to whole body gamma-radiation with single dose of 6 Gy induces significant increases in the concentration of iron and Ca in liver tissue while, a significant

decrease in Ca level was observed in the kidney and spleen tissues compared to control group.

Recently, whole body gamma irradiation at 6.5 Gy was reported to induce significant elevation of iron level in liver and spleen and significant decrease in kidney, lung, intestine and brain. Radiation-induce significant reduction in copper level in all tissue organs and the levels of calcium were significantly increased in liver, spleen, lung and brain, while it significantly decreased in kidney and intestine tissues **(Nada et al., 2008)**.

2.11. Protection Against Reactive Oxygen Species Toxicity

Sustained oxidative stress from a heavy cumulative burden of oxidants may deplete the body's antioxidant reserves to a point of "distress," beyond which the individual's antioxidant defenses are overwhelmed. The resultant negative antioxidant balance begins to compromise life functions **(Kidd, 1991)**.

Experimental studies have shown that the extent of damage caused by free radicals might be modified through three dietary intervention strategies: (a) caloric restriction and thus a depression in free radicals arising due to normal metabolism; (b) minimizing the intake of components that increase free radicals such as polyunsaturated fats; and (c) supplementation with one or more anti-oxidants **(Van den Berg et al., 2001)**.

Fruits and vegetables are a rich source of micronutrients including vitamins, trace minerals such as selenium, dietary fiber and many other classes of biologically active compounds. Several of these micronutrients have been shown to prevent the disease process by serving as antioxidants in stopping the free radical chain of events. Use

of plant extracts, food supplement which augment major cellular endogenous antioxidants following chronic administration has been identified as a promising therapeutic approach to combat oxidative stress **(Vicky et al., 2004).** Wheat germ oil, extracted from the germ of the wheat kernel, has received much attention in recent decades in treatment of diseases involving oxidative damage **(Attila et al., 2001)**.

Wheat *(Triticum aestivum Linné*) was one of the first of the grains domesticated by humans. Wheat germ is a food source and is part of the breads and cereals food group. Wheat germ oil is nutritional oil derived from expeller-pressed wheat germ that contains lipids and liposoluble vitamins. Lipids consist largely of essential fatty acids (72%), i.e. linoleic, linolenic and arachidonic acids. Supplying the body with these essential fatty acids (EFAs) enables it to combat excessive cholesterol and atherosclerosis. Indeed, EFAs are necessary for synthesis of the prostaglandins that prevent cholesterol and saturated fats from being deposited on the walls of arteries, thereby having atherosclerosis-preventing properties **(Cesare et al., 2006)**.

Wheat germ oil is also very rich in vitamin E. It is a powerful natural antioxidant that protects EFAs against oxidation and combats the formation of toxins in the body. It also helps to reduce blood cholesterol level. The combined presence of EFAs and vitamin E situates wheat germ oil in the category of a preventer of arteriosclerosis and cardiovascular diseases and useful for the treatment of excessive cholesterol **(Cesare et al., 2006)**.

Octacosanol is a long-chain fatty alcohol derived from wheat germ oil and is responsible for many of the benefits of wheat germ oil. Octacosanol has been found to improve energy storage in muscles,

enhance performance, endurance and stamina, even at high altitudes, and improve oxygen utilization balance metabolism, and increase ability to handle stress. It is also a good source of omega-3 fatty acids and zinc **(Kim et al., 2006)** and relatively high levels of carotenoids and phytosterols (**Zhou et al., 2005**).

Paranich et al., (2000) showed that in oral administration wheat germ oil efficiently saturates the body with vitamin E which improves the flow of blood and strengthening the veins and capillaries. Wheat germ oil significantly increased the regeneration of platelets. It reduced platelet aggregation, thrombus formation and protected the red blood cells membranes from oxidative damage **(Lass and Sohal, 2000)**.

Wheat germ oil serves to lower marker of lipid peroxidation and stimulates antioxidant capacity of erythrocytes in radiated rats. Thus, the susceptibility of blood cells to peroxidation is decreased so the blood picture is improved. Wheat germ oil contains some B complex vitamins (B_6, B_{12} and folic acid) that are essential in the formation of red blood cells **(Vicky et al., 2004)**.

Paul et al., (2001) found that, a diet rich in wheat germ oil protects from alcohol-induced duodenal ulceration. Wheat germ oil was reported to increase endurance and to assist muscular dystrophies and other neuromuscular disorders **(Vicky et al., 2004)**. It was claimed to be anti-inflammatory and described as a suitable natural antioxidant due to its high content of vitamin E **(Paranich et al., 2000)**. The oil was reported, also, to be a valuable source of essential fatty acids, including linoleic acid and linolenic acid, whose insufficiency was observed to cause tiredness, dry skin, immune insufficiency, anorexia, indigestion and cardiovascular disorders **(Mohamed et al., 2005)**.

Said and Azab, (2006) reported that supplementation of rats with wheat germ oil (81 mg/ kg body wt) for 10 successive days before and 7 successive days after whole body gamma irradiation, significantly ameliorated serum lipid profile levels and reduced the severity of changes in the activity of serum CPK and modulated the alteration in activities of LDH and its isoenzymes patterns when comparing with irradiated rats**.** Moreover, guinea pigs receiving wheat germ oil did not develop muscular dystrophy and showed normal creatine values **(Nobuko et al., 2008)**.

3- MATERIALS AND METHODES

3.1. Experimental Animals

Male Swiss Albino rats (100-120 g) purchased from the Egyptian Organization for Biological Products and Vaccines were used for the different investigations carried out in this work. Animals were housed in specially designed cages and maintained in conditions of good ventilation, normal temperatures and humidity ranges and kept under observation for one week prior to experimentation. The rats were fed on standard pellets, containing all nutritive elements (proteins, fats, carbohydrates, vitamins, salts and minerals). Drinking water and food were provided *ad libitum* throughout the study.

All animal studies were conducted in accordance with criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

3.2. 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 ensured a homogeneous distribution of irradiation. The dose rate was 0.61 Gy/minute during the experimental periods. Animals were whole body exposed to 5 Gy delivered as a single shot dose.

3.3. Preparation of Wheat Germ Oil

Wheat germ oil (WGO) was supplied as a soft gel and was provided by Sigma Chemical Company. WGO was freshly prepared just before the application. WGO (300mg/5ml) were suspended in corn oil and administered to animals by gavage at doses of 54 mg/kg body weight. The doses used in this study were selected on the basis of the reports of previous studies **(Reddy et al., 2000 and Paranich et al., 2000)**.

3.4. Experimental Design

Experimental animals have been sorted into four groups (each of 18 rats) as follows:

1. **Control group:** Rats of that group received 0.1 ml corn oil for 21 successive days.

2. **WGO-treated group:** Rats of that group received daily wheat germ oil (54 mg/ kg body weight dissolved in 0.1ml corn oil), for 21 successive days.

3. **Irradiated group:** Rats of that group were exposed to whole body gamma irradiation (5 Gy shot dose).

4. **WGO-treated irradiated group**: Rats of that group received wheat germ oil for 14 successive days before and 7 successive days after exposure to whole body gamma radiation.

3.5. Preparation of Samples

Six rats from each group were sacrificed 1, 2 and 3 weeks post-irradiation. Immediately after sacrifice, samples of liver and skeletal

muscle of femur bone were rapidly excised from the body of each animal and divided into two parts. One part of each sample was weighed and stored at -20˚C until the assay of trace elements were performed. The other part was homogenized in normal 0.9% saline for biochemical studies.

3.6. Biochemical Analysis

3.6.1. Determination of Superoxide Dismutase Activity

Measurement of the activity of superoxide dismutase (SOD) in the liver and skeletal muscle tissues was done based on the method of **Minami and Yoshikawa, (1979)**. The assay relies on the ability of the enzyme to inhibit the phenazine methosulfate (PMS) mediated reduction of nitroblue tetrazolium (NBT) dye. The increase in absorbance at 560 nm due to the formation of reduced NBT was recorded in a spectrophotometer.

One unit of SOD activity is defined as the amount of the enzyme causing half the maximum inhibition of NBT reduction. The activity was expressed as U/g fresh tissue.

3.6.2. Determination of Catalase Activity

Catalase activity (CAT) in tissues was determined according to the method of **Aebi, (1983)**, in which the disappearance of peroxide is followed spectrophotometrically at 240 nm. The method is based on the catalytic function of the enzyme where catalase catalyzed the following reaction:

$$2\ H_2O_2 \text{ -------Catalase-----} \rightarrow 2H_2O + O_2$$

One unit of catalase activity is defined as the amount of enzyme required to decompose one micromole of hydrogen peroxide per minute. The activity was expressed as U/g fresh tissue.

3.6.3. Determination of Reduced Glutathione Content

Reduced glutathione (GSH) content in tissue was determined according to the method of **Beutler et al., (1963)**. The method depends on the fact that both protein and non-protein SH-groups (sulfhydryl groups) react with Ellman's reagent 5, 5'dithiobis- 2-nitro-benzoic acid (DTNB) to form a stable yellow color of 5-thio-2-nitro-benzoic acid, which can be measured at 412 nm. The level of GSH is expressed as mg/g fresh tissue**.**

3.6.4. Determination of Glutathione Peroxidase Activity

The activity of glutathione peroxidase (GSH-Px) in the tissue was determined according to the method of **Paglia and Valentine, (1967)**. The principle of this method is shown diagrammatically as follows:

$$2GSH + H_2O_2 \text{ --- glutathione peroxidase} \rightarrow GSSG + 2H_2O$$

The deep yellow color of oxidized glutathione (GSSG) formed by the action of hydrogen peroxide H_2O_2 and GSH-Px can be measured spectrophotometrically at 340 nm. The activity of GSH-Px was expressed as mg consumed reduced glutathione/min/g fresh tissue.

3.6.5. Determination of Glucose-6-phosphate Dehydrogenase Activity

The activity of glucose-6-phosphate dehydrogenase (G-6-PDH) in the tissue was determined according to the method of **Kornberg and Horecker, (1955).** Glucose-6-phosphate dehydrogenase catalyzes the

oxidation of glucose-6-phosphate (G-6-P) with the reduction of nicotineamide adenine dinucleotide phosphate (NADP) to reduced nicotineamide adenine dinucleotide phosphate (NADPH) and gluconate-6-phosphate (gluconate-6-P) according to the following reaction:

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{Gluconate-6-P} + \text{NADPH} + H^+$$

The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the G-6-PDH activity in the sample. The activity of G-6-PDH was expressed as U/g fresh tissue.

3.6.6. Determination of Creatine Phosphokinase Activitiy

The activity of creatine phosphokinase (CPK) was determined according to the method of **Meiattini et al., (1978)**. The method is based on the catalytic function of CPK which catalyses the reaction between creatine phosphate and adenosine diphosphate (ADP), giving creatine and adenosine triphosphate (ATP). ATP, in presence of glucose and hexokinase (HK), forms ADP and glucose-6-phosphate (G-6-P). G-6-P is converted to 6-phosphogluconate (6-PG) with G-6-PDH and NADP is reduced to NADPH. The rate of NADPH transformation is determined photometrically.

Creatine phosphokinase catalyzes the following reactions:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CPK}} \text{Creatine} + \text{ATP} \qquad (1)$$

$$\text{ATP} + \text{glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G-6-P} \qquad (2)$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-PG} + \text{NADPH} + H^+ \qquad (3)$$

The rate of $NADP^+$ reduction is proportional to the CPK activity. International enzyme unit (U) is defined as activity of enzyme that

converts one micromole of substrate in one minute under standard conditions. The activity of CPK was expressed as U/g fresh tissue.

3.6.7. Determination of Lactate Dehydrogenase Activity

Lactate dehydrogenase (LDH) activity can be measured as the conversion of lactate to pyruvate or of pyruvate to lactate. LDH activity was determined according to the method of **Amador and Wacker, (1965)**. The method depends on the catalytic activity of LDH which catalyses reduced nicotineamide adenine dinucleotide (NADH) in presence of pyruvate to nicotineamide adenine dinucleotide (NAD) and lactate**.** LDH catalyzes the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \text{ ------ LDH -----}\rightarrow \text{ Lactate} + NAD^+$$

The rate of NADH oxidation is proportional to LDH activity. The activity of LDH was expressed as international unit U/g fresh tissue.

3.6.8. Determination of Glutamate Dehydrogenase Activity

Glutamate dehydrogenase activity (GDH) was determined according to the method of **Plummer, (1999)**. The method depends on the catalytic activity of GDH on 2-oxoglutarate and ammonium (NH_4^+**)** as substrates and reduced nicotineamide adenine dinucleotide (NADH) as coenzyme as in the following reaction:

$$\text{2-oxoglutrate} + \text{NADH} + NH_4^+ \xleftrightarrow{\text{GDH}} \text{L- glutamate} + NAD^+ + H_2O$$

The decrease in absorbance due to the oxidation of NADH is the measure of catalytic activity of GDH. The activity of GDH is expressed as international unit U/g fresh tissue.

3.6.9. Determination of Thiobarbituric Acid Reactive Substances Levels

The extent of lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to **Yoshioka et al., (1979)**. The method is based on the determination of malondialdehyde (MDA) an end product of lipid peroxidation, which can react with thiobarbituric acid in acidic medium to yield a pink colored trimethine complex exhibiting an absorption maximum at 532 nm. The concentration of TBARS in the sample was calculated as nmole/g fresh tissue.

3.6.10. Determination of Xanthine Oxidoreductase System Activities

The xanthine oxidoreductase (XOR) system, which consists of xanthine dehydrogenase (XDH) and xanthine oxidase (XO) were assayed using the method of **Kaminski and Jezewska, (1979)**. The xanthine oxidoreductase activity was measured spectrophotometrically as formation of uric acid and NADH at 302 and 340 nm respectively. The reaction mixture contained 50 mM xanthine, 150mM tris-HCL and the protein samples. The total activity (XDH+XO) was determined at the same wavelength by adding NAD (150 mM) to the reaction mixture. The reaction mixture (without tissue extract) was mixed and incubated at 25˚C for 5 min. The reaction was started by adding the enzyme (tissue extract) and the progress of the reaction was monitored after an initial delay of 2 min.

The activity of XDH was calculated by subtracting the XO activity from the total activity (XDH+XO). One unit of enzyme activity was defined as amount of enzyme required to catalyze the formation of

1μmol of uric acid per minute at 25℃. The enzyme activity was expressed as mU/mg protein.

3.6.11. Determination of Minerals Content

In the present study, total iron, copper and calcium were estimated in liver and skeletal muscle using atomic absorption spectrophotometer. The method is based on the fact that atoms of an element in the ground or unexcited state will absorb light of the same wave length that they emit in the excited state. The wave lengths of that light or the resonance lines are characteristic for each element. No two elements have identical resonance lines.

Fresh tissue samples were washed thoroughly with deionized water and then dried on filter paper. Known weight of liver and skeletal muscle was digested in kjeldahl flasks using a mixture of nitric acid and perchloric acid **(Dawson et al., 1968)**. Digested samples were cooled and diluted using deionized water.

The selected elements were then estimated quantitatively by atomic absorption spectrophotometer using Pye unicam SP 191. Concentration of each element was calculated against standard solution using a calibration curve.

3.6.12. Determination of Total Protein

Protein content was determined in tissue according to the Biuret reaction **(Doumas, 1975)**.

Biuret test is used for the quantitative photometrical determination of total protein concentration. Peptides and proteins (long-chain polypeptides) react with Cu^{2+} in alkalinity to create a blue violet colored chelate complex with an absorbance maximum at 540

nm. The intensity of the color produced in the Biuret reaction is proportional to the number of peptide bonds participating in the reaction.

3.7. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS/PC) computer program was used for statistical analysis of the results. Data were analyzed using one way analysis of variance (ANOVA). The data were expressed as mean ± standard deviation (SD). Differences were considered significant at $P \leq 0.05$.

4- RESULTS

4.1. Role of Wheat Germ Oil in the Xanthine Oxidoreductase System of Liver Tissue

4.1.1. Liver xanthine oxidase

Liver xanthine oxidase (XO) activity for the four animal groups are presented in table (1) and illustrated by figure (6). The results obtained indicated the followings:

In the group of control rats: XO activity ranged from 1.39 ± 0.11 to 1.32 ± 0.14 mU/mg protein. There were no significant changes recorded for liver XO activity throughout the period of the experiment (21 days).

In the group of WGO-treated rats: There were no significant changes for liver XO activity recorded throughout the experimental period, compared to control values.

In the group of gamma irradiated rats: XO activity was significantly increased ($P<0.05$) by +113, +106 and +139%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: XO activity showed significant increases ($P<0.05$) of +53, +46 and +61%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that WGO treatment significantly decreased liver XO activity, compared to their corresponding values in irradiated rats.

Table 1: Liver xanthine oxidase activity (mU/mg protein) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	1.39± 0.11 [a]	1.35± 0.09 [a]	1.32± 0.14 [a]
WGO-treated % of change from control	1.27± 0.08 [a] -9	1.29± 0.09 [a] -4	1.30± 0.10 [a] -2
Irradiated % of change from control	2.96± 0.21 [b] +113	2.78± 0.15 [c] +106	3.16± 0.12 [d] +139
WGO-treated irradiated % of change from control	2.13± 0.15 [e] +53	1.97± 0.12 [f] +46	2.13± 0.11 [e] +61

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

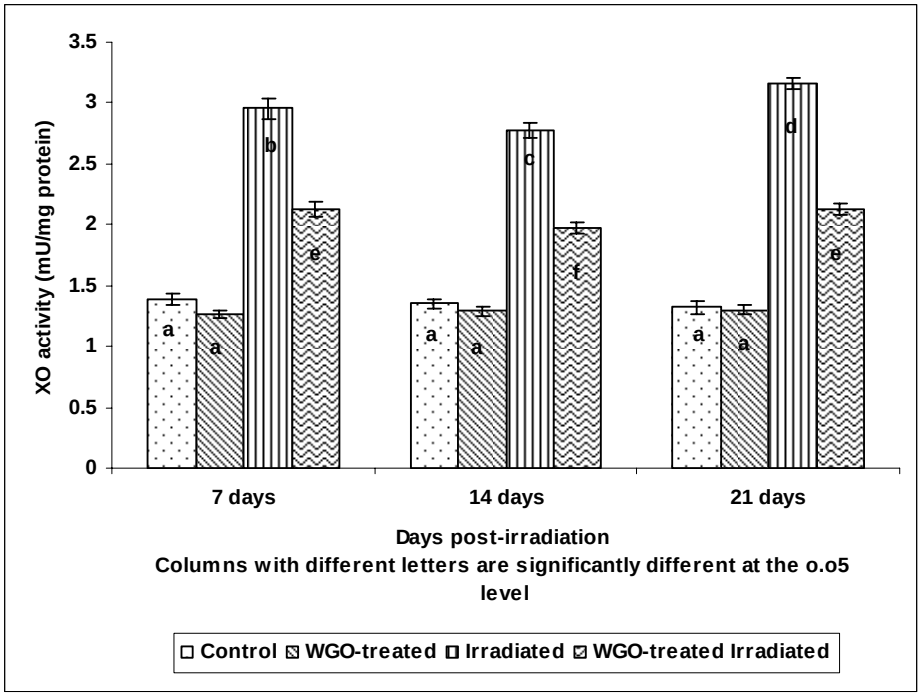

Figure 6: Liver xanthine oxidase activity (XO) (mU/mg protein) in different rat groups.

4.1.2. Liver xanthine dehydrogenase

Liver xanthine dehydrogenase (XDH) activity is presented in table (2) and illustrated by figure (7).

