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Enzymatic activity of liver and kidney altered by the different dose of organophosphate pesticide Malathion in albino rats

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Abstract

Organophosphate pesticides are commonly accepted by the agriculture system in India. Malathion is one of the most usable pesticides in the organophosphate group. It is generally found in the market by the trade name of cythion. In the Indian scenario farmers and occupational workers are not aware of the toxic effects of pesticides. Pesticide after entering inside blood comes directly into the liver hence the hepatic system is the main target organ system. Our study is based on the effects of different doses of Malathion pesticide on Enzymatical parameters like blood sGOT, sGPT, alkaline phosphatase, and acid phosphatase. These four parameters indicate the behavior of the liver and kidney of albino rats. We selected four doses of Malathion as 25 mg/kg body weight, 50 mg/kg body weight, 75 mg/kg body weight, and 100 mg/kg body weight per day for 7 days and 15 days of treatment. We find that in normal condition the value of sGOT was 38 ± 2.77 mg/dl, sGPT 30.25 ± 5.09 IU/dl, Acid phosphatase was 2.99 ± 1.98 mmol / g/h, and the value of alkaline phosphatase 33.5 ± 4.98 mol/ g/h with different doses of Malathion after 7 and 15 days the value of sGOT significantly increases up to 44.75 ± 5.17 mg/dl at the higher dose. The sGOT value significantly increased up to 36.75 ± 3.80 IU/dl, the value of Acid phosphatase was increased significantly up to 6.94 ± 1.21 mmol/ g/h. and alkaline phosphatase also increased significantly 48.37 ± 4.65 mmol/ g/h. The fluctuation in parameters represents the abnormal behavior of the liver and kidney of albino rats due to the toxicity of Malathion.

Keywords: sGOT, sGPT, acid phosphatase, alkaline phosphatase, and malathion

Introduction

Today we are living on the edge of poison because all necessary things have come with toxicants. Pesticides are one of the main agents that can visibly be exposed to them. Cases of organophosphate toxicity caused by different pesticides have been reported in large numbers from different parts of the globe. Most of the organophosphate's non-degradable pesticides leave residues in various living systems for prolonged periods of the life span and are personally responsible for a variety of unknown toxic symptoms. The organochlorine pesticides found in drinking groundwater of North Indian regions and cause illness related to type -2 diabetes and other metabolic dysfunction^[1].

However, human beings, spray men, farmers, and the population, in general, are exposed to them and run the risk of their toxicity. Pesticides are exposed to farmers in various ways especially dermal and inhalation during handling in the field^[2]. The organophosphorus groups of pesticides are more toxic due to persistent irreversible cholinesterase inhibition resulting in acute illness or even death. Even when present in minute quantities their variety, toxicity, and persistence have adverse effects on ecological systems such as birds, fishes, and trees, with which human welfare is inseparably bound. Investigations have proved that rapid uses of pesticides have effects on various physiological and endocrinological functions of the body and central nervous systems of human beings. Pesticides cause adverse effects like hyperactivity disorders in adult and adolescent stages in man^[3]. Pesticides cause adverse effects on farmers in Bijnor district and cause diabetic condition^[4]. Generally in India farmers commonly used organophosphate pesticides like Malathion. Formulation of Malathion includes emulsifiable concentrate, retable power, and ultra-low volume (ULV) liquid and smoke tablets. It is most commonly used on household and leaf-eating pesticides in the form of a spray to kill cockroaches' mosquitoes and house fly etc. The aim of present study is to spread awareness related to toxicity of pesticides used in farming system.

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Materials and Methods

Pesticide Selection: Malathion is used in wide-area treatment such as public health mosquito control, fruit fly control and boll weevil erode caution programs. Malathion is also used as a head lice treatment. The renewal toxic test method was used to find out the LC50 concentration .

Experimental Animals: Sexually mature albino rats (*Rattus norvegicus*) of the controlled breed were procured from Hundred Delhi and kept under controlled conditions.

