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Effect of Sublethal Dose of Ethion on Selected Biochemical Parameters in the Pancreas of Albino Rat

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Ethion is an organophosphorus (OP) insecticide that was specifically developed as an effective solution for controlling insects, mites, and eggs on both plants and animals. Its primary application is as an insecticide, acaricide, and ovicide. The purpose of this study was to examine the impact of ethion on protein metabolism in the pancreas of Albino rats by assessing its sublethal effects. During the course of the experiment, adult male Albino rats of the Wistar strain were given ethion orally. The dosage administered was 1/5th of the LD50 value, which equates to 42mg/kg of their body weight. The rats were subjected to this treatment for a duration of 30 days, with a 48-hour interval between each administration. The rats were randomly divided into four groups for the experiment. The first group of rats served as the control, receiving no ethion treatment. The second group of animals was treated with ethion for a duration of 10 days. The third and fourth groups of rats were administered ethion for 20 and 30 days, respectively. In groups administered with ethion,

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the levels of total proteins exhibited a decrease. However, all the parameters analyzed in this study demonstrated an overall increase. Notably, the increase observed in the 30-day administered group was more prominent compared to the groups administered for 20 and 10 days. Ethion exposure led to severe alterations in all the parameters studied in Albino rats during the present investigation. Histopathological studies were conducted to evaluate the extent of damage caused. Examination of the pancreas in Albino rats exposed to Ethion revealed various histological observations. These included Cloudy Swelling with Granulated Cytoplasm (CSGC), Denatured nucleus (DN), Hemorrhage (H), and Congestion (C). The study findings indicate that the administration of Ethion has negative consequences on pancreatic functions, resulting in impairments to its normal physiological functioning. The study suggests that Ethion's toxic impact is manifested by disrupting various aspects of protein metabolism within the pancreas of Albino rats.

Keywords: Albino rats; ethion; pancreas; protein metabolism; biochemical changes; histopathological changes.

1. INTRODUCTION

For several decades, organophosphorus (OP) compounds have found extensive application in agriculture for safeguarding crops and controlling pests. In this regard, numerous OP compounds have undergone rigorous evaluation, with more than a hundred of them subsequently being made available in the market for these specific purposes. Organophosphorus compounds, which belong to the most widely utilized group of insecticides, are favored due to their exceptional effectiveness and relatively low persistence in the environment [1]. The indiscriminate use of OP pesticides is leading to harmful exposure for humans and other non-target species. These compounds currently hold the record for being the most commonly used pesticides worldwide [2]. In a recent development, activated agricultural waste microstructure and metal-organic framework adsorbents have been utilized to successfully remove ethion and other organophosphorus insecticides from water. This groundbreaking achievement marks a significant milestone in the field of water purification. Exposure to OP (organophosphorus) compounds can have severe detrimental effects on vital organs, including the liver, kidney, heart, nervous system, and reproductive system, as documented by Ferah Sayim in 2007 [3]. Introduced in 1956, Ethion is a potent OP compound utilized as an insecticide, acaricide, and ovicide on plants and animals. However, its extensive use in agriculture has raised significant apprehension about its detrimental impact on the environment and public health. This compound has been identified as a prominent environmental contaminant found across different regions globally. Typically, Ethion is employed in oil solutions and can be blended with other chemical substances. Ethion, with its formulation and concentration, can exhibit varying levels of toxicity. It has been observed that workers engaged in the harvesting of grapes and peaches have reported instances of poisoning caused by Ethion. This compound specifically targets the pancreas, one of the vital organs in the body. Commonly practiced techniques such as clinical biochemistry tests and histopathological evaluations are utilized to identify and evaluate the precise consequences of OP exposure on various organs. Bhatti et al. [4] found that when Ethion was administered in vivo, it caused oxidative damage to the membranes of erythrocytes in rats. While there are numerous clinical reports on pancreas impairment caused by acute poisoning from organophosphorus compounds, there is limited literature available on the toxicity of Ethion specifically in the pancreas of rats. Therefore, the objective of this study is to examine the potential harmful effects of Ethion on the pancreas when subjected to organophosphate (OP) stress.

2. MATERIALS AND METHODS

2.1 Test Chemical

Crystalline Ethion (92.5% purity) was acquired from Hyderabad Chemical Limited, located in Hyderabad, Andhra Pradesh, India.