In the group of control rats: XDH activity ranged from 3.02 ± 0.15 to 3.01 ± 0.17 mU/mg protein. There were no significant changes recorded for liver XDH activity throughout the period of the experiment (21 days).

In the group of WGO-treated rats: There were no significant changes for liver XDH activity recorded throughout the experimental period, compared to control values.

In the group of gamma irradiated rats: XDH activity was significantly decreased ($P<0.05$) by -52, -51 and -55% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: XDH activity showed significant decreases ($P<0.05$) of -20, -23 and -24% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to corresponding control values.

The results obtained in the present study showed that WGO treatment significantly increased liver XDH activity, compared to their corresponding values in irradiated rats.

Table 2: Liver xanthine dehydrogenase activity (mU/mg protein) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	3.02± 0.15 [a]	3.02± 0.13 [a]	3.01± 0.17 [a]
WGO-treated % of change from control	3.11± 0.14 [a] +3	3.07± 0.18 [a] +2	3.07± 0.15 [a] +2
Irradiated % of change from control	1.44± 0.14 [b] -52	1.49± 0.08 [b] -51	1.36± 0.14 [b] -55
WGO-treated irradiated % of change from control	2.41± 0.07 [c] -20	2.34± 0.11 [c] -23	2.29± 0.10 [c] -24

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

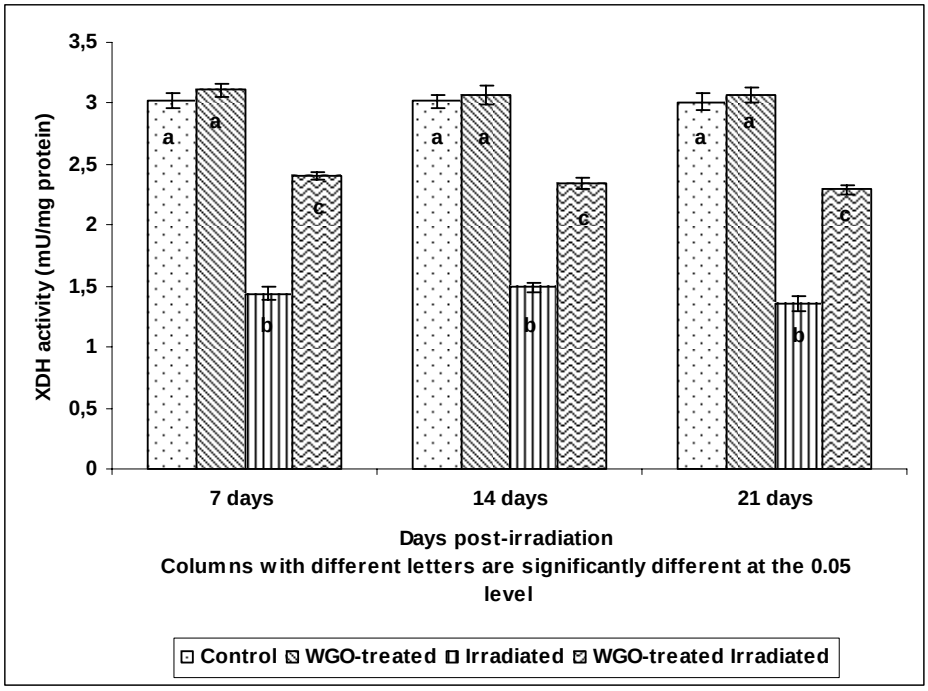

Figure 7: Liver xanthine dehydrogenase activity (XDH) (mU/mg protein) in different rat groups

4.2. Role of Wheat Germ Oil in the Antioxidant Status of Liver Tissue

4.2.1. Liver superoxide dismutase

Liver superoxide dismutase (SOD) activity is presented in table (3) and illustrated by figure (8).

In the group of control rats: SOD activity ranged from 135 ± 6.2 to 131 ± 6.0 U/g fresh tissue. There were no significant changes recorded for liver SOD activity throughout the period of the experiment (21 days).

In the group of WGO-treated rats: There were no significant changes recorded for the liver SOD activity throughout the experimental period, compared to control values.

In the group of gamma irradiated rats: SOD activity were significantly decreased ($P<0.05$) by -47, -32 and -43%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: SOD activity showed significant decreases ($P<0.05$) of -30, -14 and -24%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that WGO treatment significantly increases liver SOD activity, compared to their corresponding values in irradiated rats.

Table 3: Liver superoxide dismutase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	135± 6.2 [a]	133 ± 7.5 [a]	131 ± 6.0 [a]
WGO-treated % of change from control	139± 15.8 [a] +3	137± 7.1 [a] +3	136± 9.2 [a] +4
Irradiated % of change from control	72 ± 14.9 [b] -47	91 ± 18.1 [c] -32	75 ± 8.7 [b] -43
WGO-treated irradiated % of change from control	95± 11.0 [c] -30	115 ± 14.3 [d] -14	99 ± 13.2 [c] -24

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

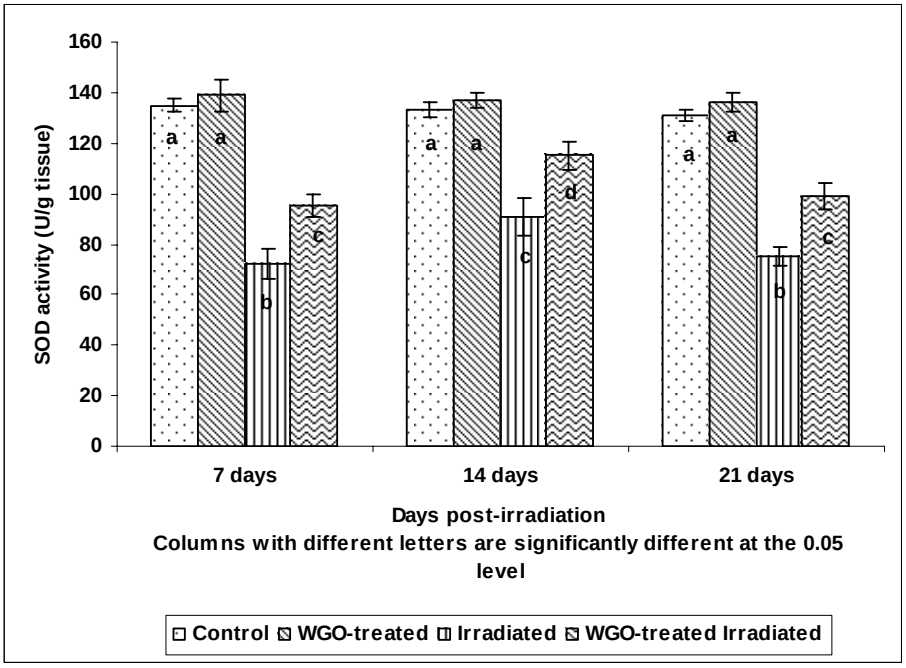

Figure 8: Liver superoxide dismutase activity (SOD) (U/g fresh tissue) in different rat groups

4.2.2. Liver catalase

Liver catalase activity (CAT) is presented in table (4) and illustrated in figure (9).

In the group of control rats: Liver CAT activity ranged from 83.9 ± 7.6 to 82.9 ± 4.7 U/g fresh tissue, with no significant changes recorded throughout the period of the investigation.

In the group of WGO-treated rats: There was no significant change recorded for liver CAT activity throughout the period of experiment, compared to corresponding values of control groups.

In the group of gamma irradiated rats: CAT activity showed significant decreases ($P<0.05$) of -57, -41 and -50 % on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: CAT activity showed significant decreases ($P<0.05$) of -38, -25 and -34% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that WGO administration significantly increased liver CAT activity compared to their corresponding values in irradiated rats.

Table 4: Liver catalase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	83.9 ± 7.6 [a]	84.5 ± 7.7 [a]	82.9 ± 4.7 [a]
WGO-treated % of change from control	85.0 ± 4.0 [a] +1	86.0 ± 4.1 [a] +2	84.0± 5.6 [a] +1
Irradiated % of change from control	36.4 ± 2.5 [b] -57	50.0 ± 4.4 [c] -41	41.0 ± 4.5 [b] -50
WGO-treated irradiated % of change from control	52.0 ± 5.1 [c] -38	63.0 ± 4.2 [d] -25	55.0 ± 5.3 [c] -34

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

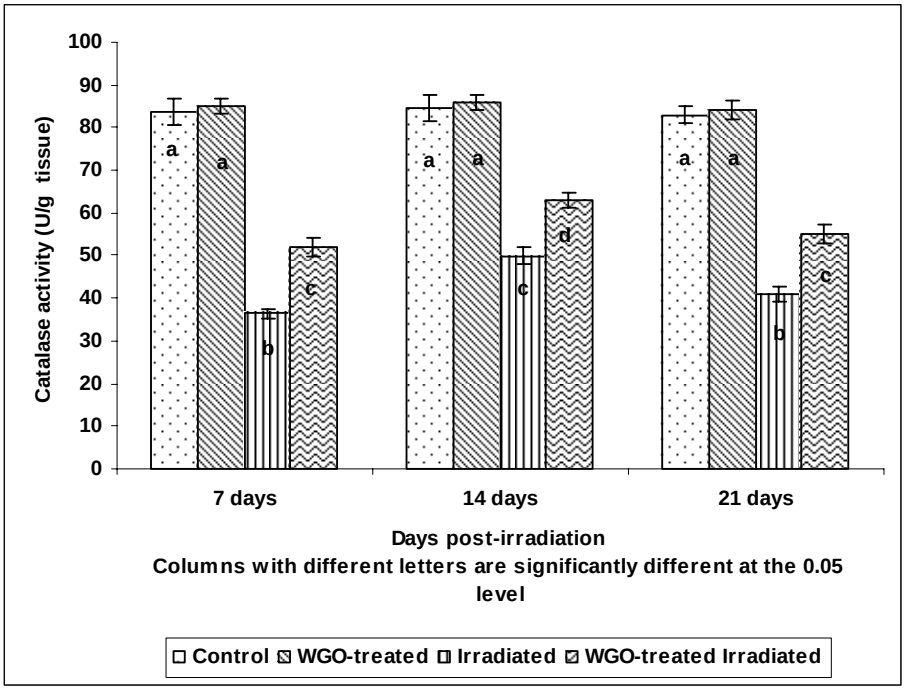

Figure 9: Liver catalase activity (U/g fresh tissue) in different rat groups

4.2.3. Liver glutathione peroxidase

Liver glutathione peroxidase activity (GSH-Px) is presented in table (5) and illustrated in figure (10) respectively. The results indicate that:

In the group of control rats: GSH-Px activity ranged from 5.7 ± 0.52 to 5.6 ± 0.54 mg consumed glutathione/min/g fresh tissue, with no significant changes recorded throughout the period of the investigation.

In the group of WGO-treated rats: There were no significant changes recorded for GSH-Px activity at all the experimental times, compared to control values.

In the group of gamma irradiated rats: GSH-Px activity showed significant decreases ($P<0.05$) of -32, -23 and -39 %, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated: GSH-Px activity showed significant decreases ($P<0.05$) of -20, -18 and -22 % on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results demonstrated that WGO treatment significantly increased GSH-Px activity, compared to their corresponding values in irradiated rats.

Table 5: Liver glutathione peroxidase activity (mg consumed glutathione/min/g fresh tissue) in different groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	5.7± 0.52 [a]	6.0 ± 0.55 [a]	5.6 ± 0.54 [a]
WGO-treated % of change from control	5.8 ± 0.33 [a] +2	6.3± 0.48 [a] + 5	5.7± 0.39 [a] +2
Irradiated % of change from control	3.9 ± 0.88 [b] -32	4.6 ± 0.43 [c] -23	3.4 ± 0.49 [b] -39
WGO-treated irradiated % of change from control	4.6± 0.44 [c] -20	4.9 ± 0.67 [c] -18	4.4 ± 0.62 [c] -22

Values are expressed as means of 6 records ± standard deviation

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

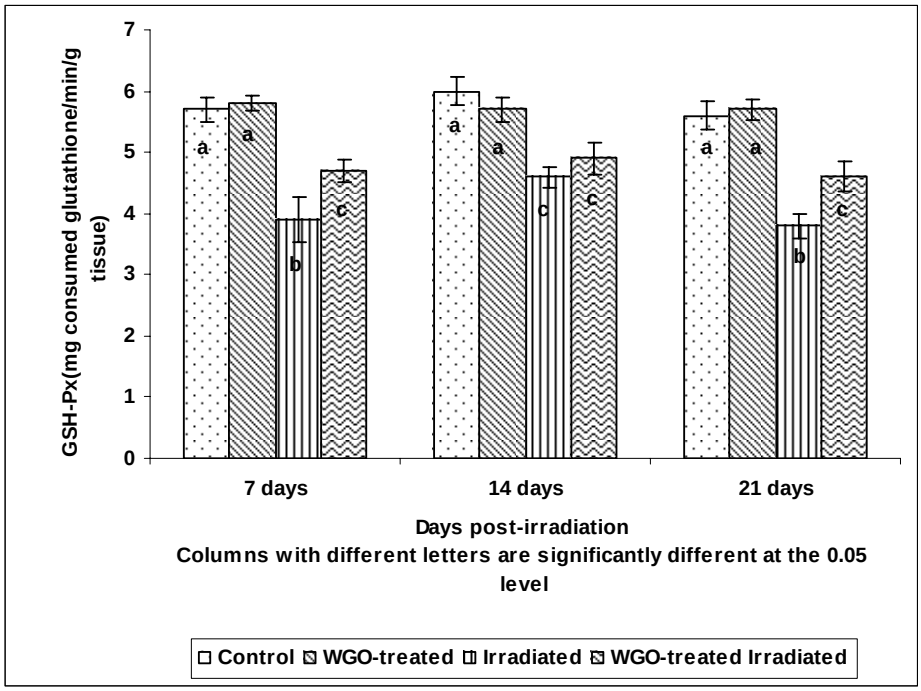

Figure 10: Liver glutathione peroxidase activity (GSH-Px) (mg consumed glutathione/min/g fresh tissue) in different rat groups

4.2.4. Liver glutathione

Liver glutathione content (GSH) is presented in table (6) and illustrated in figure (11).

In the group of control rats: GSH content ranged from 24.1 ± 2.3 to 23.5 ± 0.5 mg /g fresh tissue, with no significant changes recorded throughout the period of the investigation.

In the group of WGO-treated rats: There were no significant changes recorded for GSH content at all the experimental times, compared to control values.

In the group of gamma irradiated rats: GSH content showed significant decreases ($P<0.05$) of -46, -39 and -45% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated: GSH content showed significant decreases ($P<0.05$) of -34, -30 and -27 % on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results demonstrated that WGO treatment significantly increased GSH content, compared to its corresponding values in irradiated rats.

Table 6: Liver glutathione content (mg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	24.1± 2.3 [a]	22.8 ± 0.9 [a]	23.5 ± 0.5 [a]
WGO-treated % of change from control	24.6 ± 0.7 [a] +2	23.9± 0.9 [a] +5	24.0± 2.3 [a] +2
Irradiated % of change from control	13.0 ± 2.1 [b] -46	14.0 ± 2.4 [b] -39	12.8 ± 1.1 [b] -45
WGO-treated irradiated % of change from control	15.9± 1.7 [c] -34	16.0 ± 1.3 [c] -30	17.1 ± 1.3 [c] -27

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

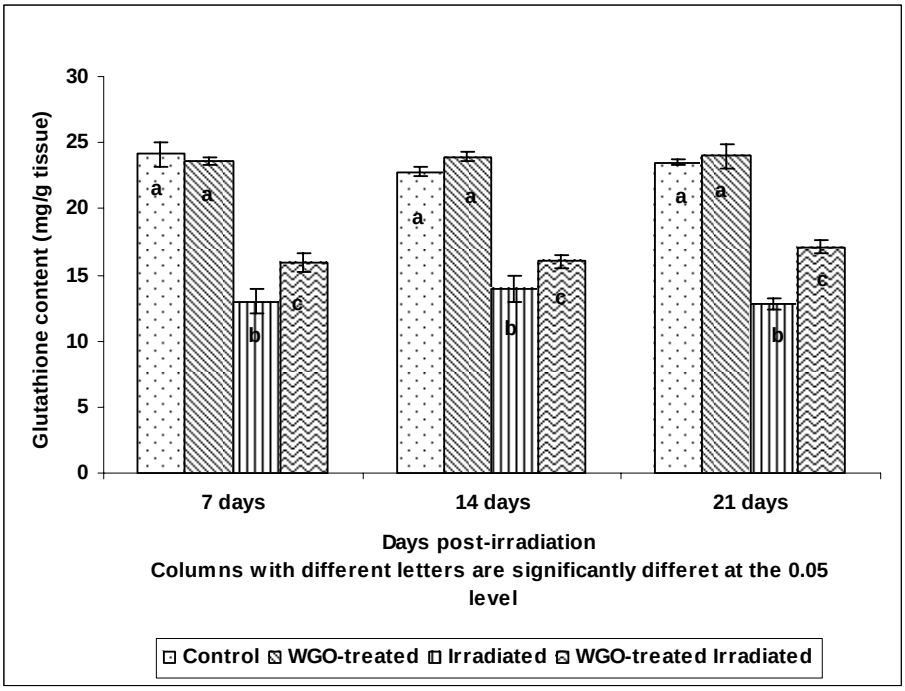

Figure 11: Liver glutathione content (mg/g fresh tissue) in different rat groups

4.2.5. Liver thiobarbituric acid reactive substances

Liver thiobarbituric acid reactive substances (TBARS) levels are presented in table (7) and illustrated in figure (12).

In the group of control rats: TBARS levels ranged from 236 ± 11.0 to 233 ± 6.6 nmole/g fresh tissue, with no significant changes recorded throughout the period of the investigation.

In the group of WGO-treated rats: There was no significant change recorded throughout the experiment time, compared to the corresponding values of control groups.

In the group of gamma irradiated rats: TBARS levels showed significant increases ($P<0.05$) of 64, 29 and 57% 7, 14 and 21 days post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: TBARS levels were significantly increased ($P<0.05$) by 28, 15 and 29 %, 7, 14 and 21 days post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that WGO administration significantly diminished the levels of TBARS in liver tissues, compared to the corresponding values in irradiated rats.

