# Aggravation of Irradiation Induced Impairment in Protein Metabolism in Albino Rats Subjected to Oral Injection of Kelthane Miticide

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## خلاصـــة

إستهدف البحث دراسة تأثير الجرعات المتجزئة لإشعاعات جاما على الجسم الكلى لذكور الجرذان المعطاه المبيد الحشرى «كلثان» الملوث للبيئة . وقد تم إعطاء مادة الكلثان عن طريق الفم بجرعة أسبوعية مقدارها ١٠٠ ملليجرام/كجم من وزن الجسم مقسمة إلى ثلاثة أجزاء أما بالنسبة للمعاملة بالتشعيع الجامى فقد عرضت الجـــرذان لجرعة أسبوعية مقدارها ٣ جراى مجزئة على جرعات بمستوى ١ جراى لكل يومين حيث بلغت الجرعات المتراكمة تحت الاختبار ٣ ، ٦ ، ٩ ، ١٢ ، ١٥ جراى . وشملت الدراسة البيوكيميائية قياس مستوى البروتين الكلى والأحماض الأمينية فى الدم والبول والفصل الكهربى لبروتينات الدم ومستوى اليوريا فى الدم . ولقد أظهرت النتائج تغيرات معنوية فى معايير البروتين المختارة وذلك نتيجة لتعرض الجسم الكلى للتشعيع . كما أوضحت الدراسة أن هذه التغيرات ترتبط طرديا بالوقت والجرعة حيث قد بلغت أقصاها فى نهاية فترة التجربة (خمسة أسابيع) وأن هذه التغيرات كانت أكثر وضوحا فى مجموعة حيوانات التجارب المعرضة لكل من الإشعاع والمبيد الحشرى .

## Abstract

The combined effect of both whole body gamma radiation exposure and administration of organochlorine miticide "kelthane" on protein metabolism was investigated in male albino rats. Kelthane was orally given at a dose level of 100 mg/kg body weight over a period of seven days. Irradiation process permitted the rats to receive one Gray every other day at a weekly cumulative dose of 3 Gy up to a total dose of 15 Gy. The biochemical assays included total proteins, protein fractions, free amino acids (FAAS) and urea level in blood serum as well as protein content and its FAAS in urine. The data revealed significant changes in the protein parameters due to whole body gamma irradiation. These changes were

shown to be dose and time dependent which reached their maximum at the end of the experimentation period. The alterations were more pronounced in animal groups exposed to gamma radiation and received kelthane pesticide.

## 1- Introduction

It has been assumed that a number of occupational radiation workers and patients subjected to radiodiagnosis and /or radiotherapy, are occasionally exposed to certain environmental stresses prevailing at the different agro-industrial complexes. Among these, the exposure to chemical pollutants, contaminating air, water and food commodities. Therefore, it is worthy to investigate the radiation tolerance of experimental subjects living under stresses of toxic chemical substance namely the organochlorine miticide "kelthane". This is commonly applied in Egypt for controlling mites invading vegetables and other agricultural field crops. Since it is lipophilic compound, it is expected to find residues of the pesticide and many of its metabolites in adipose tissue of humans, birds and fishes. These types of compounds can accumulate at relatively high concentration in adipose tissues when ingested by various species at low dose rate over prolonged periods of time [1,2,3].

In Egypt radioactive labeled insecticide residues in crude cotton seed oil were estimated after spraying cotton plants under simulated condition of agricultural practice. It has been found that the refined oil still contained 0.2 to 1.3 ppm of insecticide [4,5].

The present research is designed to study the response of rats ingesting "kelthane" to cumulative dose of whole body gamma radiation. The investigated biochemical parameters related to protein metabolism include total proteins and their electrophoretically separated fractions in serum, free amino acids in serum and urine, urea levels in serum and protein content in urine.

## 2- Materials and Methods

Male Swiss albino rats (145 -150 g) were provided with a pellets of concentrate diet (coop-feed mix) which was supplemented with vitamin mixture. The animals had free access to water and diet.

**Animals were classified into four groups :**

1- Untreated normal control rats.