2.2 Animal and Experimental Design

Male adult Albino rats, around 7 weeks old and weighing approximately 200 ± 20g, were obtained from the Indian Institute of Science (I.I.Sc.) in Bengaluru. The rats were kept in controlled environmental conditions, with an ambient temperature of 28 ± 2°C, a 12-hour light/dark cycle, and a minimum humidity of 40%. They were provided with a commercial pellet diet

from Sai Durga Feeds and Foods, Bengaluru, India, and had unrestricted access to water. A total of four groups were formed, comprising six rats each. The first group served as the control group. The second group received ethion orally via gavage at a dosage of 1/5th of the LD50 (lethal dose for 50% of the population), which equated to 42mg/kg body weight, administered for a duration of 10 days. The third and fourth groups were subjected to the same ethion treatment but for 20 and 30 days, respectively, with a 48-hour interval between administrations. The allocation of rats to the groups was randomized.

2.3 Biochemical Estimations

The Lowry et al. [5] method was utilized to determine the total protein content. To estimate the amount of free amino acids, the Colowick and Kaplan [6] method was employed. The protease activity was assessed by measuring the released free amino acids from the protein substances, following the methodology of Moore and Stein (1954). For aspartate aminotransferase (AST) activity, the procedure outlined by Bergmeyer and Bernt [7] was used. Similarly, the activity of alanine aminotransferase (ALAT) was determined using the Bergmeyer and Bernt [7] method. The assessment of glutamate dehydrogenase (GDH) activity involved the usage of the Lee and Lardy [8] technique. Ammonia levels were estimated using the Bergmeyer [7] method. Lastly, the concentration of urea was measured using the diacetyl monoxime method as described by Natelson [9].

2.4 Histopathological Studies

Samples of pancreatic tissue from both the control and Ethion-administered rats were isolated. To prepare for light microscopic examination, the tissues were gently washed with physiological saline solution (0.9% NaCl) to eliminate any blood or debris. Subsequently, they were fixed in a 5% formalin solution for a duration of 24 hours. The fixative was then removed by rinsing the tissues with running tap water overnight. After undergoing dehydration in a series of alcohol solutions, the tissues were cleared using methyl benzoate and subsequently embedded in paraffin wax. Thin sections measuring 6µm in thickness were cut from the embedded tissues. These sections were subjected to staining using Harris hematoxylin (Harris, 1900) and counterstained with eosin, which was dissolved in 95% alcohol. Following dehydration and clearing, the sections were mounted in Canada balsam and examined under a microscope.

2.5 Statistical Treatment

The data was subjected to a One-way Analysis of Variance (ANOVA) and subsequent post-ANOVA tests. Specifically, the student- Newman-Keuls (S-N-K) test was employed using SPSS version 21 on a personal computer. A significance level of $p < 0.01$ was considered to be statistically significant.

3. RESULTS

3.1 Biochemical Changes

Table 1 presents the findings of the study on Albino rats exposed to Ethion. The results revealed a decrease in protein content in these rats. Furthermore, all the enzymes analyzed in this investigation, as well as the levels of ammonia and urea in the pancreas, displayed an increase in Ethion-exposed rats compared to the control group. Notably, the increase was more prominent in Albino rats exposed for 30 days compared to those exposed for 20 days and 10 days.

3.2 Histopathological Changes

Histopathological examinations of animals exposed to ethion revealed significant pathological changes in the pancreas. In rats administered ethion for 10 days, the pancreas displayed denatured nuclei (DN) along with cloudy swelling and granulated cytoplasm (CSGC). After 20 days of ethion administration, the pancreas exhibited Islets of Langerhans (I), acinar cells (A), glandular acini Langerhans (GAL), and cytoplasm with vacuolation (VC). Finally, in rats administered ethion for 30 days, the pancreas showed acinar cells (A), cloudy swelling with granulated cytoplasm (CSGC), denaturednuclei (DN), hemorrhage (H), congestion (C), and cytoplasm with vacuolation (VC).

Table 1. Biochemical and enzymatic alterations in the pancreas of Albino rats exposed to Ethion toxicity

