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EFFECT OF SUBLETHAL DOSE OF ETHION ON SELECTED BIOCHEMICAL PARAMETERS IN THE KIDNEY OF ALBINO RAT

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ABSTRACT

Ethion[(*O,O,O′*,*O′*-tetraethyl *S,S′*-methy bis(phosphorodithioate))] an organophosphorous (OP) insecticide was introduced seven decades ago for use on plants and animals as an insecticide, acaricide and ovicide.The aim of present study was to investigate the sub lethal effects of ethion induced alterations in protein metabolism in the kidney of Albino rat. Adult male *Albino rats* of Wistar strain were orally administered ethion ($1/5^{th}$ of LD_{50} *i.e.* 42mg/kg body weight) for 30 days with an interval of 48h. Animals were randomly divided into four groups. The first group served as control. Second group of animals was treated with ethion for 10 days, third and fourth groups of animals were administered for 20 days and 30 days respectively. Total proteins decreased in ethion administered groups while all the parameters studied in the present investigation showed an increase and this increase was more in 30 days when compared to 20 days and 10 days administered groups. All the parameters studied in the present investigation were severely altered in Ethion exposed *Albino rats*. The severity of the damage was also assessed by histopathological studies.The histological observations of the kidney in the ethion exposed *Albino rats* reveal flattened tubular cells, normal arrangement of kidney cortical tubules are disturbed, glomeruli are atrophied and are loosely attached in Bowman's capsule. Vacuoles were also found prominent with loss of glomerulus. The results of the present study suggest that Ethion adversely affects kidney functions leading to its physiological impairment. Ethion might have affected protein metabolism and detoxification system in the kidney. The present study suggests that ethion exerts its toxic effect by altering all the parameters of protein metabolism in the kidney of the albino rat.

KEY WORDS: Albino Rat, Ethion, Kidney, Protein Metabolism, Biochemical Changes, Histopathological Changes.

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INTRODUCTION

Organophosphorus (OP) compounds have been widely used for a few decades in agriculture for crop protection and pest control, thousands of these compounds have been screened and over one hundred of them have been marketed for these purposes[1]. OP pesticides are often used indiscriminately resulting in detrimental exposure to humans and other nontarget species. Currently OP compounds are the most frequently used pesticides worldwide[2]. All vital organs like liver, kidney, heart, nervous and reproductive systems are seriously impaired under OP exposure[3]. Ethion [(*O,O,O′*,*O′*-tetraethyl *S,S′*-methylene bis(phosphorodithioate))] an OP compound was introduced in 1956 for use on plants and animals as an insecticide, acaricide and ovicide. Ethion, is a major environmental contaminant in many parts of the world and poses a significant threat to environmental and public health. Among many OP compounds ethion is one of the substances that were approved for use in agricultural crops. Ethion is an insecticide that is used in a variety of forms and in several oil solutions and combinations with other chemicals. As a result, the acute toxicity values vary considerably. Ethion poisoning has been reported in workers harvesting grapes and peaches. Next to liver, kidney is the major organ which is targeted by OP compounds. Hence, in the present investigation the renal toxicity was studied under OP stress. The commonly used methods for detecting organ specific effects related to OP exposure are clinical biochemistry tests and histopathological evaluations. Hence, in the present study, we have examined various constituents and enzymes related to protein metabolism in the kidney of ethion exposed *Albino rats*. Bhatti[4] reported that *in vivo* administration of ethion results in oxidative damage to erythrocyte membranes in the rats. There are many clinical reports on impaired kidneys caused by acute organophosphorus compound poisoning, however, there is limited literature pertaining to ethion toxicity in the kidney of rats. Hence in the present investigation various parameters were evaluated in the kidney of ethion exposed *Albino rats*. The present study clearly indicates that ethion exposure seriously impairs protein metabolism in the kidney of *Albino rats*.

MATERIALS AND METHODS

Test Chemical

Ethion (92.5%) pure in crystalline form was obtained from Hyderabad chemical limited, Hyderabad, A.P. India.