2- Irradiated rats received fractionated doses of gamma irradiation at one Gray increment, delivered every other day with a weekly total cumulative dose of 3 Gy up to total cumulative doses of 3, 6, 9, 12 and 15 Gy.
3- Kelthane group : Animals received kelthane insecticide orally at a dose level of 100 mg/kg body weight divided into three fractions administered over a period of seven days.
4- Combined treatment : Animals received kelthane and whole body gamma irradiation treatment .

Animals receiving the cumulative irradiation doses of 3, 6, 9, 12 and 15 Gy were sacrificed at the end of $1^{st}$, $2^{nd}$, $3^{rd}$, $4^{th}$ and $5^{th}$ week . The results were recorded at the end of each experimental interval.

**The irradiation process :**

It was performed by a Gamma Cell-40 biological irradiator achieved by Egypt's National Center for Radiation Research and Technology (NCRRT). It is a caesium-137 irradiation unit as manufactured by the Atomic Energy of Canada. The dose rate was 0.821 Gy/min. at the time of experimentation.

Animals after overnight fast were subjected to light anaesthesia, blood samples were obtained by heart puncture and serum was separated by centrifugation at 3000 rpm for 20 min. Twenty four hours urine samples for protein and amino acids analyses, were collected by housing the animals in metabolic cages. Serum and urine proteins were assayed colorimetrically using Biuret reagent prepared by Bio-Merieux diagnostic kit according to the method of Gornalla [6].

The level of urea concentration was estimated according to the method described by Fawcett and Scott [7] and modified by Patton and Crouch [8].

Serum protein fractions were determined according to the method described in Beckman instructions 015-083630-B, microzone electrophoresis Beckman Instruments, Inc, Fullerton, California, CA92534, USA.

Qualitative and quantitative estimations of amino acids were conducted by gas liquid chromatography : Protein was removed from blood serum and urine samples by using 80% ethanol according to Richard et al. [9] and the derivatization of ion exchange cleaned sample was carried out according to the method of Gehrke and Takada [10].

## 3- Results

### Protein constituents :

As shown in Table 1 whole body gamma irradiation resulted in decreased levels of serum total proteins and the different serum protein fractions. The most pronounced effect has been shown by gamma globulin fraction which was the only studied parameter that showed a slight tendency for radiation dose dependance. The only exceptions have been expressed by the $\alpha_1$ and $\alpha_2$ fractions which showed a retarded increase as recorded on the 5th week post radiation exposure. The increase was more pronounced with the serum $\alpha_2$ globulin fraction.

The ingestion of "kelthane" (Table 1) exerted non significant changes in serum total proteins and in albumin and globulin fractions apart from a transitory initial rise in albumin fraction and elevation of gamma globulin.

Combined treatment by kelthane and whole body gamma irradiation (Table 1) showed a common drop in the studied parameters. Only the $\alpha_1$, $\alpha_2$ and $\beta$ fractions showed retarded elevation on the 5th week post treatment. The magnitude of rise being more pronounced with the $\alpha_2$ fraction.

**Table 1 : Effect of whole body gamma irradiation and/or kelthane administration on serum total proteins and its electrophoretically separated fractions (g/dl).**