Pancreas	Control	10 days	20 days	30 days	F value
Total Proteins	156.228	122.732a	109.948a	87.074	30.653*
(mg/g. wet wt. of tissue)	14.793	10.033	13.410	7.426	
		(-21.44)	(-29.62)	(-44.26)	
Free amino acids	71.118	82.361	90.020	99.989	22.766*
(µmoles of tyrosine/g. wet wt. of tissue)	5.295	5.272	7.790	9.808	
		(15.81)	(26.58)	(40.59)	
Protease	1.363	1.490	1.710	1.937	16.377*
(µmoles of tyrosine/mg protein/h)	0.125	0.053	0.140	0.215	
		(9.32)	(25.45)	(42.11)	
Aspartate Amino transferase	1.336	1.409	1.730	1.963	14.253*
(µmoles of pyruvate/mg protein/h)	0.141	0.207	0.103	0.211	
		(5.46)	(29.49)	(46.93)	
Alanine Amino transferase	7.804	8.938	9.720	11.168	18.458*
(µmoles of pyruvate/mg protein/h)	0.848	0.596	0.695	1.274	
		(14.53)	(24.55)	(43.11)	
Glutamate dehydrogenase	0.494	0.610	.668	0.778	48.932*
(µmoles of formazan/mg protein/h)	0.041	0.038	0.043	0.032	
		(23.48)	(35.22)	(57.49)	
Ammonia	6.593	7.590	8.091	9.217	8.897*
(µmoles of ammonia/g. wet weight of tissue)	0.711	0.5356	0.532	0.868	
		(9.16)	(16.37)	(32.56)	
Urea	2.948	3.522	3.810	4.379	190.304*
(µmoles of urea/g. wet weight of tissue)	0.091	0.158	0.085	0.084	
		(19.47)	(29.24)	(48.54)	

*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*

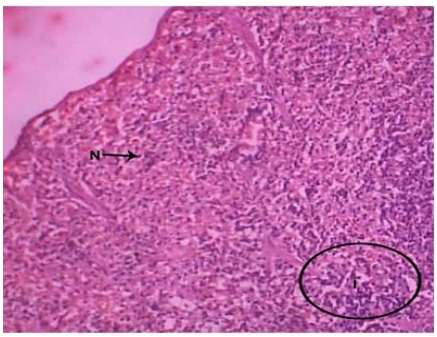

Plate 1. Control rat pancreas showing nucleus (N)

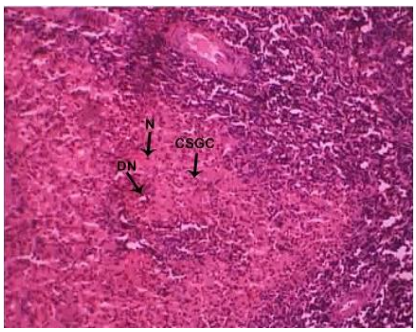

Plate 2. 10 days Ethion administered rat pancreas

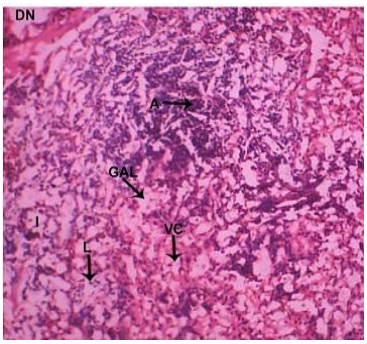

Plate 3. 20 days Ethion administered rat pancreas Showing Glandular Acini Langerhans (GAL) & histological degeneration

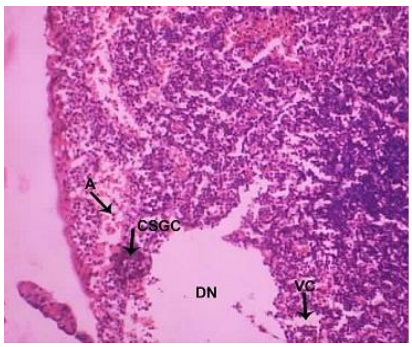

Plate 4. 30 days Ethion administered rat pancreas showing marked pathological changes Vacuolated Cytoplasm (VC)

4. DISCUSSION

Typical signs of organophosphate (OP) toxicity were observed when exposed to sublethal doses of ethion. The present study revealed significant changes in all the parameters examined under OP-induced stress. The impact of ethion varied with time, wherein rats exposed for 30 days exhibited greater effects compared to those exposed for 20 or 10 days. Over the past three decades, the widespread use of OP compounds in agriculture has resulted in severe consequences for non-target animals. Regrettably, the majority of these chemicals lack high selectivity, resulting in their proven toxicity to both humans and other beneficial life forms that share the environment. Improper application of these pesticides, therefore, poses serious illness and death. According to the study conducted by Ncibi et al. [11] when the membranes of various organs sustain damage, a significant portion of enzymes such as AST, ALT, and alkaline phosphatase are released into the bloodstream. In the study conducted by Eraslan et al. [11], it was found that serum enzymes can serve as indicators of organ damage. On the other hand, Safi et al. [12] and Ben Amara et al. [13] discovered that OP exposure led to increased levels of ALT and AST in rats. Interestingly, these findings coincide the results obtained in our current investigation. Furthermore, Yahya et al. [14] concluded that OP toxicity has detrimental effects on the morphology of various organs. During extended periods of stress, Total Proteins serve as the primary source of energy for animals. This is because stress conditions necessitate additional energy to both eliminate harmful substances and combat the effects of stress. The findings from the current study revealed an elevation in amino acid levels, suggesting that it occurs as a consequence of protein breakdown to fulfill the energy demands and a disruption in amino acid synthesis [15]. Stress conditions commonly trigger an increase in the transamination pathway, leading to elevated levels of catabolic products such as ammonia and urea. Any disruption or stress in the metabolism of amino acids can have significant consequences, resulting in a serious disturbance to normal metabolic processes.