Table 7: Liver thiobarbituric acid reactive substances levels (TBARS) (nm/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	236 ± 11.0 [a]	233 ± 11.3 [a]	233 ± 6.6 [a]
WGO-treated % of change from control	232 ± 15.5 [a] -2	235± 9.3 [a] +1	231± 9.0 [a] -1
Irradiated % of change from control	388 ± 47.6 [b] + 64	301 ± 37.6 [c] + 29	366 ± 29.2 [b] + 57
WGO-treated irradiated % of change from control	302± 10.1 [c] + 28	268 ± 12.7 [d] + 15	300 ± 19.2 [c] + 29

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

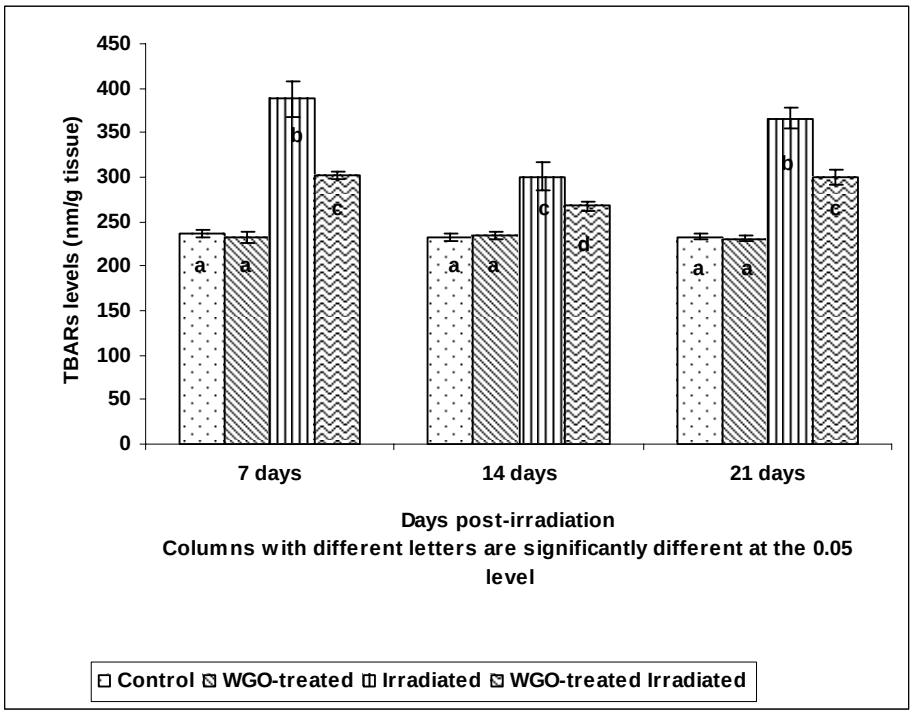

Figure 12: Liver thiobarbituric acid reactive substances levels (TBARS) (nmol/g fresh tissue) in different rat groups

4.3. Role of Wheat Germ Oil in the Metabolic Enzymes of Liver Tissue

4.3.1. Liver glucose-6-phosphate dehydrogenase

Liver glucose-6-phosphate dehydrogenase activity (G-6-PDH) is presented in table (8) and illustrated by figure (13). The results obtained indicate that:

In the group of control rats: liver G-6-PDH activity ranged from 112 ± 4.3 to 113 ± 3.3 U/g fresh tissue.

In the group of WGO-treated rats: No significant changes in liver G-6-PDH activity were recorded throughout the experiment time, as compared to control group.

In the group of irradiated rats: Liver G-6-PDH activity showed significant decrease of -38, -34 and -49%, 7, 14 and 21 days post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: Liver G-6-PDH activity showed significant decreases of -24, -14 and -23%, 7, 14 and 21 days post-irradiation, respectively, compared to the control values.

The results obtained in the present study showed that WGO treatment significantly increased liver G-6-PDH activity, compared to their corresponding values in irradiated rats.

Table 8: Liver glucose-6-phosphate dehydrogenase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	112± 4.3 [a]	113 ± 3.8 [a]	113 ± 3.3 [a]
WGO-treated % of change from control	113± 3.1 [a] +1	114± 3.7 [a] +1	116± 2.5 [a] +3
Irradiated % of change from control	70 ± 3.1 [b] -38	75 ± 6.0 [c] -34	57 ± 5.8 [d] -49
WGO-treated irradiated % of change from control	85± 4.3 [e] -24	97 ± 9.9 [f] -14	87 ± 3.2 [e] -23

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

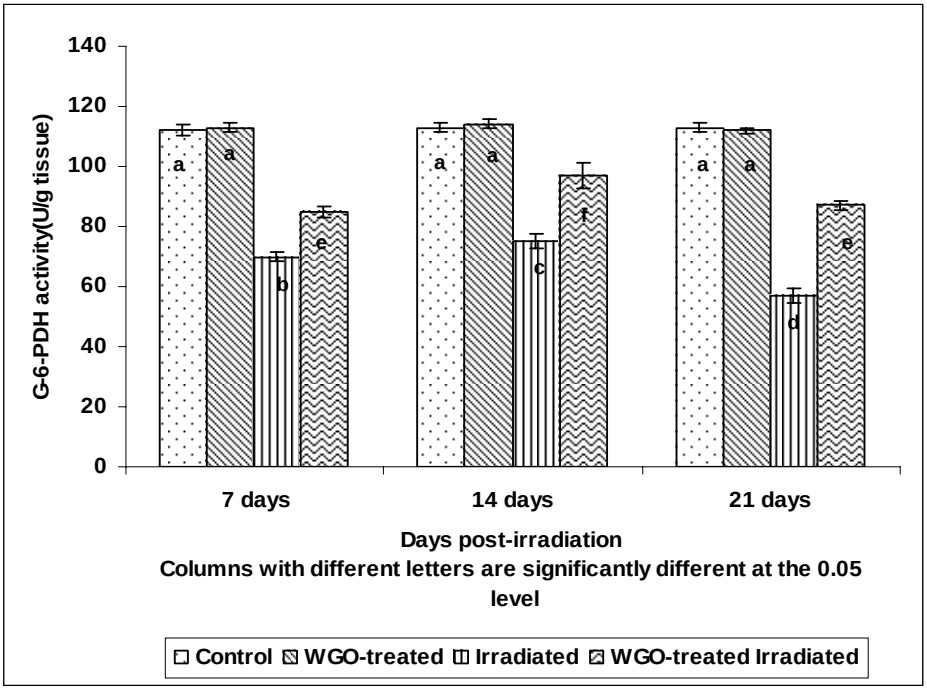

Figure 13: Liver glucose-6-phosphate dehydrogenase activity (G-6-PDH) (U/g fresh tissue) in different rat groups

4.3.2. Liver glutamate dehydrogenase

Glutamate dehydrogenase activity (GDH) of liver tissue is presented in table (9) and illustrated in figure (14). The results obtained indicated the followings:

In the group of control rats: Liver GDH activity ranged from 22.0 ± 2.8 to 21.3 ± 3.3 U/g fresh tissue, with no significant changes recorded throughout the period of investigation.

In the group of WGO-treated rats: No significant changes were recorded in liver GDH activity at all the experimental times, compared to control groups.

In the group of gamma irradiated rats: GDH activity was significantly decreased ($P<0.05$) by -46, -50 and -53% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: GDH activity showed significant decreases ($P<0.05$) of -30, -24 and -30%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that wheat germ oil treatment significantly ameliorated the radiation-induced alterations in liver GDH activity.

Table 9: Liver glutamate dehydrogenase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	22.0± 2.8 [a]	21.5 ± 3.3 [a]	21.3 ± 3.3 [a]
WGO-treated % of change from control	22.7± 2.9 [a] +3	22.8± 3.3 [a] +6	22.2± 3.3 [a] +4
Irradiated % of change from control	11.8 ± 1.9 [b] -46	10.7 ± 1.4 [b] -50	10.0 ± 2.4 [b] -53
WGO-treated irradiated % of change from control	15.3± 2.2 [c] -30	16.3 ± 1.7 [c] -24	15.0 ± 2.4 [c] -30

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

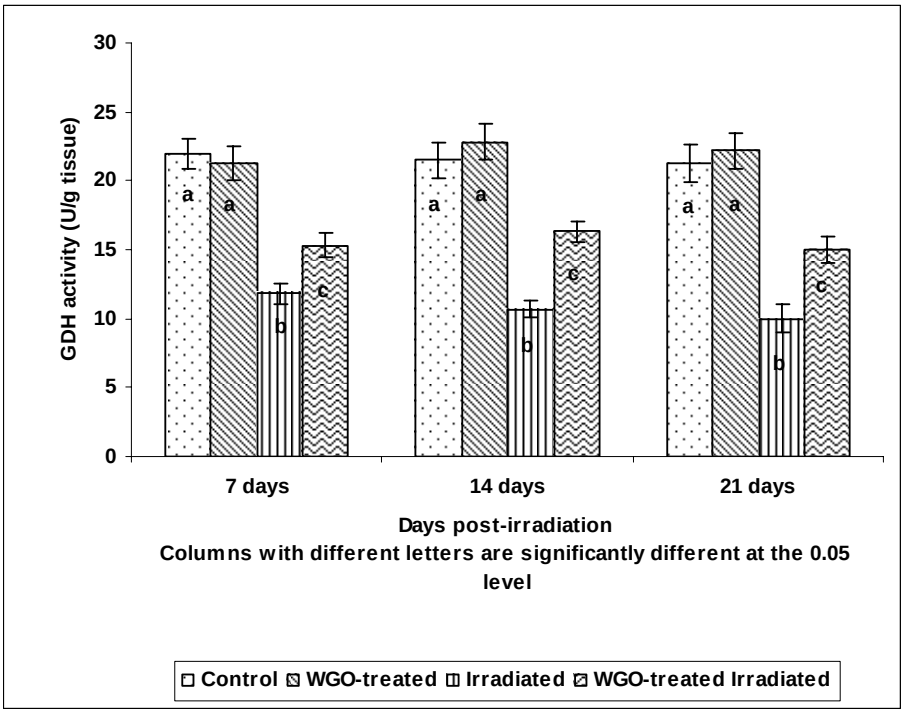

Figure 14: Liver glutamate dehydrogenase activity (GDH) (U/g fresh tissue) in different rat groups

4.3.3. Liver lactate dehydrogenase

Liver lactate dehydrogenase (LDH) activity is presented in table (10), and illustrated by figure (15).

In the group of control rats: liver LDH activity ranged from 145 ± 11.4 to 142 ± 12.5 U/g fresh tissue.

In the group of WGO-treated rats: There was no significant change in liver LDH activity recorded throughout the experimental period, compared to control values.

In the group of irradiated rats: Liver LDH activity showed significant increases ($P<0.05$) of 56, 46 and 89%, 7, 14 and 21 days post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: liver LDH activity showed significant increases ($P<0.05$) of 27, 18 and 37% 7, 14 and 21 days post-irradiation, respectively, compared to control values.

The results obtained showed that wheat germ oil treatment significantly reduced the radiation-induced increase in liver LDH activity.

Table 10: Liver lactate dehydrogenase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	145± 11.4 [a]	142± 11.7 [a]	142± 12.5 [a]
WGO-treated % of change from control	142± 9.0 [a] -2	144± 10.3 [a] +1	143± 7.1 [a] +1
Irradiated % of change from control	227± 7.6 [b] +56	207± 6.4 [c] +46	268± 12.3 [d] +89
WGO-treated irradiated % of change from control	184± 8.9 [e] +27	167± 12.0 [f] +18	194± 7.3 [e] +37

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

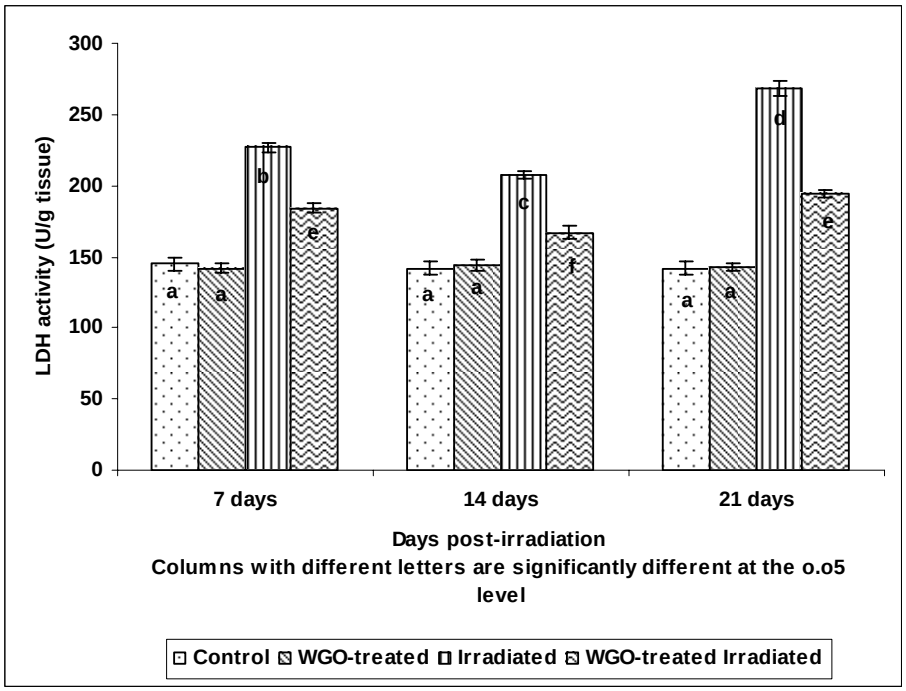

Figure 15: Liver lactate dehydrogenase activity (LDH) (U/g fresh tissue) in different rat groups

4.3.4. Liver creatine phosphokinase

Liver creatine phosphokinase activiy (CPK) in the four animal groups is presented in table (11) and illustrated by figure (16). The results obtained indicate that:

In the group of control rats: Liver CPK activity ranged from 175± 12.0 to 172 ± 15.4 U/g fresh tissue.

In the group of WGO-treated rats: There was no significant change recorded throughout the experiment time, compared to the corresponding values of control groups**.**

In the group of irradiated rats: Liver CPK activity showed significant decreases of -43, -28 and -43% 7, 14 and 21 days post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: Liver CPK activity showed significant decreases of -26, -21 and -23%, 7, 14 and 21 days post-irradiation, respectively, compared to the control values.

The results obtained in the present study showed that wheat germ oil treatment significantly increased the radiation-induced decrease in liver CPK activity.

Table 11: Liver creatine phosphokinase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	175± 12.0 [a]	174± 11.9 [a]	172± 15.4 [a]
WGO-treated % of change from control	177± 12.0 [a] +1	178± 11.0 [a] +2	175± 12.0 [a] +2
Irradiated % of change from control	100± 16.7 [b] -43	125± 5.1 [c] -28	98± 18.2 [b] -43
WGO-treated irradiated % of change from control	130± 6.6 [c] -26	138± 8.7 [c] -21	133± 6.6 [c] -23

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

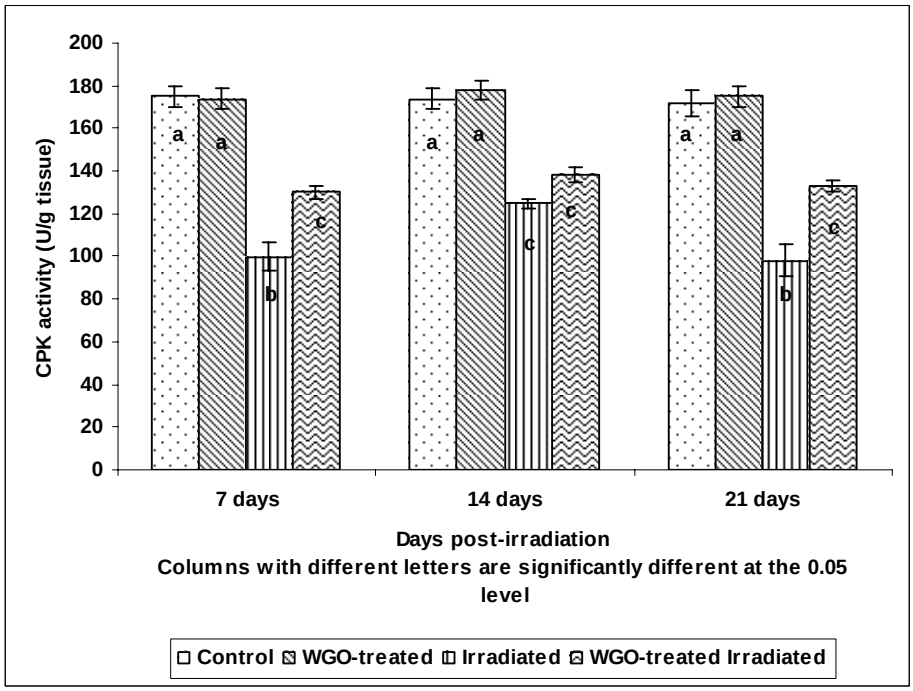

Figure 16: Liver Creatine phosphokinase activity (CPK) (U/g fresh tissue) in different rat groups

4.4. Role of Wheat Germ Oil in Minerals Level of Liver Tissue

4.4.1. Liver total iron level

Role of wheat germ oil on the liver total iron content is presented in table (12) and illustrated by figure (17).

In the group of control rats: Liver total iron level ranged from 124 ± 6.0 to 129 ± 6.5 μg/g fresh tissue.

In the group of WGO-treated rats: No significant changes in total iron level were recorded throughout the experimental times, compared to control values.

In the group of gamma irradiated rats: Liver total iron level showed significant increases ($P<0.05$) of 45, 66 and 43%, on the 7th, 14th and 21st day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Liver total iron level showed significant increases ($P<0.05$) of 27, 42 and 26%, on the 7th, 14th and 21st day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment significantly reduced liver total iron level, compared to the corresponding values in irradiated rats.

Table 12: Liver total iron level (µg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7th days	14th day	21st day
Control	124± 6.0 [a]	126± 6.9 [a]	129± 6.5 [a]
WGO-treated % of change from control	130± 7.3 [a] +5	128± 6.0 [a] +2	127± 7.7 [a] -2
Irradiated % of change from control	180± 5.3 [b] +45	209± 4.7 [c] +66	185± 8.0 [b] +43
WGO-treated irradiated % of change from control	157± 8.7 [d] +27	179± 6.1 [b] +42	163± 4.5 [d] +26

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

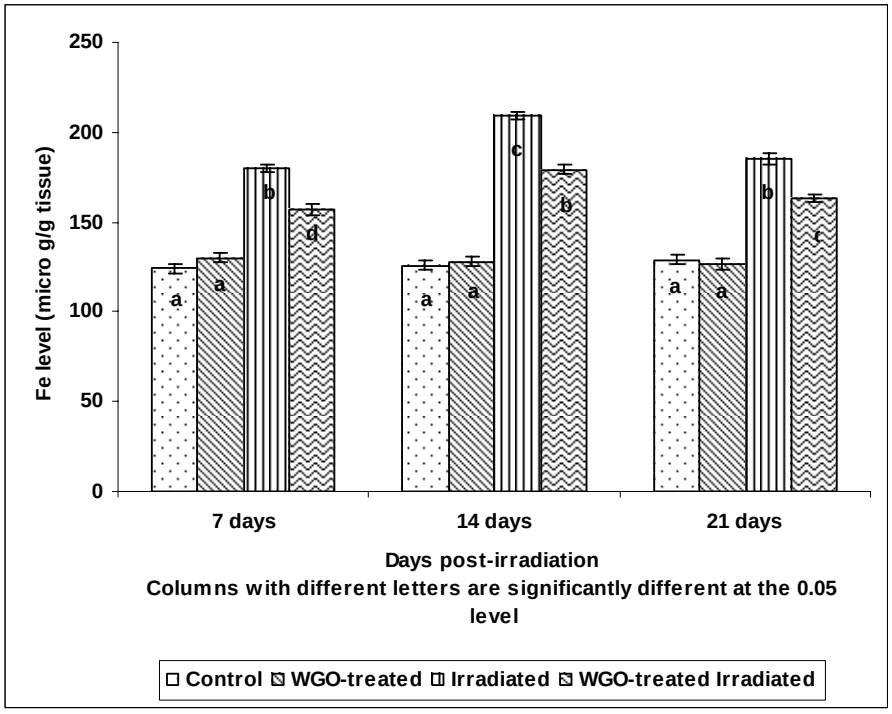

Figure 17: Liver total iron level (Fe) (µg/g fresh tissue) in different rat groups

4.4.2. Liver total copper level

Role of wheat germ oil on the total copper level of liver tissue is presented in table (13) and illustrated by figure (18).