Animals usually weighing between 60-200 gm. were used for the experiments. Before commencement of the experiment, the animals were acclimatized under laboratory conditions for a week on a normal diet and water ad libitum. Properly acclimatized animals were divided into five experimental groups containing 16 rats in each group for each experiment. Out of these groups, one group was treated as a control, and the remaining four groups were treated as experimental groups for 7 days and 15 days of administration of malathion in a different dose.

Experimental groups received a definite dose of malathion in combination with olive oil; Different doses of malathion were given for different time durations.

The Malathion was administered orally in fixed doses (25 mg./kg./ body weight/day 50 mg./kg. body weight/day; 75 mg./kg. body weight/day and 100 mg./kg. body weight/day) for 7 and 15 days respectively in each group. The treated animals were sacrificed at regular intervals: i-e 8 animals of each group were sacrificed after 7 days and 15 days of treatment to study the Enzymatic changes in the blood. After the completion of one experiment, it was repeated to confirm the findings of previous experiments.

During experimentation, all possible efforts were made to keep the animals at a uniform temperature. During extreme summers and winters, the rat houses were called with the air conditioner or heated with room heaters respectively. Necessary precautions were taken to keep the animals free from any apparent disease as directed by competent authorities from CPCSEA Chennai.

Sample collection

Autopsy Schedule

Twenty-four hours after administration of the last dose, the animals were weighed and autopsied under ether anesthesia and the Parameters were assessed at autopsy.

Bio-Chemical Studies

The estimation of GOT and GPT activity using the method was done by calorimeter using standard chemistry^[5]. The estimation of alkaline and acid Phosphatase activity was done by the method^[6].

The alkaline acid substrate was prepared by dilute sodium Phenyl Phosphates, distilled water, and CHCl_3 (Chloroform). Anhydrous sodium carbonate and sodium bicarbonate were required for preparing alkaline buffer solutions. The acid buffer was prepared with crystalline citric acid, sodium hydroxide, and distilled water. CHCl_3 was used as a preservative in buffer solutions.

$$T - C \times 4 = \text{Result mol/g/h}$$

Where C = Control Value

T = Test Value

For testing of acid Phosphates activity, 1 ml substrate was

taken in the test tube. This test tube was kept in an incubator for 3 minutes, after adding 1 ml acid buffer, 0.02 ml homogenate of tissue, and 1 ml Potassium Ferricyanide were mixed in the test tube. Reading was noted using a calorimeter. The final value was calculated by the formula:-

$$T - C \times 2 = \text{Result mol/g/h}$$

Where C = Control Value

T = Test Value

(d) Glutamyl oxaloacetic transaminase activity (GOT)

Estimation of GOT was done by autoanalyzer using standard chemistry and using Kit.

$$T - C \times 3 = \text{Result IU/dl}$$

T = Test Value

C = Control Value

(e) GlutamyI Pyruvate Transaminase activity (GPT)

Estimation of GPT was done by autoanalyzer using standard chemistry through Kit.

$$T - C \times 5 = \text{Result IU/dl}$$

T = Test Value

C = Control Value

Statistical analysis: Standard Deviation

An attempt was made for a comprehensive analysis of the data collected for this research project. To assess the changes and to find out the statistical significance of the result obtained, the 'Students 't' test was used. The 't' test is the statistical test of choice for testing the significance of the difference in this type of study.

Results and Discussion

Our findings related to enzymatic tests in the blood serum of albino rats are given in Table I. The Enzymatic activity of SGOT, SGPT, acid phosphatase, and Alkaline phosphatase was affected by different doses of Malathion. The present study was undertaken to observe the alteration in serum and liver transaminases with Malathion. Average Serum transaminase (SGOT) and Serum pyruvic transaminase (SGPT) activity are moderately increased in comparison with control animals. 38 ± 2.77 to 38.5 ± 5.67 mg/dl after 7 days of treatment. These increases were moderately significant. A similar observation was found after 15 days of treatment at 38.75 ± 2.18 . Malathion treatment caused an increase in SGOT activity after administration of 50mg, 75mg, and 100 mg doses (Table-1 and Fig 1). The value of sSGOT were recorded as 39.62 ± 2.50 , 40.25 ± 5.49 and 43 ± 3.89 . Our findings were similar as noticed in rats with doses of Malathion which cause a significant increase of GOT, GPT, Acid phosphatase, and alkaline phosphatase but more than a month dose of Malathion administered in rats cause damage to liver and kidney structure^[7].