Exposure to Ethion, a pesticide, has a significant toxic effect on the pancreas. In Albino rats exposed to Ethion, notable elevation in the levels of free amino acids was observed in the pancreas. The rapid increase in free amino acid content is believed to be caused by heightened proteolysis (breakdown of proteins) or enhanced synthesis of free amino acids through transaminase activity. This rise in the pool of free amino acids may potentially help the rats cope with the stress induced by Ethion exposure. n the Albino rats exposed to Ethion, there was a notable rise in GDH activity, suggesting heightened glutamate oxidation. GDH plays a crucial role in generating substrates for protein synthesis and carbohydrate metabolism. The observed increase in GDH activity likely contributed to elevated glutamate oxidation, leading to the generation of ammonia. These findings are further supported by alterations in transaminase activity. An elevation in GDH activity can also be attributed to mitochondrial permeability or lysosomal damage. Since GDH is an enzyme primarily located within mitochondria, any changes in mitochondrial structure may result in fluctuations in enzyme activity. There was a noteworthy elevation observed in the levels of AST and ALT activities in rats that were exposed to Ethion. This hepatotoxic affinity of ethion to the liver and its enzymes has been earlier reported by Abdel-Gawad et al. [16] who posited that ethion causes liver damage in rats. The findings of the current study indicate that exposure to Ethion has led to considerable damage to the pancreas. Pesticides can alter plasma urea, uric acid, and creatinine levels Mansour et al., [17]. Ammonia and urea, which are byproducts of protein metabolism and require elimination from the body, showed a noticeable increase. This increase serves as an indicator of impaired pancreas function. Additionally, animals exposed to OP displayed elevated levels of urea and creatinine, as observed in the study conducted by Garba et al. in [18]. In a study conducted by Bhatti et al. [19], the researchers examined the hepatoxicity caused by exposure to ethion in rats. The findings revealed that ethion toxicity resulted in a notable elevation in the enzyme activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in the pancreas. Additionally, the researchers observed that ethion induced damage to the pancreatic tissue. Moreover, a reduction in the activity of glutathione reductase was observed in the rats that were administered ethion, in comparison to the control group.

Studying the tissue histology is crucial in comprehending the toxicological impacts of pesticides. The degree of pancreatic damage is influenced by the concentration of the toxicant, as well as the duration of exposure. The severity

of the damage is further influenced by the toxic potentiality of the specific compound or pesticide present in the tissue [20]. The pancreas, being a significant exocrine and endocrine center, is commonly vulnerable to toxic effects.

Histopathological changes were observed in the pancreas of Albino rats exposed to ethion during light microscopy examinations. The histopathological studies of animals exposed to ethion revealed significant pathological alterations in the pancreas. Specifically, after 10 days of ethion administration, the pancreatic tissue showed denatured nuclei (DN) and cloudy swelling with granulated cytoplasm (CSGC). After 20 days of exposure, the pancreas displayed islets of Langerhans (I), acinar cells (A), glandular acini of Langerhans (GAL), and cytoplasm with vacuolation (VC). Lastly, after 30 days of ethion exposure, the pancreas exhibited acinar cells (A), cloudy swelling with granulated cytoplasm (CSGC), denatured nuclei (DN), hemorrhage (H), congestion (C), and cytoplasm with vacuolation (VC) [21].

5. CONCLUSION

Based on the findings of this study, it has been observed that the utilization of Ethion can have adverse consequences on the proper functioning of the pancreas, leading to physiological impairment. Ethion is thought to disturb protein metabolism and the detoxification system within the pancreas, thereby exerting toxic effects. The research proposes that Ethion-induced toxicity is evident through modifications in different aspects of protein metabolism within the pancreas of Albino rats.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee at S.V. University (Registration Number 438/01c/ CPCSEA) approved the study protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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