In the group of control rats: Total copper level ranged from 4.11 ± 0.11 to 4.12 ± 0.13 μg/g fresh tissue.

In the group of WGO-treated rats: There was no significant changes in total copper level recorded throughout the experiment time, compared to the corresponding values of control groups**.**

In the group of gamma irradiated rats: Liver total copper level showed significant increases of 71, 63 and 57% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively ,compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Total copper level showed significant increases of 30, 32 and 16% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment induced significant reduction in the level of total liver copper, compared to the corresponding values in irradiated rats.

Table 13: Liver total copper level (µg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	4.11± 0.11 [a]	4.07± 0.09 [a]	4.12± 0.13 [a]
WGO-treated % of change from control	4.14± 0.13 [a] +1	4.16± 0.10 [a] +2	4.20± 0.12 [a] +2
Irradiated % of change from control	7.03± 0.12 [b] +71	6.62± 0.34 [c] +63	6.47± 0.45 [c] +57
WGO-treated irradiated % of change from control	5.34± 0.37 [d] +30	5.36± 0.29 [d] +32	4.78± 0.32 [e] +16

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

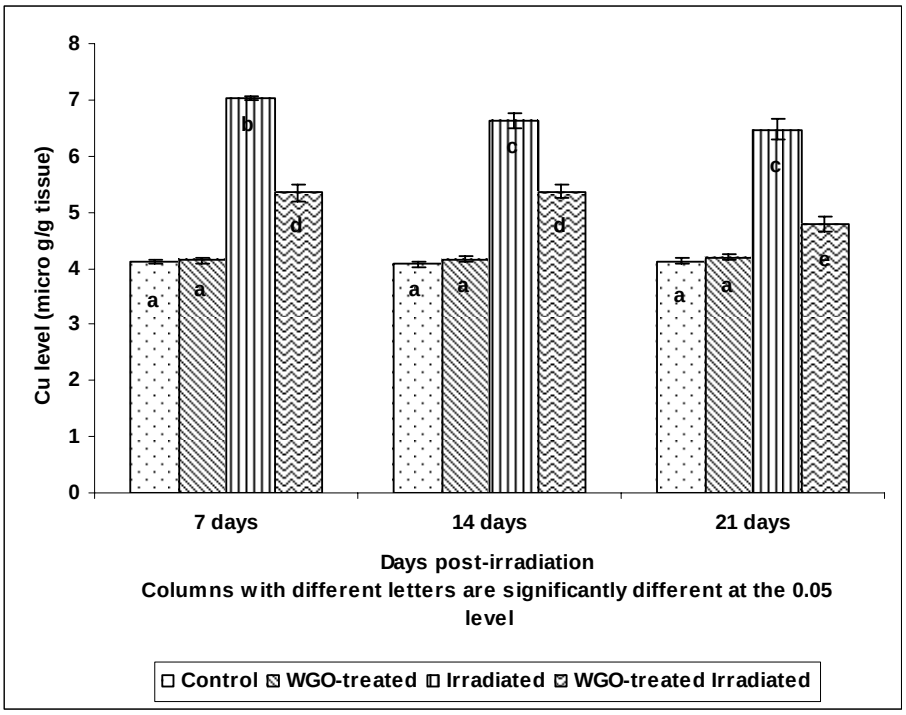

Figure 18: Liver total copper level (Cu) (µg/g fresh tissue) in different rat groups

4.4.3. Liver total calcium level

Effect of wheat germ oil (WGO) on total liver calcium level is presented in table (14) and illustrated by figure (19).

In the group of control rats: Total calcium level ranged from 126 ± 4.6 to 126 ± 4.8 µg/g fresh tissue.

In the group of WGO-treated rats: There was no significant changes in total calcium level recorded throughout the experiment time, compared to control groups**.**

In the group of gamma irradiated rats: Liver total calcium level showed significant increases ($P<0.05$) of 129, 91 and 114%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Total calcium level showed significant increases ($P<0.05$) of 18, 40 and 73% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment induced significant reduction in the level of liver total calcium, compared to the corresponding values in irradiated rats.

Table 14: Liver total calcium level (µg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	126± 4.6 [a]	127± 4.2 [a]	126± 4.8 [a]
WGO-treated % of change from control	129± 3.9 [a] +2	132± 3.7 [a] +4	130± 4.5 [a] +3
Irradiated % of change from control	289± 8.6 [b] +129	243± 5.7 [c] +91	270± 12.2 [d] +114
WGO-treated irradiated % of change from control	149± 10.0 [e] +18	178± 9.4 [f] +40	218± 7.5 [g] +73

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

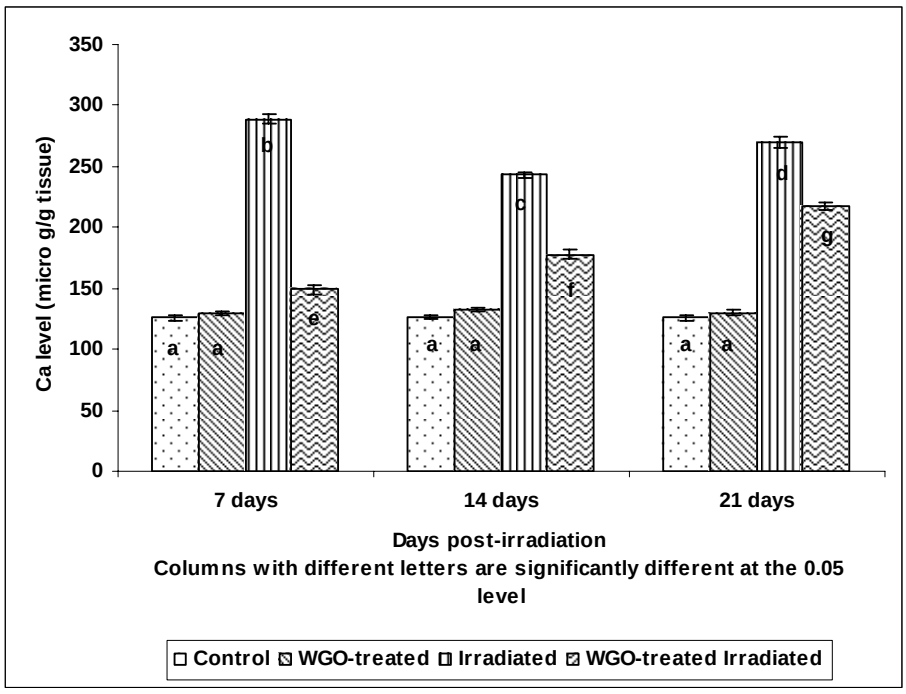

Figure 19: Liver total calcium level (Ca) (µg/g fresh tissue) in different rat groups

4.5. Role of Wheat Germ Oil in the Xanthine Oxidoreductase System of Skeletal Muscle Tissue

4.5.1. Muscle xanthine oxidase

Role of wheat germ oil (WGO) on the xanthine oxidase (XO) activity of muscle tissue is presented in table (15) and illustrated by figure (20). The results obtained indicated the followings:

In the group of control rats: XO activity ranged from 1.26 ± 0.09 to 1.25 ± 0.10 mU/mg protein. There were no significant changes recorded for muscle XO activity throughout the period of the experiment (21 days).

In the group of WGO-treated rats: There were no significant changes in XO activity recorded throughout the experimental period, compared to control values.

In the group of gamma irradiated rats: XO activity was significantly increased by 106, 80 and 111% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: XO activity showed significant increases of 17, 14 and 26% on the7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that wheat germ oil treatment significantly decreased muscle XO activity, compared to their corresponding values in irradiated rats.

Table 15: Muscle xanthine oxidase activity (mU/mg protein) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	1.26± 0.09 [a]	1.27± 0.10 [a]	1.25± 0.10 [a]
WGO-treated % of change from control	1.17± 0.10 [a] -7	1.20± 0.08 [a] -6	1.22± 0.07 [a] -2
Irradiated % of change from control	2.59± 0.09 [b] +106	2.28± 0.08 [c] +80	2.64± 0.13 [b] +111
WGO-treated irradiated % of change from control	1.47± 0.09 [d] +17	1.45± 0.08 [d] +14	1.58± 0.11 [e] +26

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

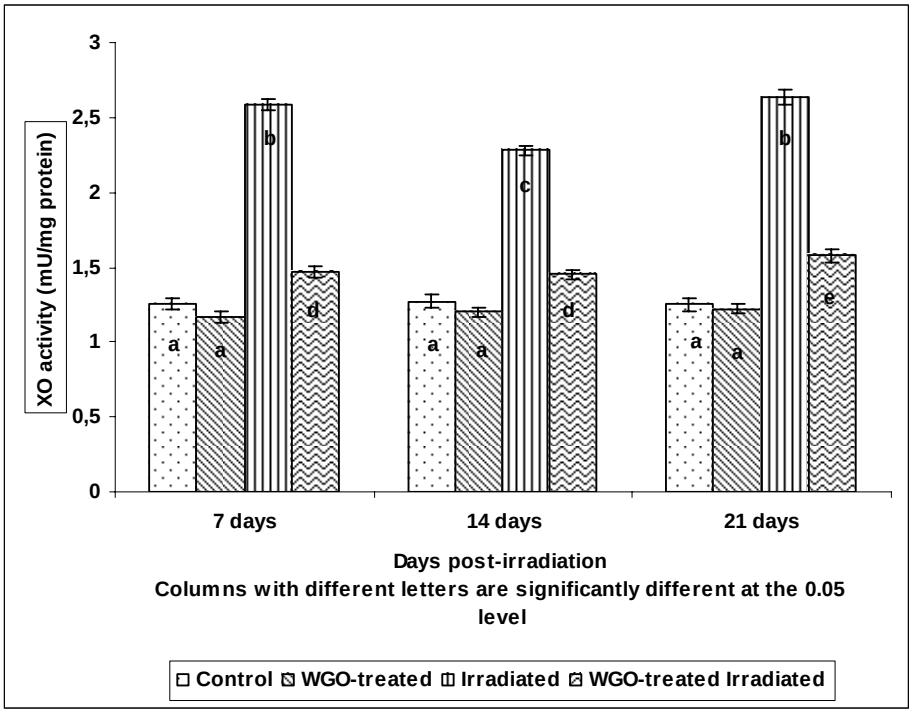

Figure 20: Muscle xanthine oxidase activity (XO) (mU/mg protein) in different rat groups

4.5.2. Muscle xanthine dehydrogenase

Effect of wheat germ oil (WGO) on xanthine dehydrogenase (XDH) activity of muscle tissue is presented in table (16) and illustrated by figure (21). The results obtained indicated the followings:

In the group of control rats: XDH activity ranged from 2.52 ± 0.13 to 2.54 ± 0.15 mU/mg protein. There were no significant changes recorded for muscle XDH activity throughout the period of the experiment (21 days).

In the group of WGO-treated rats: No significant changes were recorded throughout the experimental period, compared to control values.

In the group of gamma irradiated rats: XDH activity was significantly decreased by -46, -38 and -51%, on the7th, 14th and 21st day post-irradiation respectively, compared to control.

In the group of WGO-treated irradiated rats: XDH activity showed significant decreases of -25, -17 and -27%, on the 7th, 14th and 21st day post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that wheat germ oil treatment has significantly increased muscle XDH activity, compared to their corresponding values in irradiated rats.

Table 16: Muscle xanthine dehydrogenase activity (mU/mg protein) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	2.52± 0.13 [a]	2.50± 0.12 [a]	2.54± 0.15 [a]
WGO-treated % of change from control	2.57± 0.17 [a] +2	2.55± 0.16 [a] +2	2.56± 0.16 [a] +1
Irradiated % of change from control	1.36± 0.09 [b] -46	1.56± 0.12 [c] -38	1.24± 0.05 [b] -51
WGO-treated irradiated % of change from control	1.90± 0.09 [d] -25	2.08± 0.10 [e] -17	1.86± 0.11 [d] -27

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

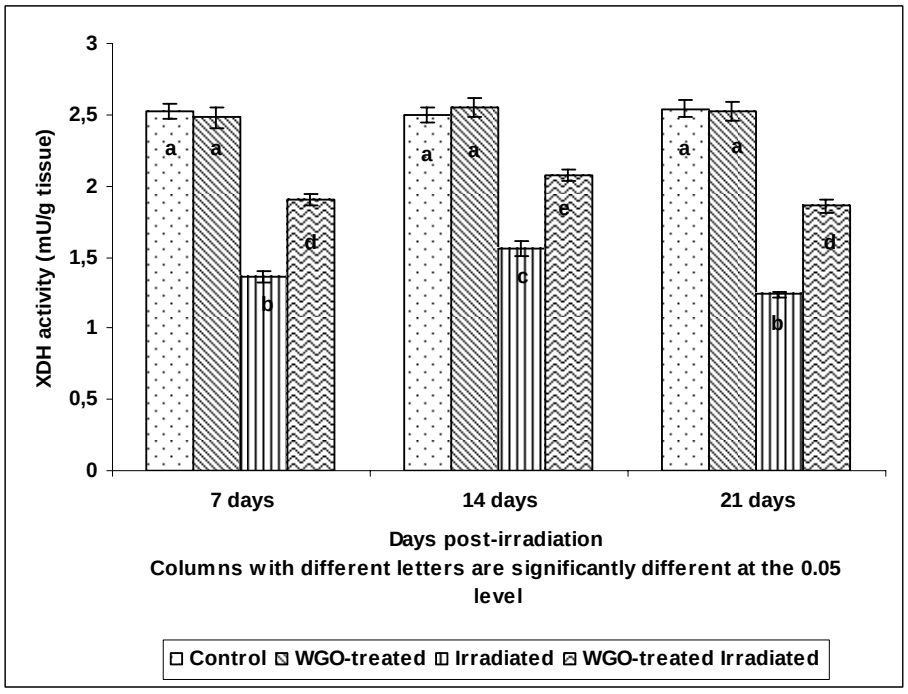

Figure 21: Muscle xanthine dehydrogenase activity (XDH) (mU/mg protein) in different rat groups

4.6. Role of Wheat Germ Oil in the Antioxidant Status of Skeletal Muscle Tissue

4.6.1. Muscle superoxide dismutase

Effect of wheat germ oil (WGO) on superoxide dismutase (SOD) activity of muscle tissue is presented in table (17) and illustrated by figure (22). The results obtained indicated the followings:

In the group of control rats: SOD activity ranged from 154 ± 9.4 to 153 ± 9.4 U/g fresh tissue, with no significant changes recorded throughout the period of investigation.

In the group of WGO-treated rats: No significant changes in muscle SOD activity were recorded at all the experimental times, compared to control groups.

In the group of gamma irradiated rats: Muscle SOD activity was significantly decreased by -44, -41 and -56% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Muscle SOD activity showed significant decreases ($P<0.05$) of -25, -13 and -26% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that wheat germ oil treatment significantly ameliorated the radiation-induced alterations in muscle SOD.

Table 17: Muscle superoxide dismutase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	154± 9.4 [a]	148 ± 11.4 [a]	153 ± 9.4 [a]
WGO-treated % of change from control	158± 6.9 [a] +3	154± 8.3 [a] +4	156± 10.6 [a] +2
Irradiated % of change from control	87 ± 6.4 [b] -44	88 ± 7.0 [b] -41	68 ± 7.0 [c] -56
WGO-treated irradiated % of change from control	116± 8.9 [d] -25	129 ± 12.9 [e] -13	113 ± 13.0 [d] -26

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

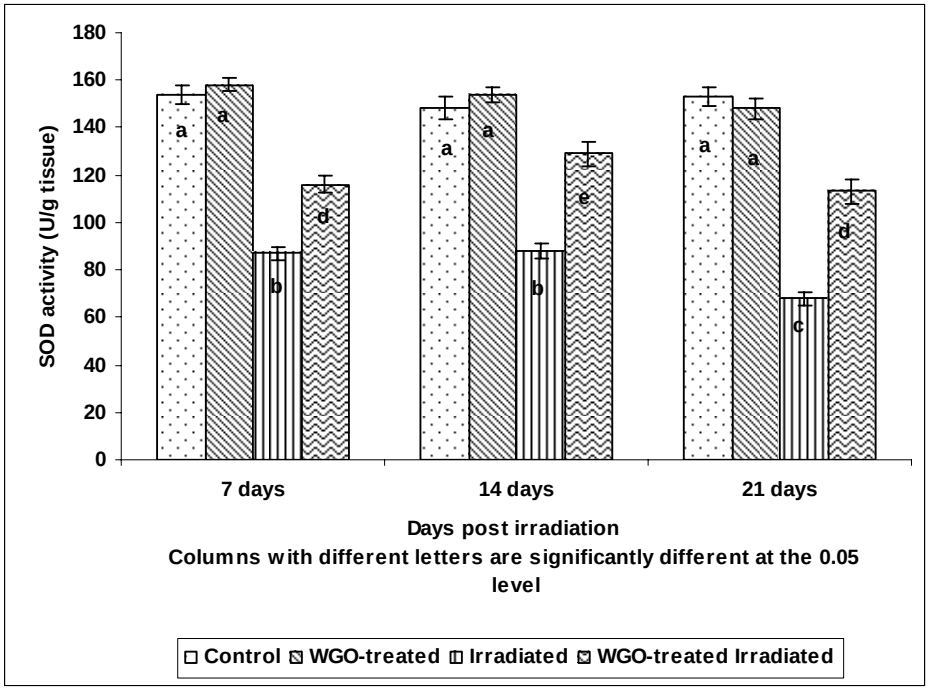

Figure 22: Muscle superoxide dismutase activity (SOD) (U/g fresh tissue) in different rat groups

4.6.2. Muscle catalase

Effect of wheat germ oil (WGO) on muscle catalase activity (CAT) is presented in table (18) and illustrated in figure (23).

In the group of control rats: Muscle CAT activity ranged from 29.8 ± 1.7 to 30 ± 1.4 U/g fresh tissue, with no significant changes recorded throughout the period of investigation.

In the group of WGO-treated rats: There was no significant change in CAT activity recorded throughout the experiment time, compared to control groups.

In the group of gamma irradiated rats: CAT activity showed significant decreases of -56, -50 and -67% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: CAT activity showed significant decreases of -39, -30 and -40% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation respectively, compared to the corresponding control values.

Wheat germ oil administration significantly ameliorated the radiation induced changes in muscle CAT activity, compared to their corresponding values in irradiated rats.