| Serum protien fractions | Experimental period (weeks) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Irradiated | | | | | Kelthane | | | | | Kelthane irradiation | | | | |
| | | 1st | 2nd | 3rd | 4th | 5th | 1st | 2nd | 3rd | 4th | 5th | 1st | 2nd | 3rd | 4th | 5th |
| Total proteins | 6.84 ± 0.58 | 6.51 ± 0.10 | 5.38** ± 0.28 | 4.91**# ± 0.19 | 5.56** ± 0.24 | 5.33** ± 0:47 | 6.94 ± 0.25 | 6.73 ± 0.30 | 6.34 ± 0.13 | 6.76 ± 0.18 | 6.19 ± 0.35 | 6.09 ± 0.37 | 5.63* ± 0.47 | 5.55** ± 0.23 | 5.18* ± 0.78 | 5.80* ± 0.41 |
| Albumin | 3.53 ± 0.33 | 3.67 ± 0.13 | 3.05* ± 0.11 | 2.70** ± 0.23 | 2.94* ± 0.25 | 2.33**# ± 0.22 | 3.89 ± 0.36 | 3.10 ± 0.23 | 3.21 ± 0.08 | 3.00** ± 0.11 | 2.41**# ± 0.12 | 3.40 ± 0.36 | 2.94* ± 0.23 | 2.90** ± 0.15 | 2.61** ± 0.25 | 2.34**# ± 0.16 |
| $\alpha_1$ globulin | 0.94 ± 0.10 | 0.88 ± 0.03 | 0.58**# ± 0.07 | 0.61** ± 0.12 | 0.79 ± 0.17 | 1.03 ± 0.02 | 0.80 ± 0.12 | 0.89 ± 0.04 | 0.86 ± 0.10 | 0.90 ± 0.11 | 0.76** ± 0.12 | 0.78 ± 0.13 | 0.71** ± 0.04 | 0.63** ± 0.12 | 0.69* ± 0.12 | 1.06 ± 0.12 |
| $\alpha_2$ globulin | 0.35 ± 0.06 | 0.34 ± 0.03 | 0.17**# ± 0.06 | 0.22** ± 0.05 | 0.30 ± 0.09 | 0.50 ± 0.16 | 0.33 ± 0.02 | 0.61**# ± 0.02 | 0.34 ± 0.07 | 0.40 ± 0.02 | 0.40 ± 0.03 | 0.29 ± 0.04 | 0.31 ± 0.05 | 0.26 ± 0.08 | 0.34 ± 0.09 | 0.57**# ± 0.06 |
| β - globlin | 1.42 ± 0.17 | 1.25 ± 0.07 | 1.23 ± 0.10 | 1.07 ± 0.22 | 1.16 ± 0.19 | 1.29 ± 0.08 | 1.38 ± 0.14 | 1.60 ± 0.06 | 1.27 ± 0.16 | 1.46 ± 0.10 | 1.52 ± 0.07 | 1.12 ± 0.08 | 1.18 ± 0.18 | 1.41 ± 0.11 | 1.20 ± 0.28 | 1.64 ± 0.19 |
| γ- globlin | 0.65 ± 0.08 | 0.37*# ± 0.043 | 0.35**# ± 0.090 | 0.31**# ± 0.050 | 0.37*# ± 0.050 | 0.15**# ± 0.035 | 0.54 ± 0.11 | 0.53 ± 0.19 | 0.66 ± 0.06 | 1.00*# ± 0.13 | 1.10**# ± 0.12 | 0.50* ± 0.06 | 0.49** ± 0.07 | 0.35**# ± 0.02 | 0.34** ± 0.10 | 0.19**# ± 0.04 |

- Irradiation dose level 3 Gy/week
- Each value reported mean ± SD of 6 determinations.
- (*) $p < 0.05$ (**) $p < 0.01$ (**#) $p < 0.001$

The data given in Table 2 showed a significant increase of protein in urine of irradiated rats at the end of the 3rd week which recorded a value of 32.56% as compared with the control level.

The excretion of protein in the irradiated group was increased up to the end of the 4th week recording a value of 134.88% and showed a mild drop of 118.60% at the end of the 5th week compared with control.

In case of rats received kelthane alone, non significant increase was observed up to the end of the 4th week followed by a significant increase recorded at the end of the 5th week (34.88%, $P < 0.05$). Rats received the combined treatment recorded increased values of 27.90, 58.14, 88.37 and 160.47% as recorded at the end of the 2nd, 3rd, 4th and 5th week re spectively.

**Non-protein nitrogenous compounds :**

**a) Serum urea level**

As shown in Table 2, the results indicate significant decrease in serum urea level of irradiated group amounting to - 21.50, - 38.40, - 36.46, and -31.68% recorded at the end of the 2nd, 3rd, 4th and 5th week respectively as compared with values of the normal group.