Animal and Experimental Design

The protocol was approved by the Institutional Animal Ethics Committee, S.V. University (Regd. NO. 438/01c/CPCSEA). Male adult *Albino rats* of 7 weeks old and aged 200 ± 20g. was obtained from Indian Institute of Science (I.I.Sc.), Bangalore. They were housed in an ambient temperature 28 ± 2°C in a 12 h light/dark cycle and a minimum humidity of 40%. The animals had free access to commercial pellet diet supplied by Sai Durga Feeds and Foods, Bangalore, India and water *ad libitum*. All the male healthy adult male *Albino rats* were randomly divided into four groups having six rats per group. The first group animals were considered as control animals. Second group of animals was treated with ethion via oral gavage ($1/5^{th}$ of LD_{50} *i.e.* 42mg/kg body weight) for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively.

Biochemical estimations

The total protein content was estimated by the method of Lowry[5]. Free amino acid content was estimated by the method described by Colowick and Kaplan[6].Protease activity was estimated by the method of Moore and Stein[7]considering the amount of free amino acids liberated from the protein substances as a measure of proteolytic activity. The activity of aspartate aminotransferase (AST) was assayed by the method described by Bergmeyer and Bernt[8]. The activity of alanine amino transferase (ALAT) was assayed by the method described by Bergmeyer and Bernt[8]. The activity of glutamate dehydrogenase (GDH) was assayed by the method of Lee and Lardy[9]. Ammonia was estimated by the

method of Bergmeyer[10].Urea was estimated by the diacetylmonoxime method[11].

Histopathological studies

For light microscopic examination, samples of kidney from the control and Ethion administered rats were fixed in Buoin's fluid. After a routine processing, paraffin–embedded tissue samples were sectioned at 3 – 5µm thickness and stained with Mayer's haematoxylin and eosin.

Statistical treatment

The data were subjected to One way Analysis of Variance (ANOVA) and post ANOVA tests (S-N-K test) using SPSS (ver. 21) in the personal computer and $p < 0.01$ was considered as statistically significant.

RESULTS

Biochemical changes

The results are presented in the Table 1. Ethion exposed *Albino rats* showed a decrease in the protein content. All the enzymes studied in the present investigation and ammonia and urea content in the kidney of Ethion exposed rats showed an increase when compared to the control. The increase was more in 30 days exposed *Albino rats* when compared to 20 days and 10 days.

Histopathological changes

Histopathological studies of ethion exposed animals reveal flattened tubular cells, normal arrangement of kidney cortical tubules are disturbed, and glomeruli are atrophied and are loosely attached in Bowman's capsule. Vacuoles were also found prominent with loss of glomerulus.

Table 1

Biochemical and enzymatic changes in the kidney of Ethion intoxicated Albino rats

Kidney	Control	10 days	20 days	30 days	F value
Total Proteins (mg/g. wet wt. of tissue)	139.809 12.867	118.999 6.888 (-14.96)	101.783 4.795 (-27.20)	86.244 5.508 (-38.31)	45.199*
Free amino acids (µmoles of tyrosine/g. wet wt. of tissue)	62.415 4.007	69.989 4.181 (11.33)	77.429 5.082 (23.16)	83.235 5.487 (32.40)	18.527*
Protease (µmoles of tyrosine/mg protein/h)	0.726 0.061	0.816 0.042 (12.39)	0.917 0.118 (26.30)	1.146 0.081 (57.85)	21.018*
Aspartate Amino transferase (µmoles of pyruvate/mg protein/h)	0.681 0.082	0.805 0.124 (18.20)	0.864 0.085 (26.87)	0.930 0.076 (36.56)	7.008*
Alanine Amino transferase (µmoles of pyruvate/mg protein/h)	7.228 1.193	8.260 0.298 (14.28)	9.287 0.683 (28.49)	10.212 0.992 (41.28)	12.046*
Glutamate dehydrogenase (µmoles of formazon/mg protein/h)	0.515 0.031	0.555 0.030 (7.77)	0.643 0.032 (24.85)	0.768 0.038 (49.12)	58.983*
Ammonia (µmoles of ammonia/g. wet weight of tissue)	5.964[a] 0.512	6.207[a] 0.422 (4.07)	7.051 0.190 (18.23)	7.964 0.324 (33.53)	22.747*
Urea (µmoles of urea/g. wet weight of tissue)	1.617[b] 0.104	1.645[b] 0.098 (1.73)	1.983 0.142 (22.63)	2.246 0.144 (39.00)	25.792*

Values are expressed in Mean ± SD of six individual observations. Values in parenthesis indicate % change cover control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test. *P < 0.01