Table 18: Muscle catalase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	29.8 ± 1.7 **a**	29.8 ± 1.5 **a**	30.0 ± 1.4 **a**
WGO-treated % of change from control	30.9 ± 1.0 **a** +4	30.1 ± 1.0 **a** +1	30.6± 1.2 **a** +2
Irradiated % of change from control	13.0 ± 2.4 **b** -56	15.0± 1.4 **c** -50	9.8 ± 0.9 **d** -67
WGO-treated irradiated % of change from control	18.0± 0.9 **e** -39	21.0 ± 1.9 **f** -30	18.0 ± 1.4 **e** -40

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

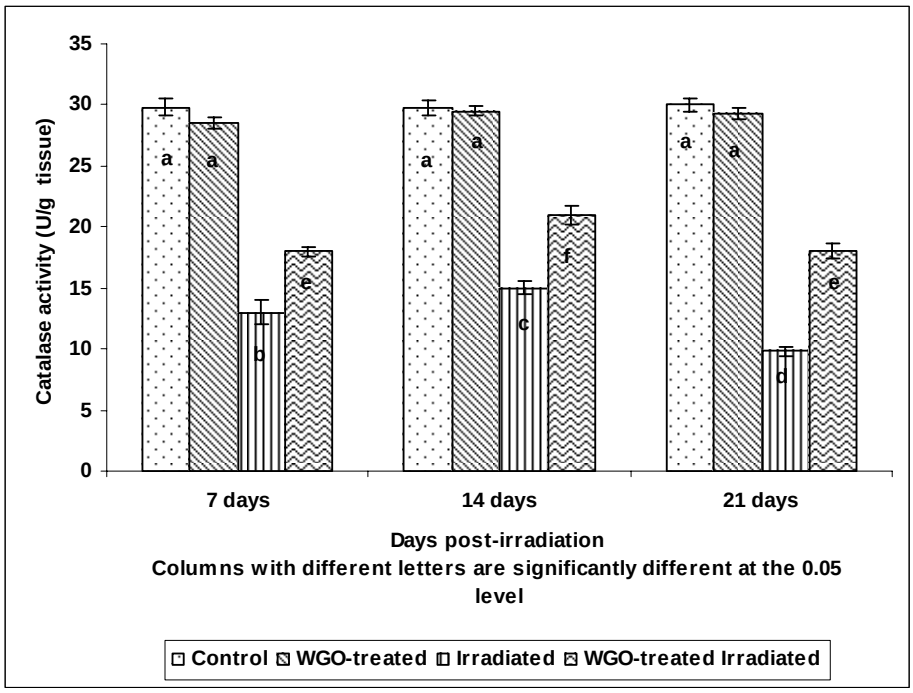

Figure 23: Muscle catalase activity (U/g fresh tissue) in different rat groups

4.6.3. Muscle glutathione peroxidase

Role of wheat germ oil (WGO) on muscle glutathione peroxidase activity (GSH-Px) is presented in table (19) and illustrated in figure (24).

In the group of control rats: Muscle GSH-Px activity ranged from 5.2 ± 0.63 to 5.4 ± 0.78 mg consumed glutathione/min/g fresh tissue.

In the group of WGO-treated rats: There was no significant change in muscle GSH-Px activity recorded at all the experimental times, compared to the control groups.

In the group of gamma irradiated rats: Muscle GSH-Px activity showed significant decreases ($P<0.05$) of - 36, -37 and -43%, on 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Muscle GSH-Px activity showed significant decreases ($P<0.05$) of -19, -15 and -17 %, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment significantly diminished the decrease in muscle GSH-Px activity, compared to their corresponding values in irradiated rats.

Table 19: Muscle glutathione peroxidase activity (mg consumed glutathione/min/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	5.2± 0.63 [a]	5.2 ± 0.68 [a]	5.4 ± 0.78 [a]
WGO-treated % of change from control	5.5± 0.29 [a] +6	5.4± 0.35 [a] +4	5.5± 0.58 [a] +2
Irradiated % of change from control	3.3 ± 0.64 [b] -36	3.3 ± 0.76 [b] -37	3.1 ± 0.39 [b] -43
WGO-treated irradiated % of change from control	4.2± 0.69 [c] -19	4.4 ± 0.37 [c] -15	4.5 ± 0.43 [c] -17

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

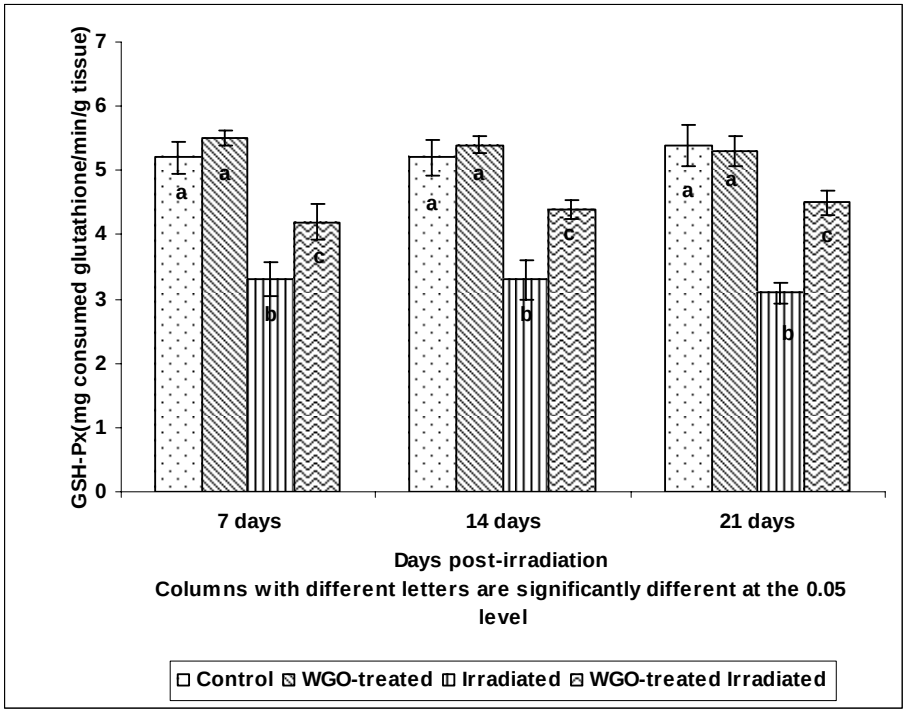

Figure 24: Muscle glutathione peroxidase activity (GSH-Px) (mg consumed glutathione/min/g fresh tissue) in different rat groups

4.6.3. Muscle glutathione

Effect of wheat germ oil (WGO) on glutathione content (GSH) in muscle tissue is presented in table (20) and illustrated in figure (25), respectively. The results indicate that:

In the group of control rats: GSH content ranged from 26.5 ± 0.9 to 26.0 ± 1.2 mg/g fresh tissue, with no significant changes recorded throughout the period of the investigation.

In the group of WGO-treated rats: There were no significant changes recorded for GSH content at all the experimental times, compared to control values.

In the group of gamma irradiated rats: GSH content showed significant decreases ($P<0.05$) of -51, -40 and -52% 7, 14 and 21 days post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated: GSH content showed significant decreases ($P<0.05$) of -27, -16 and -23 %, 7, 14 and 21 days post-irradiation respectively, compared to the corresponding control values.

The results demonstrated that wheat germ oil treatment significantly increased in GSH content, compared to their corresponding values in irradiated rats.

Table 20: Muscle glutathione content (mg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	26.5± 0.9 [a]	26.3 ± 1.1 [a]	26.0 ± 1.2 [a]
WGO-treated % of change from control	28.3 ± 2.0 [a] +7	27.6± 2.4 [a] +5	27.1± 1.1 [a] +4
Irradiated % of change from control	13.1 ± 1.7 [b] -51	15.7 ± 0.8 [c] -40	12.4 ± 2.7 [b] -52
WGO-treated irradiated % of change from control	19.3± 1.2 [d] -27	22.1 ± 0.4 [e] -16	20.0 ± 2.4 [d] -23

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

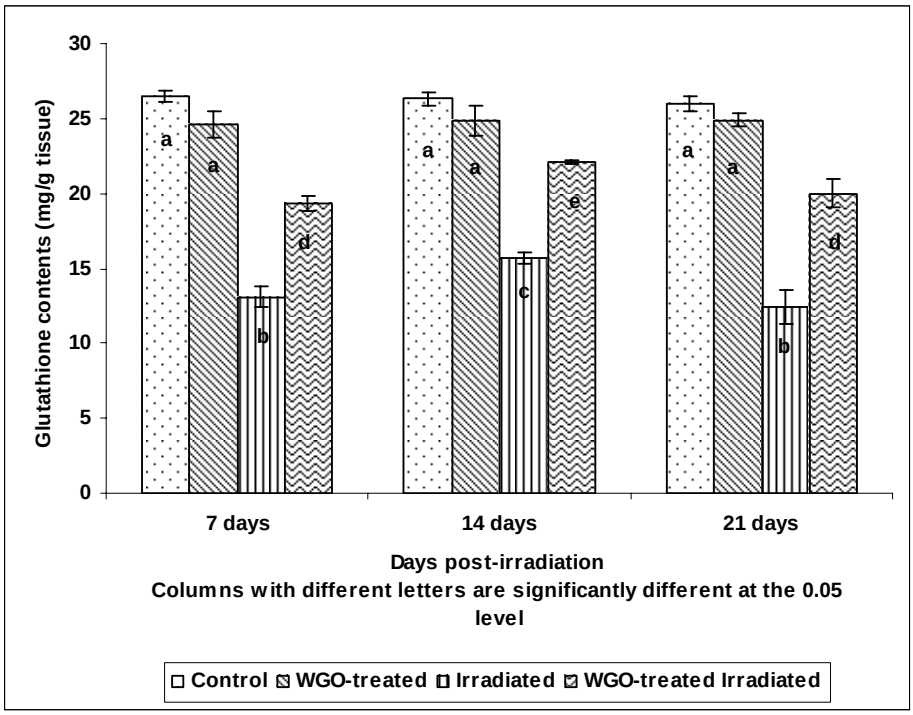

Figure 25: Muscle glutathione content (mg/g fresh tissue) in different rat groups

4.6.4. Muscle thiobarbituric acid reactive substances

Role of wheat germ oil (WGO) on the levels of muscle thiobarbituric acid reactive substances (TBARS) is presented in table (21) and illustrated by figure (26).

In the group of control rats: Muscle TBARS levels ranged from 161 ± 6.9 to 165 ± 11.2 nmole/g fresh tissue.

In the group of WGO-treated rats: There was no significant change recorded throughout the experimental times, compared to the corresponding control values.

In the group of gamma irradiated rats: Muscle TBARS levels showed significant increases ($P<0.05$) of 86, 43 and 90%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Muscle TBARS levels showed significant increases ($P<0.05$) of 25, 18 and 33% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that WGO treatment significantly reduced muscle TBARS levels, compared to the corresponding values in irradiated rats.

Table 21: Muscle thiobarbituric acid reactive substances levels (nm/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	161± 6.9 [a]	162 ± 8.6 [a]	165 ± 11.2 [a]
WGO-treated % of change from control	154 ± 12.1 [a] -4	153± 8.1 [a] -6	157± 9.6 [a] -5
Irradiated % of change from control	300 ± 11.2 [b] +86	232 ± 8.1 [c] +43	314 ± 9.8 [b] +90
WGO-treated irradiated % of change from control	201± 21.1 [d] +25	191 ± 22.9 [d] +18	220 ± 13.5 [c] +33

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

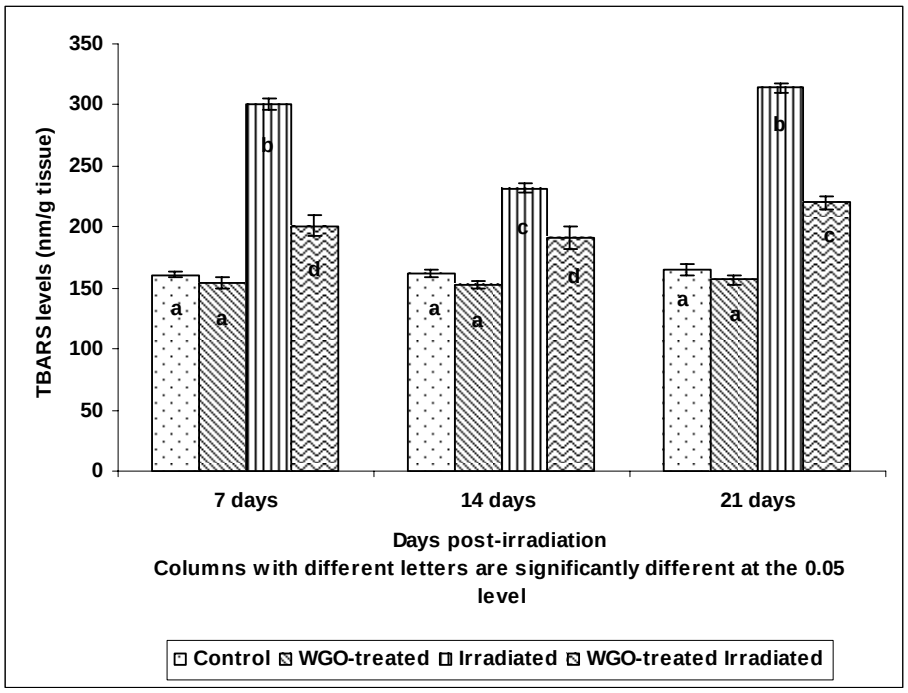

Figure 26: Muscle thiobarbituric acid reactive substances levels (TBARS) (nmol/g fresh tissue) in different rat groups

4.7. Role of Wheat Germ Oil in The Metabolic Enzymes of Skeletal Muscle Tissue

4.7.1. Muscle glucose-6-phosphate dehydrogenase

Role of wheat germ oil on glucose-6-phosphate dehydrogenase (G-6-PDH) activity of muscle tissue is presented in table (22) and illustrated by figure (27). The results obtained indicated the followings:

In the group of control rats: G-6-PDH activity ranged from 29.5 ± 4.8 to 30.7 ± 4.4 U/g fresh tissue. There were no significant changes recorded for muscle G-6-PDH activity throughout the period of the experiment (21 days).

In the group of WGO-treated rats: No significant changes in G-6-PDH activity were recorded throughout the experimental period, compared to control values.

In the group of gamma irradiated rats: G-6-PDH activity was significantly decreased by -48, -41 and -49% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: G-6-PDH activity showed significant decreases ($P<0.05$) of -22, -23 and -28%, 7, 14 and 21 days post-irradiation, respectively, compared to the corresponding control values.

The results obtained in the present study showed that wheat germ oil treatment significantly increased muscle G-6-PDH activity, compared to their corresponding values in irradiated rats.

Table 22: Muscle glucose-6-phosphate dehydrogenase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	29.5± 4.8 [a]	28.5 ± 5.9 [a]	30.7 ± 4.4 [a]
WGO-treated % of change from control	29.7± 3.7 [a] +1	29.6± 3.3 [a] +4	32.8± 2.5 [a] +7
Irradiated % of change from control	15.3 ± 1.9 [b] -48	16.7 ± 2.2 [b] -41	15.6 ± 2.2 [b] -49
WGO-treated irradiated % of change from control	22.9± 1.6 [c] -22	21.9 ± 2.9 [c] -23	22.1 ± 2.7 [c] -28

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

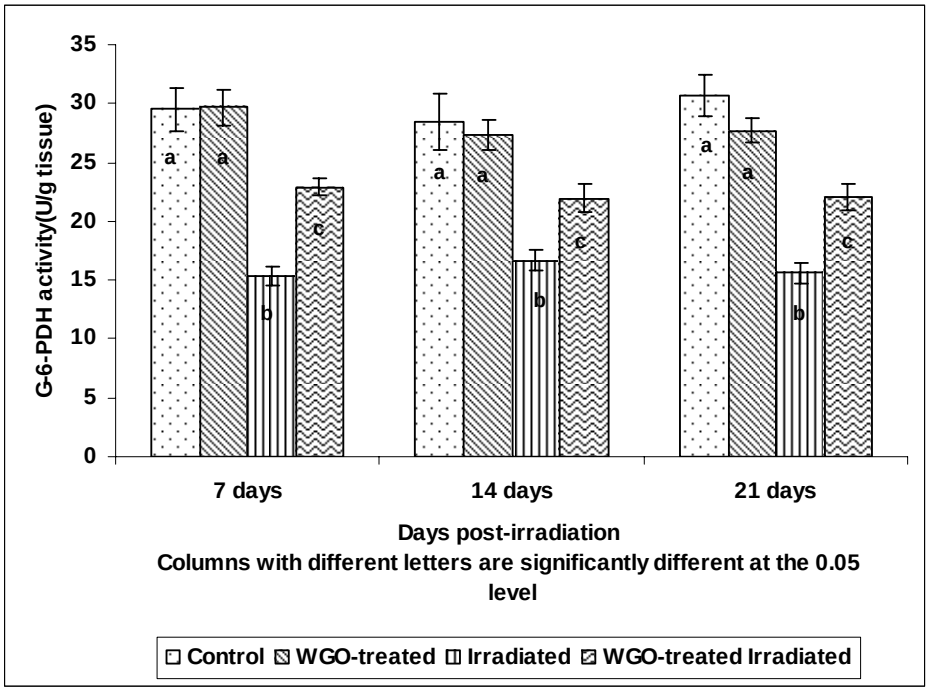

Figure 27: Muscle glucose-6-phosphate dehydrogenase activity (G-6-PDH) (U/g fresh tissue) in different rat groups

4.7.2. Muscle glutamate dehydrogenase

Muscle glutamate dehydrogenase activity (GDH) for the four animal groups are presented in table (23) and illustrated in figure (28).

In the group of control rats: Muscle GDH activity ranged from 8.7 ± 0.41 to 8.6 ± 0.29 U/g fresh tissue, with no significant changes recorded throughout the period of the investigation.

In the group of WGO-treated rats: There was no significant change recorded for muscle GDH activity throughout the period of experiment, compared to control values.

In the group of gamma irradiated rats: GDH activity showed significant decreases ($P<0.05$) of -72, -77 and -79 %, 7, 14 and 21 days post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: GDH activity showed significant decreases ($P<0.05$) of -51, -48 and -35%, 7, 14 and 21 days post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that WGO administration significantly increased muscle GDH activity, compared to their corresponding values in irradiated rats.

Table 23: Muscle glutamate dehydrogenase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	8.7± 0.41 [a]	8.4 ± 0.26 [a]	8.6 ± 0.29 [a]
WGO-treated % of change from control	9.1± 0.58 [a] +5	8.6± 0.49 [a] +2	8.7± 0.38 [a] +1
Irradiated % of change from control	2.4 ± 0.89 [b] -72	1.9 ± 0.39 [b] -77	1.8 ± 0.55 [b] -79
WGO-treated irradiated % of change from control	4.3± 0.27 [c] -51	4.4 ± 0.69 [c] -48	5.6 ± 0.66 [d] -35

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

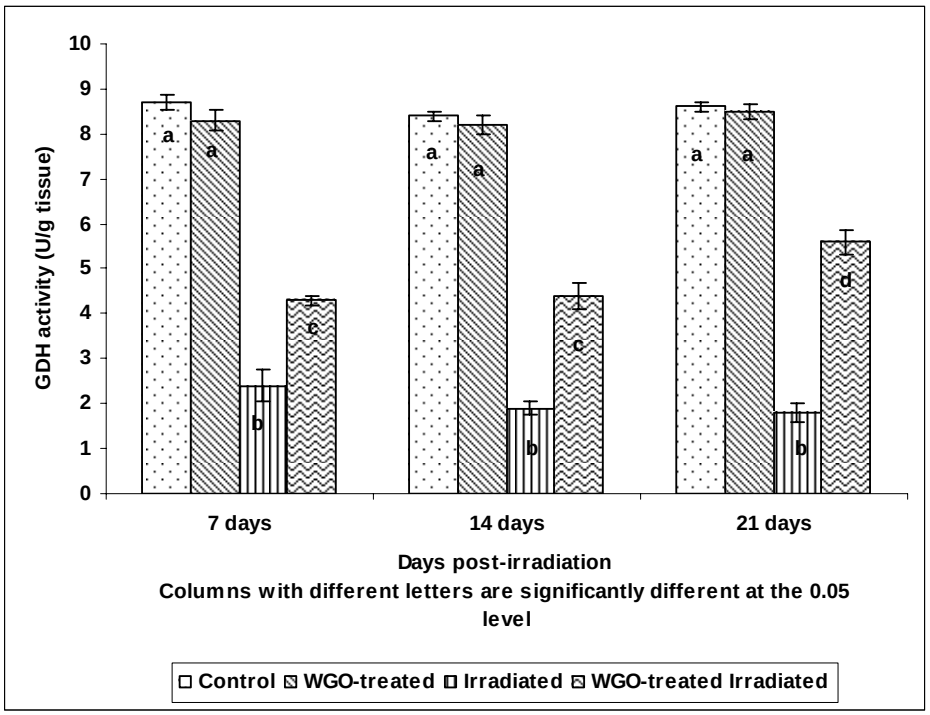

Figure 28: Muscle glutamate dehydrogenase activity (GDH) (U/g fresh tissue) in different rat groups

4.7.3. Muscle lactate dehydrogenase

Role of wheat germ oil (WGO) on muscle lactate dehydrogenase activity (LDH) is presented in table (24) and illustrated by figure (29).