**Table 2 : Effect of whole body gamma irradiation and/or kelthane administration on serum urea concentration (mg/dl) and urine protein content (g/l)**

| Experimental period | Experimental parameters | Experimental groups | | | |
|---|---|---|---|---|---|
| | | Control | Irradiated | Kelthane | Kelthane + Irradiation |
| 1st week | Serum urea | 48.78±4.52 | 45.88±7.690 | 47.31±8.000 | 49.71±9.16 |
| | Urine protein | 0.43±0.091 | 0.50±0.108 | 00.37±0.075 | 0.48± 0.072 |
| 2nd week | Serum urea | 47.16±5.30 | 37.01±8.720* | 44.23±3.550 | 36.05±8.03* |
| | Urine protein | 0.43±0.091 | 00.47±0.048 | 00.42±0.097 | 0.55±0.085* |
| 3rd week | Serum urea | 51.02±8.12 | 31.43±3.930** | 39.40±10.160 | 37.65±3.81** |
| | Urine protein | 0.43±0.091 | 00.57±0.065* | 00.41±00.097 | 0.68±0.108** |
| 4th week | Serum urea | 49.53±5.91 | 31.47±5.940** | 40.01±5.390* | 38.76±5.59** |
| | Urine protein | 0.43±0.091 | 01.01±0.110** | 00.50±0.103 | 0.81±0.098** |
| 5th week | Serum urea | 49.53±5.91 | 33.84±4.720** | 39.30±5.36* | 39.31±4.59** |
| | Urine protein | 0.43±0.091 | 00.94±0.089** | 0.58±0.129* | 1.12±0.139** |

- Designed as Table 1.

In case of kelthane group, significant decrease in serum urea level was observed at the end of the $4^{th}$ and $5^{th}$ week recording values of -19.22 and -20.65% respectively.

A similar response was also observed in animals received combined treatment. The values of -23.56, -26.20, -21.74 and -20.63% were recorded at the end of the $2^{nd}$, $3^{rd}$, $4^{th}$ and $5^{th}$ week.

**b) Serum and urine amino acids:**

The data recorded in Tables 3 & 4 revealed that, exposure to fractionated doses of gamma irradiation and/or kelthane, significantly affected the concentration of serum and urine amino acids.

The results indicated that, both serum and urine of rats received combined treatment were characterized by the appearance of prominant concentration of glycine at the end of the $4^{th}$ week. The recorded levels of serum glycine were 121.50, 101.03 and 98.59 mg/dl while in urine were 124.60, 131.77 and 42.81 mg/dl for the irradiation, irradiation together with kelthane and kelthane alone respectively as compared with the control value of serum (35.68 mg/dl) and that of urine (2.77 mg/dl).

Marked increase in serum level of cysteine, α-aminobutyric acid, valine, threonine, histidine, proline, isoleucine and tryptophane was noticed in irradiated rats together with slight to moderate elevation in concentration of leucine, hydroxyproline, methionine, aspartic, ornithine, tyrosine and arginine.

Rats received combined treatment showed marked increase in the levels of α-aminobutyric acid, threonine, isoleucine, leucine, cysteine, proline, methionine, arginine, tryptophane. While moderate increase was recorded in valine, histidine, hydroxyproline, aspartic and ornithine. In case of rats exposed to kelthane only, the concentration of α-aminobutyric acid, valine, threonine and proline showed marked elevation. The total percentage change in serum amino acids level as compared with control was 147.30, 111.83 and 78.36 for irradiation, irradiation in addition to kelthane, and kelthane only respectively.

The results of urine amino acids concentration revealed that, a massive loss of amino acids was encountered. This was indicated by marked increase in urine levels of all amino acids which recorded total percentage changes of 1599, 1316 and 765 for irradiated group and those received either both treatments or kelthane alone respectively, in comparison with control levels.