DISCUSSION

Exposure to sublethal doses of ethion induced typical signs of OP toxicity. All the parameters studied in the present investigation were significantly altered under OP stress. The effect of ethion was time dependent and 30 days exposed rats showed more effect when compared to 20 and 10 days exposed animals. During the last three decades, extensive use of OP compounds in agriculture has led to drastic effects on non-target animals. Majority of these chemicals are unfortunately not highly selective and therefore have been proved highly toxic to man and other desirable forms of life that co-inhabits the environment. Improper application of these pesticides, therefore, poses serious illness and death. When the membranes of any organs are damaged, majority of the enzymes like AST, ALAT and alkaline phosphatase are secreted into the blood[12]. The serum enzymes are markers of organ damage[13]. Safi[14]and Ben Amara[15] reported that OP raises ALT and AST levels in rats. This is inconsistent with the results obtained in the present investigation. Yahya[16] reported that the morphology of the various organs is damaged under OP toxicity. Proteins are the source of energy during chronic period of stress. During these stress conditions the animal requires more energy to detoxify the toxicants and to overcome stress. In the present investigation an increase in the amino acids was observed indicating that it is the result of the breakdown of protein for energy requirement and impairment of amino acid synthesis[17].It is well known that stress conditions induce elevation in the transamination pathway. Any abnormality or stress in the amino acid metabolism has its own consequences by elevating the catabolic products like ammonia and urea. This causes a serious disruption in the normal metabolism.

Pesticides and their metabolites are excreted mainly by the kidneys. Marked elevation in the free amino acid content was noticed in the kidney of ethion exposed *Albino rats*. The rapid rise in the free amino acid contents is attributed to stepped up proteolysis or increased synthesis of free amino acids by transaminase action. The increase in the free amino acid pool may also be useful to the rats to overcome stress under ethion exposure. The GDH activity in the ethion exposed *Albino rats* showed a significant increase which indicates an increased oxidation of glutamate. GDH catalyzes the key reactions which provide substrates for either protein synthesis or carbohydrate metabolism. The increased GDH activity in the present investigation might have led to an increased oxidation of glutamate with a consequent production of ammonia. This is augmented by the changes in the transaminase activity. An increase in the GDH activity may also be due to the mitochondrial permeability or lysosomal damage and since GDH is a mitochondrial enzyme, any alteration in the organization of mitochondria may lead to the alteration in the enzyme activity. AAT and ALAT activities showed a significant increase in ethion exposed rats. Transaminase activity is reported to increase during pathological conditions. In the present investigation it can be suggested that kidney has been significantly damaged under ethion exposure. Ammonia and urea are waste products of protein metabolism that need to be excreted by the kidney. Therefore, marked increase in ammonia and urea indicates functional damage to the kidney. An increase in the urea and creatinine was also observed in OP exposed animals[18].Bhatti[19] studied the hepatoxicity in ethion exposed rats. They reported that ethion toxicity leads to a significant increase in the activities of superoxide dismutase, catalase and glutathione peroxidase and glutathione reductase in the liver. They also reported that ethion causes damage to the liver tissue. In addition, the decrease in GR activity was observed in ethion administered rats compared to control

To understand the toxicological effects of pesticide it is essential to study the histology of the tissue. The extent of the severity of the damage to the kidney is a consequence of the concentration of the toxicant and is also time dependent. The severity of the damage also depends on the toxic potentiality of a particular compound or

pesticide accumulated in the tissue. Kidney is the major detoxification centre and this organ is frequently susceptible to nephrotoxic effects. In the light microscopic examinations, histopathological changes were observed in the kidney of ethion exposed *Albino rats*. The histological observations of the kidney in the ethion exposed *Albino rats* reveal flattened tubular cells, normal arrangement of kidney cortical tubules are disturbed, glomeruli are atrophied and are loosely attached in Bowman's capsule. Vacuoles were also found prominent with loss of glomerulus. The results of the present study suggest that ethion adversely affects kidney functions leading to its physiological impairment. Ethion might have affected protein metabolism and detoxification system in the kidney. The results of the present study suggest that ethion adversely affects kidney functions leading to its physiological impairment. Ethion might have affected protein metabolism and detoxification system in the kidney. The present study suggests that sublethal dose of Ethion exerts its toxic effect by altering all the parameters of protein metabolism in the kidney of *Albino rat*.

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