In the group of control rats: Muscle LDH activity ranged from 335 ± 10.8 to 334 ± 10.7 U/g fresh tissue.

In the group of WGO-treated rats: There was no significant change in muscle LDH activity recorded throughout the experimental period, compared to control values.

In the group of irradiated rats: Muscle LDH activity showed significant increases ($P<0.05$) of 27, 31 and 39%, 7, 14 and 21 days post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: Muscle LDH activity showed significant increases ($P<0.05$) of 12, 16 and 19%, 7, 14 and 21 days post-irradiation, respectively, compared to control values.

The results obtained showed that wheat germ oil treatment has significantly decreased muscle LDH activity, compared to their corresponding values in irradiated rats.

Table 24: Muscle lactate dehydrogenase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	335± 10.8 [a]	334± 10.0 [a]	334± 10.7 [a]
WGO-treated % of change from control	331± 12.1 [a] -1	337± 11.2 [a] +1	332± 11.9 [a] -1
Irradiated % of change from control	427± 8.1 [b] +27	437± 10.5 [c] +31	464± 17.7 [d] +39
WGO-treated irradiated % of change from control	374± 9.5 [c] +12	387± 6.5 [e] +16	397± 15.1 [f] +19

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

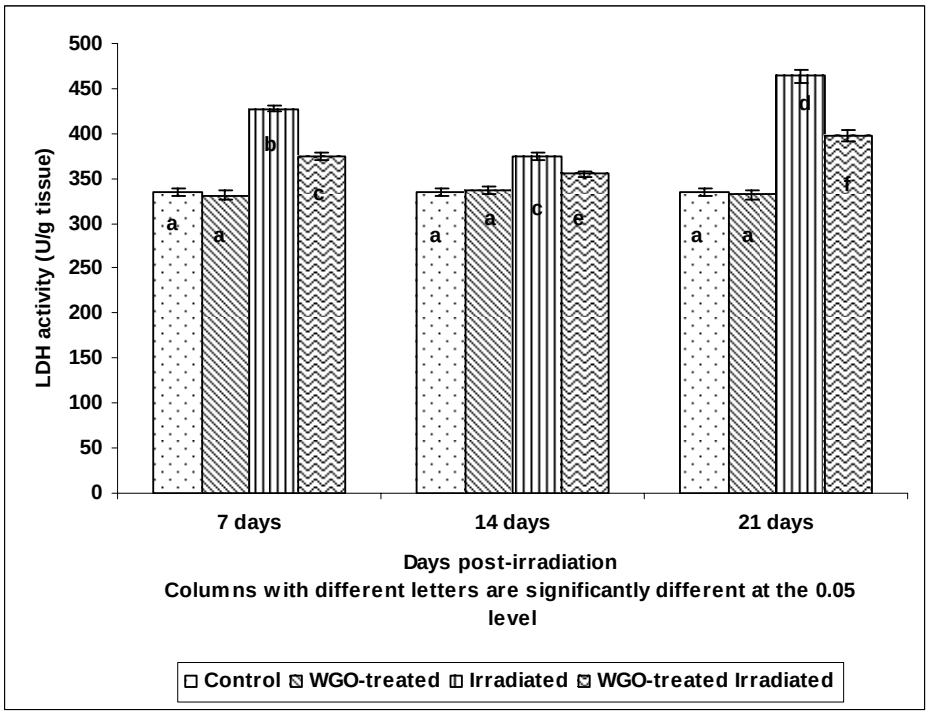

Figure 29: Muscle lactate dehydrogenase activity (LDH) (U/g fresh tissue) in different rat groups

4.7.4. Muscle creatine phosphokinase

Effect of wheat germ oil (WGO) on muscle creatine phosphokinase activity (CPK), is presented in table (25) and illustrated by figure (30). The results obtained indicate that:

In the group of control rats: Muscle CPK activity ranged from 199± 11.0 to 200 ± 12.0 U/g fresh tissue.

In the group of WGO-treated rats: No significant changes in CPK activity were recorded throughout the experimental period, compared to control values.

In the group of irradiated rats: Muscle CPK activity showed significant decreases ($P<0.05$) of -43, -49 and -61%, 7, 14 and 21 days post-irradiation, respectively, compared to control values.

In the group of WGO-treated irradiated rats: Muscle CPK activity showed significant decreases ($P<0.05$) of -26, -33 and -36 %, 7, 14 and 21 days post-irradiation, respectively, compared to the control values.

The results obtained showed that wheat germ oil administration significantly improved the radiation induced decrease of muscle CPK activity.

Table 25: Muscle creatine phosphokinase activity (U/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	199± 11.0 [a]	197± 17.4 [a]	200± 12.0 [a]
WGO-treated % of change from control	202± 16.7 [a] +2	208± 17.3 [a] +6	204± 15.6 [a] +2
Irradiated % of change from control	113± 13.9 [b] -43	100± 9.4 [b] -49	78± 10.7 [c] -61
WGO-treated irradiated % of change from control	147± 10.1 [d] -26	132± 5.2 [d] -33	127± 5.9 [d] -37

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

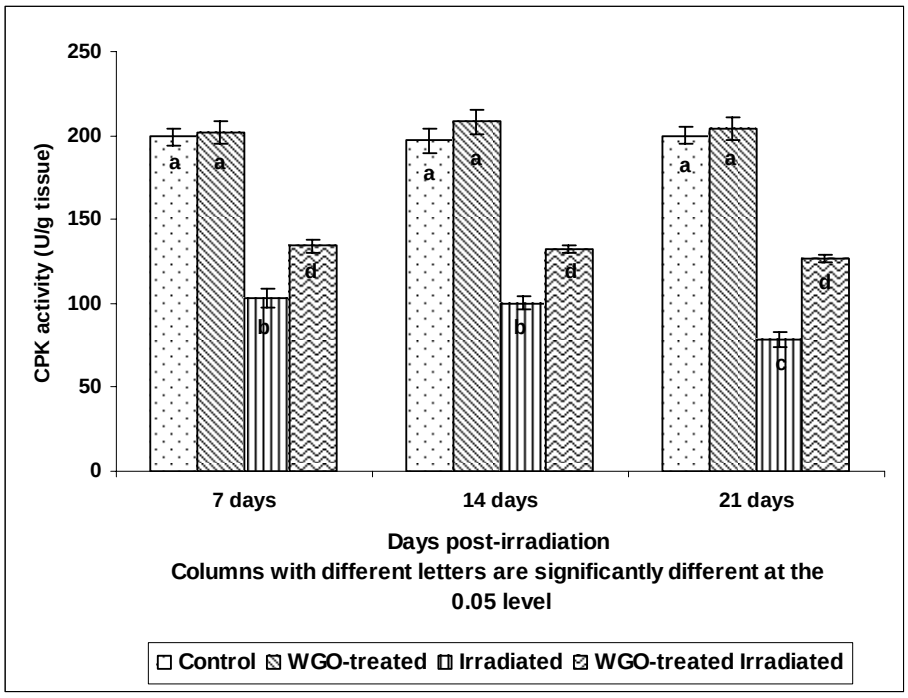

Figure 30: Muscle Creatine phosphokinase activity (CPK) (U/g fresh tissue) in different rat groups

4.8. Role of Wheat Germ Oil in Minerals Level of Skeletal Muscle Tissue

4.8.1. Muscle total iron level

Effect of wheat germ oil (WGO) on muscle total iron level is presented in table (26) and illustrated by figure (31).

In the group of control rats: Muscle total iron level ranged from 37 ± 5.5 to 37 ± 6.3 µg/g fresh tissue.

In the group of WGO-treated rats: There was no significant change in total iron level recorded throughout the experimental times, compared to control values.

In the group of gamma irradiated rats: Muscle total iron level showed significant increases ($P<0.05$) of 57, 46 and 84%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Muscle total iron level showed significant increases ($P<0.05$) of 31, 24 and 27% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment significantly reduced muscle total iron level, compared to the corresponding values in irradiated rats.

Table 26: Muscle total iron level (µg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	37± 5.5 [a]	36± 5.4 [a]	37± 6.3 [a]
WGO-treated % of change from control	39± 3.7 [a] +5	38± 4.3 [a] +6	39± 4.2 [a] +5
Irradiated % of change from control	58± 4.2 [b] +57	53± 7.2 [c] +46	68± 6.0 [d] +84
WGO-treated irradiated % of change from control	48± 4.6 [c] +31	45± 5.6 [c] +24	47± 8.4 [c] +27

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

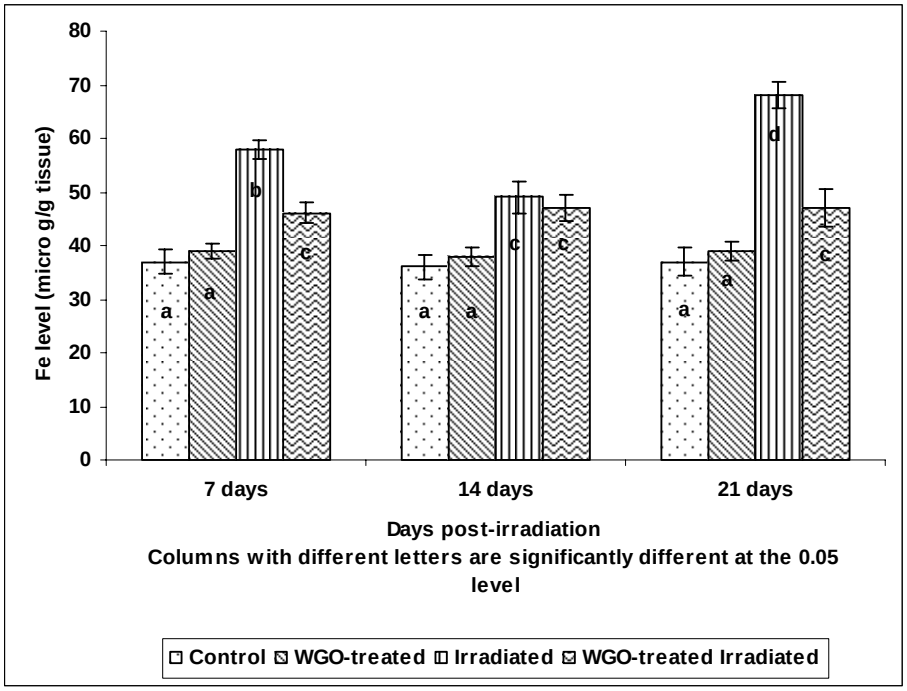

Figure 31: Muscle total iron level (Fe) (µg/g fresh tissue) in different rat groups

4.8.2. Muscle total copper level

Role of wheat germ oil (WGO) on total copper level of skeletal muscle tissue is presented in table (27) and illustrated by figures (32).

In the group of control rats: Total copper level ranged from 1.66 ± 0.09 to 1.69 ± 0.07 µg/g fresh tissue.

In the group of WGO-treated rats: There was no significant change in total copper level recorded throughout the experiment time, compared to the corresponding values of control groups**.**

In the group of gamma irradiated rats: Muscle total copper level showed significant increases ($P<0.05$) of 64, 42 and 51%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Total copper level showed significant increases ($P<0.05$) of 31, 17 and 17%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment induced significant reduction in the level of total copper, compared to the corresponding values in irradiated rats.

Table 27: Muscle total copper level (µg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	1.66± 0.09 [a]	1.71± 0.09 [a]	1.69± 0.07 [a]
WGO-treated % of change from control	1.71± 0.10 [a] +3	1.77± 0.08 [a] +4	1.75± 0.08 [a] +4
Irradiated % of change from control	2.73± 0.14 [b] +64	2.40± 0.24 [c] +40	2.56± 0.30 [c] +51
WGO-treated irradiated % of change from control	2.17± 0.09 [d] +31	1.97± 0.15 [e] +15	1.97± 0.09 [e] +17

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

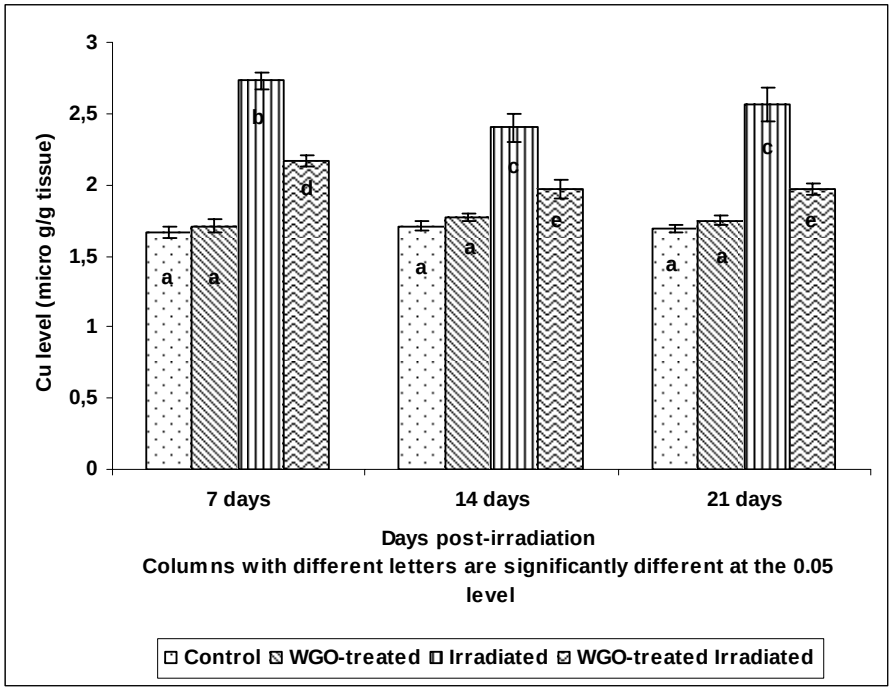

Figure 32: Muscle total copper level (Cu) (µg/g fresh tissue) in different rat groups

4.8.3. Muscle total calcium level

Effect of wheat germ oil (WGO) on total calcium level of skeletal muscle tissue is presented in table (28) and illustrated by figure (33).

In the group of control rats: Total calcium level ranged from 244 ± 7.1 to 236 ± 8.6 µg/g fresh tissue.

In the group of WGO-treated rats: There was no significant change in total calcium level recorded throughout the experiment time, compared to values of control groups.

In the group of gamma irradiated rats: Muscle total calcium level showed significant increases ($P<0.05$) of 92, 54 and 104%, on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

In the group of WGO-treated irradiated rats: Total calcium level showed significant increases ($P<0.05$) of 42, 22 and 28% on the 7^{th}, 14^{th} and 21^{st} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that wheat germ oil treatment induced significant reduction in the level of total calcium, compared to the corresponding values in irradiated rats.

Table 28: Muscle total calcium level (µg/g fresh tissue) in different rat groups

Rat groups	Days post-irradiation		
	7^{th} days	14^{th} day	21^{st} day
Control	244± 7.1 [a]	245± 8.9 [a]	236± 8.6 [a]
WGO-treated % of change from control	242± 4.3 [a] -1	241± 5.1 [a] -2	246± 9.2 [a] +4
Irradiated % of change from control	469± 8.9 [b] +92	378± 8.4 [c] +54	481± 12.4 [d] +104
WGO-treated irradiated % of change from control	347± 6.7 [e] +42	299± 6.9 [f] +22	303± 17.8 [g] +28

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

Means with different superscripts are significantly different at 0.05 level.

WGO: Wheat germ oil.

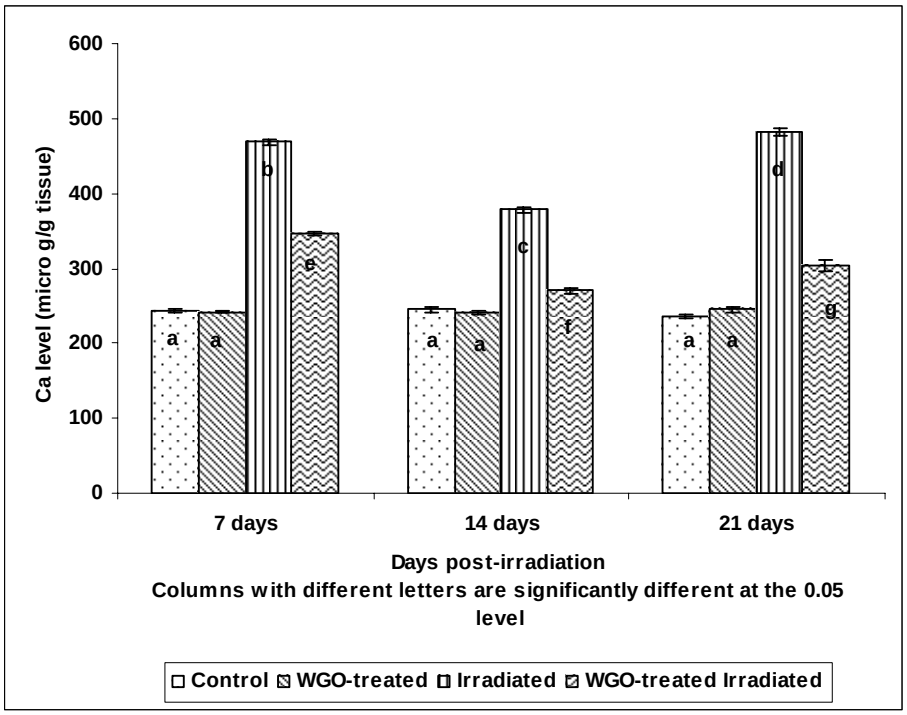

Figure 33: Muscle total calcium level (Ca) (µg/g fresh tissue) in different rat groups

5- DISCUSSION

Ionizing radiation is a physical agent that induces oxidative stress by excess generation of reactive oxygen species (ROS). The fate of these species is governed by a number of factors that vary from tissue to tissue in mammals and may be involved in the pathogenesis of disease. ROS are also invoked as agents that are important in the processes which become active in cells undergoing apoptosis **(Sandeep and Brian, 2001).**

Most of the damage induced in biological systems is mediated by ROS generated by the radiolytically products of water. These reactive species are known to cause degradation of important macromolecules including DNA and membranes (**Green et al., 1992)**. Among the sub-cellular organelles, mitochondria form some of the key components of cell killing induced by oxidative stress or radiation **(Slyshenkov et al., 1996)**.

Oxidative stress is well recognized to be a key step in the pathogenesis of radiation-associated liver and muscle injury. Mitochondria are vulnerable to oxidants because they are the major source of free radicals in the cells and are limited to their ability to cope with oxidative stress (**De Grey, 2000**). Exposure to gamma radiation alters the structure and function of mitochondria via oxidative modification of DNA, lipids and proteins. Though there are several antioxidant defenses in the form of chemicals or enzymes, including those present in the mitochondria they are overwhelmed by high levels of pro-oxidants or radiation.