**Table 3 : Qualitative and quantitative estimation of serum amino acids in different rat groups at the end of the fourth week (mg/dl).**

| Amino acids pattern | Control group | Irradiated group | Irradiation + Kelthane | Kelthane group |
|---|---|---|---|---|
| Alanine | 06.45 | 006.40 | 005.27 | 03.73 |
| α -aminobutyric acid | 00.58 | 017.43 | 005.27 | 05.36 |
| Glycine | 35.68 | 121.94 | 101.03 | 98.59 |
| Valine | 07.68 | 016.94 | 011.91 | 12.70 |
| Threonine | 03.22 | 014.58 | 014.58 | 17.97 |
| Isoleucine | 01.64 | 002.62 | 002.10 | 00.82 |
| Leucine | 01.21 | 001.90 | 003.45 | 01.11 |
| Cysteine | 01.72 | 012.81 | 010.98 | 01.83 |
| Histidine | 17.79 | 034.75 | 026.56 | 23.69 |
| Hydroxy proline | 05.02 | 008.37 | 009.62 | 06.55 |
| Proline | 01.91 | 014.19 | 007.97 | 06.25 |
| Methionine | 10.94 | 016.96 | 020.24 | 14.88 |
| Aspartic | 01.10 | 001.91 | 001.38 | 00.88 |
| Phenylalanine | 00.47 | 000.33 | 000.26 | 00.23 |
| Ornithine | 14.34 | 016.04 | 017.19 | 13.18 |
| Tyrosine | trace | 000.33 | trace | 00.33 |
| Glutamic | 00.43 | 000.43 | 000.28 | trace |
| Lysine | 00.40 | 000.37 | 000.37 | trace |
| Arginine | 12.45 | 016.86 | 022.26 | 14.20 |
| Tryptophane | 07.44 | 014.26 | 014.88 | 09.50 |
| **Total** | **129.96** | **321.39** | **275.30** | **231.80** |
| **% Change** | | **147.30** | **111.83** | **78.36** |

**Table 4 : Qualitative and quantitative estimation of urine amino acids in different rat groups at the end of the fourth week (mg/dl).**

| Amino acids pattern | Control group | Irradiated group | Irradiation + Kelthane | Kelthane group |
|---|---|---|---|---|
| Alanine | trace | 005.21 | 002.24 | 01.12 |
| α -aminobutyric acid | 1.34 | 012.50 | 005.52 | 01.00 |
| Glycine | 2.77 | 124.60 | 131.77 | 42.81 |
| Valine | trace | 008.10 | 099.26 | 42.81 |
| Threonine | 1.06 | 009.20 | 015.19 | 06.60 |
| Isoleucine | trace | 007.25 | 010.29 | 11.56 |
| Leucine | trace | 008.00 | 006.44 | 06.25 |
| Cysteine | 5.38 | 006.10 | 025.16 | 06.40 |
| Histidine | 2.63 | 107.50 | 130.00 | 42.00 |
| Hydroxy proline | 3.13 | 013.80 | 004.95 | 06.56 |
| Proline | 4.13 | 036.40 | 007.79 | 22.19 |
| Methionine | 8.37 | 051.31 | 024.06 | 57.37 |
| Aspartic | 0.67 | 014.44 | 010.25 | 11.13 |
| Phenylalanine | trace | 000.70 | 000.61 | 00.70 |
| Ornithine | trace | 001.80 | 001.15 | 02.02 |
| Tyrosine | trace | 015.50 | 005.18 | 07.25 |
| Glutamic | 0.19 | 047.96 | 007.66 | 09.30 |
| Lysine | trace | 023.13 | trace | 06.63 |
| Arginine | trace | 025.00 | 017.87 | 16.06 |
| Tryptophane | 1.95 | 013.13 | 001.50 | 08.63 |
| **Total** | **31.42** | **534** | **445** | **272** |
| **% Change** | | **1599** | **1316** | **765** |

## 4- Discussion

The present data of serum protein patterns of rats exposed to whole body gamma irradiation with or without kelthane administration revealed hypoproteinemia encountering a decrease in all serum protein fractions.

Secretion of albumin is known to be highly sensitive to the membrane damage induced by either toxic chemicals (e.g. kelthane) or exposure to ionizing radiation which causes liver injury sufficient to induce impairment of albumin synthesis [11,12]. The pathological changes in liver

structure due to whole body exposure to ionizing radiation were previously reported by many authors [13,14,15]. Excessive gostrointestinal (GI) albumin loss, in association with the loss in other serum proteins, have been demonstrated in conjunction with a number of GI disorders of diverse causes [16]. The clinical features manifested in this condition include severe hypoproteinemia, lymphocytopenia as well as reduced serum immunoglobulin level.