5.1 Radiation Effects on Pro-Oxidants in Liver and Skeletal Muscle Tissues

Pro-oxidants are chemicals that induce oxidative stress, either through creating ROS or inhibiting antioxidant systems **(Puglia and Powell, 1984)**. The oxidative stress produced by these chemicals can damage cells and tissues through its production of ROS.

Lipid peroxidation, xanthine oxidoreductase system especially xanthine oxidase (XO), iron, copper and calcium overloads are most pro-oxidants in the liver and skeletal muscle **(Bhosle et al., 2005)**.

In the present study, whole body gamma irradiation of rats with 5 Gy (one shot dose) provokes oxidative stress in the liver and skeletal muscle tissues, manifested by imbalance between oxidants and antioxidants. Radiation-induce significant increase the levels of lipid peroxides as determined by thiobarbituric acid reactive substances (TBARS) which was concomitant with significant increases in the levels of iron, copper and calcium as well as significant increases in the activity of xanthine oxidase 7, 14 and 21 days post-irradiation.

Lipid peroxidation (LP) is one of the measures to determine the cellular toxicity. The concentration of lipid peroxidation products may reflect the degree of oxidative stress**.** Lipid peroxidation mediated by free radicals is considered to be a primary mechanism of cell membrane destruction and cell damage **(Samuni et al., 1997)**.

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. Polyunsaturated fatty acids (PUFA), containing two or more double bonds are particularly

susceptible to peroxidation. Free radical induced lipid peroxidation causes a loss of cell homeostasis by modifying the structure and functions of cell membrane (**Kolanjiappan et al., 2002**).

In the present study, the significant increase of TBARS might result from the interaction of ROS radicals generated after exposure to radiation with the PUFA in the phospholipids portion of cell membranes **(Frank et al., 1998)**. It is now generally accepted that LP and its products play an important role in liver and skeletal muscle toxicity **(Lakshmi et al., 2005)**.

Bendi et al., (1998) and coworkers have suggested that ROS induced by radiation may reduce mitochondrial ATP synthesis through effects on the mitochondrial membrane. Ionizing radiation elevates mitochondrial **calcium** (Ca^{2+}) levels which can enhance mitochondrial reactive oxygen or nitrogen (ROS/RNS) generation (**Kevin et al., 2001)**. The excess of ROS may block the inner mitochondrial membrane transport pore which controls calcium passage across the inner mitochondrial membrane, which leads to a decrease in skeletal muscle mitochondrial ATP synthesis, causing muscle dysfunction and cell death **(Grace et al., 2006)**.

Low Ca^{2+} levels are necessary for muscle cell function while high intracellular Ca^{2+}, leading to Ca^{2+} accumulation within the mitochondria could be involved in loss of Ca^{2+} homeostasis and may depress mitochondrial function, and therefore decrease ATP production and consequently development of muscle cell damage (**Khuchua et al., 1994)**. In the present study, the increase of calcium level in the liver and skeletal muscles may be attributed to radiation-induced hypoxia (**Dominique and Edward, 1999**). In addition, during oxidative stress,

the inadequate generation of ATP can cause malfunctioning of calcium ATPase pumps and an increase in intracellular calcium (**Heunks et al., 1999**).

Iron is an essential nutrient with limited bioavailability. When present in excess, iron poses a threat to cells and tissues, and therefore iron homeostasis has to be tightly controlled. Iron's toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury (**Papanikolaou and Pantopoulos, 2005**). Iron overload is associated with liver and muscle toxicity. Free iron facilitates the decomposition of lipid hydroperoxides resulting in lipid peroxidation and induces the generation of $^{\bullet}OH$ radicals and also, accelerates the non enzymatic oxidation of glutathione to form $O_2^{\bullet-}$ radicals (**Gavin et al., 2004**). In the present study, the increased iron level may be due to proteolytic modification of ferritin, transferrin, and heme (**Nelson and Cox, 2005**).

Copper, a component of multiple enzymes, is involved with the regulation of gene expression, mitochondrial function/cellular metabolism, connective tissue formation, as well as the absorption, storage, and metabolism of iron. Copper levels are tightly regulated in the body. Copper is carried mostly in the bloodstream on a plasma protein called ceruloplasmin. Hepatic copper overload leads to progressive liver injury and eventually cirrhosis **(Dashti et al., 1992)**.

In the present study, the increased copper level may be due to oxidative stress inducing proteolytic modification of ceruloplasmin **(Kemal et al., 2003)**. Copper is absorbed into the intestine and transported by albumin to the liver. Any excess in copper levels is excreted into the bile mainly through a lysosome-to-bile pathway. Thus,

the increased hepatic copper level may be due to a reduction in the bile excretion of copper **(Sternlieb, 1994)**.

Radiation-induced oxidative damage to liver and muscle may be enhanced by the accumulation of iron and copper. These transition metals undergo auto-oxidation reactions, increase hydroxyl radicals thereby enhancing lipid peroxidation and increasing liver and muscle damage (**Nelson and Cox, 2005**).

In the present study, the increase of TBARS levels associated with significant increases of iron, copper and calcium levels in liver and skeletal muscle tissues provides evidence of the relationship between these parameters and increased oxidative damage.

Xanthine oxidoreductase system consists of xanthine dehydrogenase (XDH) and xanthine oxidase (XO). It is pre-dominantly present as XDH in normal tissues and converts into the free radical **generating** XO-form in the damaged tissue. Therefore, the XO-form is expected to be mainly found in radiolytically damaged tissues **(Kale, 2003)**. Ionizing radiation may help in the conversion of xanthine dehydrogenase to xanthine oxidase and produces a burst of ROS **(Srivastava et al., 2002, Said et al., 2005)**. XO activity has been demonstrated in human tissues such as liver, small intestine, heart and skeletal muscle (**Hellsten et al., 1997**).

There is evidence that during the hypoxia, ATP is degraded to hypoxanthine which is the substrate for xanthine oxidase. Once an oxygen supply is re-established, xanthine oxidase has the ability to generate the superoxide anion and hydrogen peroxide, which react in the presence of iron and other metals to form hydroxyl radicals, one of the most potent radicals known (**Cuzzocrea et al., 2001**).

In the present study the radiation-induced increase of XO activity in liver and muscle tissues may be explained according to the following concepts. During oxidative stress, the inadequate generation of ATP can cause malfunctioning of calcium ATPase pumps and an increase in intracellular calcium, which in turn activates calcium-dependent proteases. These proteases cleave a portion of xanthine dehydrogenase, converting it into xanthine oxidase.

Ionizing radiation induces endothelial cell damage and subsequent blood-spinal cord barrier breakdown. This leads to edema and hypoxia **(Yu-Qing et al., 2001)**. Hypoxia (Oxygen-deficient) may perpetuate late normal tissue injury. When a tissue becomes ischaemic (reduced blood supply), oxidation of cytochrome C fails with the result that ATP levels become depleted. The consequence is that there is a build up of hypoxanthine and xanthine which are oxidizable purine substances. During hypoxia, calcium also accumulates within cells which convert xanthine dehydrogenase to xanthine oxidase which is oxygen-dependent (**Dominique and Edward, 1999**).

5.2 Radiation Effects on Antioxidant Status In Liver and Skeletal Muscle Tissues:

Muscles are highly susceptible to oxidative stress; they are poor in antioxidant enzymes catalase and superoxide dismutase (**Marzani et al., 2004)** and contain a very high concentration of myoglobin. These heme containing proteins are known to confer greater sensitivity to free radical induced damage by conversion of hydrogen peroxide to more reactive species. Furthermore, skeletal muscle membrane contains large proportions of polyunsaturated fatty acids particularly susceptible to oxidative damage (**Rando, 2002**).

In the present study, whole body gamma irradiation of rats with 5 Gy (one shot dose) provokes significant decreases in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, as well as in glutathione content (GSH) 7, 14 and 21 days post-irradiation.

Several possible biochemical mechanisms can explain the significant decrease in the activity of antioxidant enzymes SOD, CAT and GSH-Px observed in the present study. Decrease in the activity of antioxidant enzymes might result from radiation-induced cell membrane damage and alterations in dynamic permeability of membranes due to peroxidation, which is followed by the release of intracellular enzymes to the blood stream **(Saada et al., 2003)** or might be attributed to their utilization by the enhanced production of ROS **(Said, 2004)**.

The significant decrease in the activity of glutathione peroxidase in the present study may be due to exhaustion or inactivation of the enzyme by ROS, since oxidative damage to hemoglobin and cell membrane has been reported to reduce the activity of glutathione peroxidase **(Zima et al., 2001)**.

Significant decrease in the activity of catalase could be due to less availability of NADPH. However, the gradual decrease in liver and muscle catalase activity may be due to excessive generation of $O_2^{\bullet -}$ leading to inactivation of the enzyme, as $O_2^{\bullet -}$ has been shown to reduce catalase activity **(Zima et al., 2001)**.

Moreover, the significant decrease in the activity of antioxidant enzymes in liver and muscle of irradiated rats could be due to increase in lipid peroxides which can cross-link with amino group of protein to

form intra and intermolecular cross-links thereby inactivating several membrane bound enzymes **(Zima et al., 2001)**.

Inactivation of antioxidant enzymes may result also from the direct effect of radiation energy that can break some chemical bonds in the protein molecule causing denaturation and inactivation of enzymes. In addition, $^{\bullet}OH$ and H_2O_2 resulting from water radiolysis cause oxidation of enzyme molecules and modification of their activities (**Kregel and Zhang, 2007**).

Glutathione (GSH) is an important intracellular peptide with multiple functions ranging from antioxidant defense to modulation of cell proliferation. GSH is a major endogenous antioxidant which counterbalance free radical mediated damage and it is well known that GSH is involved in the protection of normal cells structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions **(Buhl, 1994)**.

In the present study, whole body exposure of rats to gamma irradiation (5 Gy) resulted in significant depletion of liver and muscle glutathione content. Several factors contribute to the fall in GSH level in liver and muscle tissues of irradiated rats. Most important is oxidative stress, which occurred in the present study and consumes GSH. Cellular glutathione (GSH) is oxidized to GSSG that is either reduced by glutathione reductase or depleted from cells (**Deneke and Fanburg, 1989**). Therefore, glutathione can also serve as an indicator of oxidative stress.

Moreover, the depletion in GSH after exposure to gamma radiation may be due to reaction of GSH with free radicals resulting in the formation of thiyl radicals that associate to produce GSSG. Further,

normal synthesis/repair of GSH will be impaired due to damage to DNA and membranes **(Navarro et al., 1999)**.

Marked glutathione (GSH) depletion induced liver and skeletal muscle degeneration, associated with mitochondrial damage (**Rando, 2002**). GSH can, also, react with either nitric oxide (NO) or peroxynitrate ($ONOO^-$) to form S-nitrosoglutathione (GSNO). Therefore, another potential mechanism to explain the apparent loss of total glutathione in muscles would be through the increased formation of GSNO (**Roy et al., 2006**).

Liver synthesizes GSH from endogenous or dietary amino acids and supplies most of the circulating GSH (**Meister and Anderson, 1983)**. During prolonged oxidative stress, hepatic GSH efflux is increased due to the stimulation of elevated plasma glucagon and vasopressin levels **(Liu et al., 1999)**. This ensures plasma GSH homeostasis despite enhanced tissue GSH use (**Sen et al., 1994**). However, plasma and muscle GSH content may be decreased eventually during prolonged oxidative stress when hepatic GSH reserve is diminished and GSH use exceeds GSH uptake **(Sen et al., 1994).** Depletion of GSH renders the cell more susceptible to oxidative stress **(Subir and Vasudevan, 2005).**

5.3 Radiation Effects on Some Metabolic Enzymes Activity of Liver and Skeletal Muscle Tissues:

Ionizing radiation induces endothelial cell damage and this leads to hypoxia **(Yu-Qing et al., 2001)**. Under normal circumstances the blood supplies enough oxygen to the liver, but if hepatocytes (liver cells) use up more oxygen because of the breakdown of ROS, oxygen

deficits (i.e., hypoxia) can develop in some liver areas. Radiation-induced hypoxia, in turn, may impede the liver cells' ability to produce adenosine triphosphate (ATP), which is generated during the breakdown of nutrients and supplies energy needed for numerous biochemical reactions. Sufficiently high levels of ATP are essential to the survival of all cells; reduced ATP levels in the liver is a major factor contributing to liver cell death and may contribute to development of liver cirrhosis **(Baio et al. 1998)**.

Hepatic damage is associated with distortion of these metabolic functions. Therefore, damage to the liver or interference with its critical functions can be harmful or even lethal to the organism (**Wolf, 1999**). One of the factors that play a central role in many pathways of radiation-induced damage is oxidative stress. Excessive production of oxygen radicals leads to altered enzyme activity, decreased DNA repair, and impaired utilisation of oxygen, lipid peroxidation and protein oxidation. Some of these alterations induced by oxidative stress have been recognised to be characteristic features of necrosis and subsequently leading to liver and muscle damage **(Kurose et al., 1996)**.

In the present study, whole body gamma irradiation of rats with 5 Gy induced significant increases in the activity of lactate dehydrogenase (LDH) and significant decreases in creatine phosphokinase (CPK), glutamate dehydrogenase (GDH) and glucose-6-phosphate dehydrogenase (G6PD) activities 7, 14, and 21 days post-irradiation in the liver and skeletal muscle tissues.

Lactate dehydrogenase (LDH) was used as an indicator of tissue damage. LDH can be quite high with malignancies involving the liver. Significant rise in muscle and hepatic activity of LDH may be due to

muscle and hepatic toxicity as result of ROS produced from radiation exposure which induce redox imbalance. Significant elevation of LDH level seemed to be correlated with changes in the rate of protein expression secondary to DNA damage initiated by free radicals which is known to induce lipid peroxidation, oxidation of proteins, DNA damage and interference of reactive oxygen species or reactive nitrogen species with signal transduction pathways. These consequences become even more harmful when genetic variations impair protein expression **(El-Zayat, 2007)**.

Glycolysis is a series of reactions that break down the sugar glucose **(Bailey and Cunningham, 1999)** into two molecules of a compound called pyruvate. When oxygen levels in the liver tissues are too low, the immediate breakdown products of glucose, including pyruvate, accumulate in the cells because there is not enough oxygen available to further break down pyruvate via the citric acid cycle. The accumulating pyruvate subsequently is converted to lactate, which also can accumulate **(Baio et al. 1998; Van Horn and Cunningham, 1999).** In the present study, radiation-induced increase in LDH activity of liver and skeletal muscles tissues might be attributed to radiation-induced hypoxia and the consequent enhanced glycolysis.

It is well established that radiation exposure cause adversely affects both to the structure and the function of the mitochondria in liver cells, thereby interfering with the oxidative phosphorylation system. Other evidence indicates that ROS also interferes with glycolysis, resulting in reduced ATP synthesis, particularly in the presence of oxygen deficits. Researchers found that changes in the structure and function of hepatic mitochondria are early consequences of radiation exposure **(Cunningham et al. 1990; Hoek, 1994)**. For example, the

mitochondria can swell to an abnormal size following radiation exposure. ROS also can change the composition of the phospholipids that make up the mitochondrial membranes, although it is not known whether these changes influence mitochondrial functioning. Finally, radiation-indced ROS can alter the protein content of the mitochondria, and these alterations interfere with the mitochondria's ability to synthesize ATP.

Glucose-6-phosphate dehydrogenase plays a key role in the production of NADPH, utilized to maintain GSH in the reduced form; one may deduce that, in the G6PD deficient muscle, a lower level of NADPH leads to a decrease of GSH. Taking into consideration that G6PD activity in normal muscle is the lowest compared to the other body tissues; thus a deficiency of that enzyme may lead to a dramatic fall in the steady-state concentration of NADPH and GSH (**Mohit et al., 2003**).

In the present study, it could be suggested that in irradiated rats the alterations of the redox state of the cell results in a decreased formation of NADPH, which in turn inactivates glucose-6-phosphate dehydrogenase (**Pari and Venkateswaran, 2003)**.

Glutamate dehydrogenase (GDH), performs a key role in aerobic energy metabolism, and acts as a preventive antioxidant against oxidative stress, reducing mitochondrial ROS generation. GDH a glutamate oxidative deamination enzyme is a specific mitochondrial marker enzyme. In the present study, the decrease of GDH might be due to its release after alteration of mitochondria membrane permeability resulting from increased lipid peroxidation **(Saada and Azab, 2001**) or

loss of its active configuration due to the destruction of mitochondrial membrane (**Guido et al., 2007)**.

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

The decrease in the activity of CPK might result from the interaction of lipid peroxidation products with protein forming cross linkages that inactivate membrane-bound enzymes (**Lakshmi et al., 2005**) in addition, to increased membrane permeability resulting from increased Ca^{2+} leakage into the muscle (**Gissel, 2000**).

According to the results obtained in the present study, it could be observed that various pathways play a role in radiation-induced tissue injury, including alteration in the pro-oxidant/antioxidant balance that leads to mitochondrial damage.

5.4. Protective effect of Wheat Germ Oil

The human diet contains an array of natural antioxidants that may contribute to the endogenous antioxidant defense system. Epidemiological studies have shown that diets rich in plant foods significantly reduce the incidence and mortality rates of degenerative diseases caused by oxidative stress **(Kelley and Bendich, (1996)**. This protective effect has been attributed to the fact that such foods may provide an optimal mix of phytochemicals **(Ames et al., 1993)**. Wheat

germ oil is rich in vegetable oil compounds, particularly vitamin E, octacosanol and omega-3 fatty acids **(Moure et al., 2001)**.

The results obtained in the present study, demonstrated that extended administration of wheat germ oil for 14 days pre and 7 days post whole body gamma irradiation at 5 Gy (single dose) has significantly minimized the severity of biochemical changes. Wheat germ oil protection against oxidative stress appeared to be mediated through decreasing the pro-oxidants and enhancement of cellular antioxidant activities. Significant reductions in the level of lipid peroxides associated to enhancement in the activity of antioxidant enzymes SOD, CAT and GSH-Px as well as in the content of reduced glutathione were recorded in the liver and skeletal muscles of WGO-treated irradiated rats. Furthermore, WGO administration has decrease the levels of total Fe, Cu and Ca which were in parallel to significant improvement in the activity of metabolic enzyme activities of GDH, CPK, G-6-PD and LDH.

The possible mechanism that may be responsible for the protection of radiation- induced liver and muscle damage by wheat germ oil may be due to its radical scavenger activity intercepting those radicals involved in water radiolysis. By trapping oxygen related free radicals wheat germ oil could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid per-oxidative processes (**Paranich et al., 2000)**.

Beneficial effects from wheat germ oil may be due to a combination of its Vitamin E, octacosanol, linoleic and linolenic acids, the precursors for the omega-6 and omega-3 essential fatty acids, respectively. These two fatty acids are of great importance in human

metabolism and cannot be synthesized by the organism. They are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes **(Zacchi et al., 2006)**. Furthermore, linoleic acid helps to eliminate cholesterol and is a precursor of cell membrane phospholipids **(Salinas, 1993).** Furthermore, studies have shown that linoleic and linolenic acids-rich wheat germ oil decreases oxidative stress in patients with mild hypercholesterolemia **(Alessandri et al., 2006)**.

Paranich et al., (2000) showed that in oral administration wheat germ oil efficiently saturates the body with vitamin E which improves the flow of blood and strengthening the veins and capillaries. Wheat germ is the richest known natural source of tocopherols (Vitamin E) and also abundant in B-group vitamins. These are free radical scavengers and might increase intracellular glutathione (GSH) stores **(Attila et al., 2001)**. B complex vitamins are essential in the formation of red blood cells (**Vicky et al., 2004**).