The present data showed that the loss of protein (proteinuria) through increased glomerular permeability (nephrotic syndrome), was due to the radiation exposure combined with kelthane administration. Polychlorinated biphenyles (PCBs) kelthane, DDA and DDT were secreted by renal proximal tubule and then reabsorded at an unspecified sites of the nephron. Since they produce alterations in cellular functions, they cause acute nephrotoxicity and induce acute renal failure which indicate that PCBs may have a direct effect on nephron function showing cloudy swelling of the convoluted tubules as observed by Mikhail et al. [17] and Koschier et al. [18].

The evaluated serum protein patterns and the levels of different protein fractions in the present study confirm the finding obtained by Roushdy et al. [19] and Ezzat et al. [20] who suggested that hypoproteinemia, hypoalbuminemia and the changes in serum proteins in irradiated rats might be due to either damage of vital biological processes or to changes in permeability of liver, kidney and other tissue cells resulting in an increase followed by leakage of proteins via the kidney.

Our findings concerning elevated levels of serum $\alpha_1$ and $\alpha_2$ globulins due to irradiation exposure which are related to acute phase protein patterns are in full agreement with the results of Aladzhov [21]. The destruction of radiosensitive tissue after exposure to lethal or sublethal radiation dose levels, might be considered as one of the most clear feature for post irradiation effect [22,23,24]. The disruption of the cellular structure was shown to cause activation and liberation of proteolytic enzymes [25]. These processes lead to autodigestion of host tissue which would induce inflammatory reactions.

The decrease in albumin and gamma globulin concentration is accompanied by a rise in $\alpha_1$ and $\alpha_2$ globulin pattern as a result of the injury that was developed after radiation exposure, which indicated a paraclinical picture of an acute inflammatory process [21].

Our present results seem to agree with those obtained by Jinping and

Liren [26] and Chlebovska and Chlebovsky [27]. They noticed that, two groups of proteins named acute phase reactive proteins $\alpha_1$ and $\alpha_2$ were increased during acute phase of the disease after exposure of dogs to gamma-rays with doses at 2.65, 3.75, 5.0 and 10 Gy.

Irradiation induced peripheral lymphocytes loss, early pathogenesis in cell ultrastructure and produced damage of different degrees in lymphatic tissue which are probably an indication of degenerative effect [28].

It has been reported that organochlorine compounds increased host susceptability to viral infection [29]. This phenomenon confirms our finding concerning the decrease in plasma gamma globulin as observed in rats at the end of the $4^{th}$ and $5^{th}$ week of the experimentation period.

Acute viral hepatitis is often accompanied by hypoalbuminemia and slight to moderate decrease of polyclonal antibodies (PG). The biochemical picture of hepatocellular injury is characterized by a reduced hepatic amino acids metabolic transformation causing accumulation of amino acids in liver and plasma and overflow amino acid urea. Moreover, hepatic capacity for the removal of toxic ammonia from blood stream is reduced, associated with low urea level. If the formation of urea is not balanced by renal retention, the plasma urea concentration declines [30].

Urine amino acids analysis of humans accidentaly exposed to different doses of gamma irradiation, was reported by Frank and Howland [31]. They stated that, patients suffered from great tissue damage, showed highest degree of amino acid urea. Furthermore, urine analysis for research workers exposed to irradiation delivered to hands and face, revealed a permanent output of glycine amounting to as much as 7210 mg/24 hrs. as compared with normal mean of 714 mg.

Moroz et al. [32], found that for increased blood amino acids concentration, the ratio of arginine to ornithine and urea to ammonia sharply dropped in animals which died later on indicating that, the urea producing function of liver was impaired and hyperammonemia was developed .

The data reported in the present study suggested that, exposure to ionizing radiation combined with organochlorine pesticide has led to histopathological changes in different body organs, specially liver, kidney and intestine resulting in impairment in their biological activities. These changes were associated with defects in biochemical pathways and subsequently impairment in blood constituents, was developed.

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