Vitamin E is an important dietary antioxidant in biological systems because of its association with the cell membrane and its ability to act directly on ROS and prevent lipid peroxidation. It is an essential micronutrient for normal muscle function. It is also, known to enhance the body's immune response, inhibit nitrosamine formation, enhances cell communication and inhibit the metabolic activation of carcinogen **(Van Poppel and Van den Berg, 1997 and Lee et al., 2005)**.

Vitamin E can effectively counteract ROS generated by toxicant or carcinogen induced oxidative damage by trapping reactive oxyradicals and preserve membrane integrity **(Sies et al., 1992)**. Its

deficiency can lead to the degeneration of muscular tissue (nutritional muscular dystrophy) in animals and humans because it is involved in regulation of the synthesis of specific proteins required for normal muscle function **(Chiarelli et al., 2004)**.

Octacosanol; an alcohol fatty acid is another active ingredient of wheat germ oil. Octacosanol had promising effects on endurance, reaction time, and other measures of exercise capacity and is helpful in cholesterol management and neurological disorders (**Reddy et al., 2000)**. A number of mechanisms have been proposed for how octacosanol could affect endurance or strength. For example, some evidence suggests octacosanol may improve the efficiency of transmission of nerve impulses or that it may increase oxygen transport or uptake.

Another theory is that wheat germ oil can boost the energy supplied to muscles (octacosanol seems to concentrate in the body in muscle tissue, as well as the liver and the digestive tract) by promoting the movement of fatty acids within muscles. Japanese researchers determined that octacosanol significantly reduced fat accumulation in the tissue of certain rats, suggesting an effect on some aspect of fat metabolism. Octacosanol ability to affect fat metabolism, cholesterol production, and blood platelet stickiness could account for cardiovascular benefits and minor tonic actions **(Carbajal et al., 1998 and Gamez et al., 2005)**.

Antioxidant principles from herbal resources are multifaceted in their effects and provide enormous scope in correcting the imbalance through regular intake of a proper diet. Thus, from the foregoing

findings, it was observed that wheat germ oil is a radio-protective agent and this activity may be due to the antioxidant chemicals present in it.

According to the results obtained in the present study it could be concluded that wheat germ oil by enhancing antioxidant activities and decreasing lipid peroxidation, may afford protection against radiation-induced oxidative stress and might preserve the integrity of tissue functions and minimize metabolic disorders induced by ionizing irradiation. Hence wheat germ oil administration prior to radiation therapy may be useful to cancer patients to prevent normal cell damage.

6- SUMMARY

Ionizing radiation is a physical agent that induces oxidative stress by excess generation of reactive oxygen species. The human diet contains an array of natural antioxidants that may contribute to the endogenous antioxidant defense system. Among the most common dietary sources of natural antioxidants, wheat germ oil is rich in vegetable oil compounds; particularly vitamin E, octacosanol, linoleic and linolenic essential fatty acids. The present study was designed to investigate the effect of wheat germ oil (WGO) on gamma radiation-induced oxidative stress in liver and skeletal muscle tissues of rats.

Male albino rats (100-120g) were used for the different investigations carried out in this work. WGO were suspended in corn oil and administered to animals by gavage at doses of 54 mg/kg body weight. The animals were divided into 4 groups; **Control group**: Rats received 0.1 ml corn oil for 21 successive days. **WGO-treated group**: Rats received WGO (54 mg/ kg body weight/day), for 21 successive days. **Irradiated group**: Rats were whole body exposed to 5Gy gamma irradiation applied as one shot dose. **WGO-treated irradiated group**: Rats received WGO for 14 successive days before and 7 successive days after exposure to whole body gamma radiation.

Liver and skeletal muscle samples were taken at time intervals 1st, 2nd, and 3rd weeks' post-irradiation exposure.

6.1. Radiation Effects on Pro-Oxidant Level on Liver and Skeletal Muscle tissues

The result revealed that exposure to ionizing gamma irradiation of rats with 5 Gy (one shot dose) induce significant increase in the

levels of thiobarbituric acid reactive substances (TBARS) which was concomitant with significant increases in the level of iron, copper and calcium as well as significant increases in the activity of xanthine oxidase and significant decreases in xanthine dehydrogenase activity 7, 14, and 21 days post-irradiation.

6.2. Radiation Effects on Antioxidant Status In Liver and Skeletal Muscle Tissues:

The results obtained in the present study showed that whole body gamma irradiation of rats with 5 Gy provoke oxidative stress in the liver and skeletal muscle tissues. Significant decrease in glutathione content as well as significant decreases in the activity of the antioxidant enzymes; superoxide dismutase, catalase and glutathione peroxidase were recorded 7, 14 and 21 days post-irradiation.

6.3. Radiation-Induced Alteration in Some Enzymes Activity

The result revealed that whole body gamma irradiation of rats with 5 Gy induced significant increases in the activity of lactate dehydrogenase and significant decreases in creatine phosphokinase, glutamate dehydrogenase and glucose-6-phosphate dehydrogenase activities 7, 14 and 21 days post-irradiation in the liver and skeletal muscle tissues.

6.4. Protective effect of Wheat Germ Oil

The results obtained in the present study, demonstrated that extended administration of wheat germ oil for 14 days pre and 7 days post whole body gamma irradiation at 5 Gy (single dose) has

significantly minimized the severity of biochemical changes. Wheat germ oil protection against oxidative stress is mediated through augmentation of a number of cellular antioxidants and by decreasing the pro-oxidants.

Experimental animals receiving 54 mg/Kg body weight wheat germ oil for 14 successive days before and 7 days after whole body exposure of rats to 5 Gy of ionizing gamma radiations showed an increased resistance to oxidative challenge.

The results showed that wheat germ oil enhances antioxidant defenses and specific metabolic activities of rat liver and skeletal muscle tissues. This was obvious by an increase in the activities of SOD, CAT and GSH-Px, compared to the corresponding values of irradiated rats. WGO treatment has significantly decrease activity of xanthine oxidase and control iron, copper and calcium overloads.

Furthermore, WGO has significantly minimized the formation of lipid peroxidation products obvious by a lower level of TBARS when compared to their corresponding values in irradiated rats. Significant amelioration in the antioxidant status of liver and skeletal muscle tissues was substantiated by the significant amelioration of lactate dehydrogenase and creatine phosphokinase, glutamate dehydrogenase and glucose-6-phosphate dehydrogenase activities.

CONCLUSION

Reactive oxygen species or oxidants formed in the body due to exogenous and endogenous factors are found to be responsible for many diseases. Day to day research is revealing the potential of photochemical antioxidants as health benefactors. This is due to their ability to neutralize the free radicals or reactive oxygen species or oxidants responsible for the onset of cell damage. Most of the natural antioxidants from plant source are safer to health and have better antioxidant activity.

Wheat germ oil is an energy-rich, unrefined vegetable oil and preserves more of the natural nutrients, provides vitamin E, octacosanol, linoleic and linolenic essential fatty acids.

According to the results obtained it could be concluded that wheat germ oil might attenuates radiation-induced oxidative organ injury and improve metabolic functions by decreasing lipid peroxidation and increasing antioxidant status that subsequently prevents the development of oxidative damage initiated by irradiation. Wheat germ oil also maintains the integrity of cell membrane, and helps in the restitution of cellular energy metabolism in irradiated rats.

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الملخص العربى

ساعدت عوامل كثيره على زيادة الجهد التأكسدى للجسم ومنها زيادة تلـوث البيئـة وكثرة الإشعاعات الذرية وأزدياد إنتشار عادم السيارات والضغوط النفسية وتناول الطعـام غير الصحي والتدخين. وترتب على زيادة عمليات الاكسدة فى الجسم إنتاج ذرات الأكسجين الحرة غير المستقرة التي تنتج بدورها الشوارد الحرة ، كما أن الشوارد الحره تنتج ثانويـا عن عملية أنتاج الطاقة في الخلية. الميتوكوندريا هي المركز النشط لأنتاج الطاقة لكل الخلية لذلك فانها تموج بعديد من الشوارد الحرة التي تسبب تلف خلاياها فيضعف انتاجها للطاقـة مما يؤدي الى تلف الأنسجة والخلايا فى الجسم وحدوث كثير من الامراض مثل الجلطـات وتجاعيد الوجه والشيخوخة المبكرة وبعض الأمراض العصبية وحتى السرطان.

ويقوم الجسم يوميا بتعويض الأنسجة والخلايا التالفة حيث خلق الله فـي أجـسامنا بعض الأساليب الدفاعية التي تقاوم هذه الشوارد الضارة وتحمينا من أخطارها مثل إنزيمات مضادات الأكسدة ومنها (جلوتاثيون بير أوكسيديز، وسوبر أكسيد ديزميوتيز، والكاتـاليز). ولكن إذا كانت سرعة تكوين الشوارد الحرة أكبر من طاقة الجسم الدفاعية ـ فإن الجـسم لا يستطيع التخلص منها وتحدث أضرار جسيمة للخلايا مما يؤثر سلبا على وظائفها وتظهـر عليه علامات المرض.

لذلك فان الحاجة الى تحديد مواد لها القدرة على تقليل هذه الاضرار يمثل ضـرورة كبيرة. ويعتبر زيت جنين القمح مصدرا غذائيا فعالا من حيث تركيبة الكيميائى حيث يحتوى على العديد من الاحماض الامينية الاساسية التى تلعب دورا رئيسيا فى تنظيم عمـل جميـع أجهزة الجسم. كما يتميز بارتفاع محتواة من مضادات الأكسدة وهذة المركبات تقلل تكـوين الخلايا السرطانية وتعمل على زيادة حيوية وكفاءة الخلايا داخل الجسم .

لذلك تهدف هذه الدراسة الى تقييم الدور الوقائى المحتمل لزيت جنين القمـح فـى تحسين الأضرار التى يحدثها التعرض للإشعاع الجامى وذلك من خلال دراسـة التغيـرات البيوكيميائية التى تحدث فى نشاط بعض الانزيمات مثل الكرياتين فسفوكينيز، جلوكـوز 6 فوسفات دى هيدروجنيز، اللكتات دى هيدروجنيز و جلوتاميت دى هيـدروجنيز والـزانثين اوكسيدو ريدكتيز . كما تم قياس التغيرات التى تحدث فى مستوى مضادات الأكسدة الداخلية مثل السوبر اكسيد ديسميوتيز، الكاتاليزو الجلوتاثيون بير اكسيديز بجانـب دراسـة بعـض التغيرات التى تحدث فى مستوى الجلوتاثيون وعملية فوق الأكسدة للدهون وبعض العناصر الشحيحة مثل الحديد، النحاس والكالسيوم وذلك فى خلايا الكبد والعضلات الهيكلية للجرذان البيضاء بعد تعريض جسمها الكلى لجرعة اشعاعية واحده قدرها 5 جراى.

وقد تم تخفيف زيت جنين القمح بإذابتة فى زيت الذره ثم تم حقنه عن طريـق الفـم فـي الجرذان يوميا لمدة 21 يوم متتالية بجرعة قدرها (54 مليجرام/كجرام وزن الجـسم). تـم تقسيم الجرذان البيضاء وزن (100- 120جرام) الى أربع مجموعات متساوية:

1- **مجموعة ضابطة:** تم حقن الجرذان بزيت الذره يوميا لمدة 21 يوم.

2- **مجموعة معرضة للإشعاع الجامى**: تتم تعريض جسم الجرذان كليا لجرعة إشعاعية قدرها 5 جراى.

3- **مجموعة معالجة بزيت جنين القمح**: تم حقن الجرذان بزيت جنين القمح عن طريق الفم بتركيز قدرة 54 ملجم/ كجم من وزن الجسم يوميا لمدة 21 يوم متتالية.

4- **مجموعة معالجة بزيت جنين القمح ومعرضة للإشعاع**: تم حقن الجـرذان بزيـت جنين القمح عن طريق الفم بتركيز قدرة 54 ملجم/ كجم من وزن الجسم لمـدة 14 يوم قبل و7 ايام بعد التعرض للإشعاع.

وقد تم اجراء القياسات البيوكيميائية فى الاسبوع الاول والثـانى والثالـث بعـد التعـرض للإشعاع. ويمكن تلخيص النتائج التى تم التوصل اليها كما يلى:

أولا: تأثير تعرض الجسم الكلى للاشعه جاما على:

1- مستوى المواد المتفاعلة مع حمض الثيوبربتيوريك ومستوى نشاط بعض مضادات الاكسده:

أظهرت نتائج هذه الدراسة أن تعرض الجسم الكلي لأشعة جاما بجرعة حـادة قـدرها (5 جراى) يؤدي الي زيادة معنوية فى مستوى المواد المتفاعلة مع حمض الثيوبربتيوريك فـى خلايا الكبد والعضلات الهيكلية للجرذان كمؤشر لزيادة عملية الفوق أكسدة للدهون. وتكـون هذه الزيادة مصحوبة بنقص معنوى فى مستوى كل من الجلوتاثيون ومستوى نشاط إنزيمات مضادات الأكسدة مثل السوبر أكسيد ديسميوتيز والكاتاليز والجلوتاثيون بير أكسيديز وذلـك في الاسبوع الأول والثانى والثالث بعد التعرض للإشعاع.

2- مستوى الزانثين اوكسيدو ريدكتيز:

توضح نتائج هذه الدراسة أن تعرض الجسم الكلي لأشعة جاما بجرعة حـادة قـدرها (5 جراى) يؤدي الي زيادة معنوية فى مستوى الزانثين اكسيديز يصاحبها نقص معنـوى فـى مستوى الزانثين دى هيدروجنيز فى خلايا الكبد والعضلات الهيكلية وذلك فى جميع أوقـات التجربة.

3- مستوى بعض العناصر الشحيحة:

أظهرت نتائج هذه الدراسة أن تعرض الجسم الكلي لأشعة جاما بجرعة حادة قدرها (5 جراى) يؤدي الي أرتفاع ملحوظ فى مستوى الحديـد والنحـاس والكالـسيوم كمؤشر لزيادة عملية تكوين الشوارد الحرة.

4- مستوى نشاط بعض إنزيمات التفاعل:

وتظهر نتائج هذه الدراسة أن تعرض الجسم الكلي لأشعة جاما بجرعة حـادة قـدرها (5 جراى) يؤدي الي خلل فى نشاط بعض الأنزيمات منها نقص فـى الكريـاتين فـسفوكينيز والجلوتاميت دى هيدروجينيز والجلكوز-6 –فوسفيت دى هيدروجنيز مع إرتفاع معنوى فى نشاط إنزيم اللكتات دى هيدروجنيز وذلك فى الاسبوع الأول والثانى والثالث بعد التعـرض للإشعاع.

ثانيا- دور زيت جنين القمح فى تقليل الأضرار الإشعاعيه

وتوضح نتائج هذه الدراسة أن حقن الجرذان بزيت جنين القمح لمدة 14 يوم قبل و7 ايام بعد التعرض للإشعاع يقلل من التغيرات البيوكيميائية الضارة التى تحدث فى خلايا كبد والعضلات الهيكلية للجرذان المعرضة للإشعاع. حيث أدى حقن الجرذان بزيت جنين القمح الى تقليل مستوى الزانثين اوكسيديز كما أدى الى نقص فى مستوى المواد المتفاعلة مع حمض الثيوبربتيوريك كمؤشر لنقص عملية الفوق أكسدة للدهون. وتوضح نتائج هذه الدراسة أيضا أن زيت جنين القمح عمل على زيادة نشاط مستوى كل من الجلوتاثيون ومستوى نشاط انزيمات مضادات الأكسدة. كما أدى حقن الجرذان المشعة بزيت جنين القمح الى تعديل الخلل الذى

ظهر فى نشاط انزيمات الكرياتين فسفوكينيز والجلوتاميت دى هيدروجينيز والجلكوز-6 – فوسفيت دى هيدروجنيز واللكتات دى هيدروجنيز. كما ساعد أيضا على تحسين مستوى العناصر الشحيحة وذلك في الأسبوع الأول والثانى والثالث بعد التعرض للإشعاع.

ومن نتائج هذه الدراسة يمكن إستنتاج أن زيت جنين القمح بما يحتويه من مضادات أكسدة طبيعية يحافظ على الوظائف الحيوية للكبد والعضلات الهيكلية من تأثير الشوارد الضارة الناتجة من تعريض الجرذان للأشعاع الجامى.

المستخلص العربى

تهدف هذه الدراسة الى تقييم الدور الوقائى المحتمل لزيت جنين القمح فى تحسين الأضرار التى يحدثها التعرض للإشعاع الجامى وذلك من خلال دراسة التغيرات البيوكيميائية التى تحدث فى نشاط الكرياتين فسفوكينيز، جلوكوز 6 فوسفات دى هيدروجنيز، اللكتات دى هيدروجنيز وجلوتاميت دى هيدروجنيز والزانثين اوكسيدو ريدكتيز. كما تم قياس التغيرات التى تحدث فى مستوى مضادات الأكسدة الداخلية ومحتوى الدهون فوق المؤكسدة ومستوى الجلوتاثيون والحديد، النحاس والكالسيوم وذلك فى خلايا الكبد والعضلات الهيكلية للجرذان بعد تعريض جسمها الكلى لجرعة اشعاعية قدرها 5 جراى وذلك في الاسبوع الأول والثانى والثالث بعد التعرض للإشعاع.

أظهرت نتائج هذه الدراسة أن تعرض الجسم الكلي لأشعة جاما يؤدي الي زيادة معنوية فى معظم المعايير البيوكيميائيه وخلل فى نشاط بعض الأنزيمات التى تحت الدراسه. وتكون هذه الزيادة مصحوبة بنقص معنوى فى مستوى كل من الجلوتاثيون ومستوى نشاط انزيمات مضادات الاكسدة وذلك في معظم اوقات التجربه. كما أن حقن الجرذان بمستخلص زيت جنين القمح عن طريق الفم في الجرذان يوميا بجرعة قدرها (54 مليجرام/كجرام وزن الجسم) لمدة 14 يوم قبل و7 ايام بعد التعرض للإشعاع يقلل من التغيرات البيوكيميائية الضارة التى تحدث فى خلايا كبد والعضلات الهيكلية للجرذان المعرضة للإشعاع.

كلية العلوم

دورزيت جنين القمح فى الحد من الإجهاد التأكسدى والتغير فى عملية أيض الطاقة فى الجرذان المعرضة كليا للأشعاع

رسالة مقدمة من

شرين محمد أبراهيم شديد

بكالوريوس علوم قسم الكيمياء الحيوية- جامعة عين شمس

2002

للحصول على درجة الماجستير
فى الكيمياء الحيوية

الى

جامعة حلوان
كلية العلوم
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بكالوريوس علوم قسم الكيمياء الحيوية - جامعة عين شمس- 2002

للحصول على درجة الماجستير
فى الكيمياء الحيوية

تحت أشـراف

الأستاذ الدكتور/ السيد محمد السيد مهدى

أستاذ الكيمياء الحيوية بقسم الكيمياء – كلية العلوم- جامعة حلوان

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أستاذ الكيمياء الفسيولوجية بقسم البيولوجيا الاشعاعية
المركز القومى لبحوث وتكنولوجيا الإشعاع- هيئة الطاقة الذرية

الأستاذ الدكتور/ أسـامة زكريا سعيد

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كلية العلوم- قسم الكيمياء
جامعة حلوان
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