After 15 days as soon as the doses were increased than the value of sGOT also increased as 38.75 ± 2.18 , 40.12 ± 6.46 , 41.87 ± 4.48 and 44.75 ± 5.17 after 25mg, 50mg, 75mg and 100mg doses given to albino rats. The parameter of sGPT was recorded decreased slightly at 25 mg/kg b.w/day dose 30.25 ± 5.09 to 29.75 ± 5.67 . the same pattern was found after 15 days of treatment as 30.25 ± 5.09 to 29.5 ± 4.50 . The value of a parameter at 50mg, 75mg, and 100mg was recorded significantly increased as given in (Table-1 fig.2). The adverse effects on biochemical parameters like Serum

Aspartate aminotransferase (AST), Alanine Amino Transferases (ALT) Alkaline Phosphatase(AP), and oxidative stress by fipronil caused in the male albino rat [8]. The effect of carbon tetrachloride in mice and show liver injury, especially in hepatocytes [9]. The liver enzymes Aspartate

aminotrans aminase (AST), Alanine amino transaminases significantly increase after administration of Dichlorvos insecticides in Wister rats [10]. The endosulfan insecticide cause a statistical increase in hematological parameters in rat [11].

Table 1: (Alteration of Enzymatical Parameter by different dose of Malathion)

| Dose /parameter | SGOT mg/dl | | SGPT IU/dl | | Acid Phos. mol/ g/h | | Alk Phos. mol/ g/h | |
|-----------------|--------------|----------------|--------------|----------------|---------------------|----------------|--------------------|-----------------|
| | 7 days | 15 days | 7 days | 15 days | 7 days | 15 days | 7 days | 15 days |
| Normal | 38 ± 2.77 | 38 ± 2.77 | 30.25±5.09 | 30.25±5.09 | 2.99 ± 1.98 | 2.99 ± 1.98 | 33.5 ±4.98 | 33.5 ±4.98 |
| 25 mg | 38.5 ± 5.67 | 38.75 ± 2.18 | 29.75±5.67 | 29.5 ± 4.50 | 2.87 ± 1.62 | 3.44 ± 1.80 | 33.5 ± 5.45 | 34.87 ± 6.62 |
| 50 mg | 39.62 ± 2.50 | 40.12 ± 6.46 | 33.25 ± 3.80 | 33.87 ± 4.25 | 4.49 ± 1.45* | 4.13 ± 1.11 | 34.62 ± 4.24 | 38 ± 3.70** |
| 75 mg | 40.25 ± 5.49 | 41.87 ± 4.48** | 34.5 ± 5.23 | 34.25 ± 5.82 | 5.22 ± 0.95** | 6.84 ± 1.09*** | 39.62 ± 3.46** | 39.62 ± 7.24 |
| 100 mg | 43 ± 3.89** | 44.75 ± 5.17** | 33.62 ± 5.28 | 36.75 ± 3.80** | 6.72 ± 1.31*** | 6.94 ± 1.21*** | 46.87 ± 4.18*** | 48.37 ± 4.65*** |

Enzymatical parameter induced by different doses of malathion

Effect of Malathion insecticide on serum alkaline phosphatase has been carried out to know the extent of damage Malathion may cause to damage the liver. Malathion treatment caused an increase in SGOT and SGPT activity. The difference in enzymatic activity between the control and Malathion-treated group was non-significant in 25 mg/kg b.wt/day. A significant rise in GOT in the liver of rats due to the administration of Thiram [12]. A significant increase in serum GOT and GPT activity in rabbits were reported by previous after Herachlorocyclohexane administration [13]. Malathion pesticides cause an increase in alkaline phosphatase, GOT, and GPT levels in rat's liver but they suggest that vit. C is used as a cure for toxicity [14]. The exposure to Malathion may cause an increase in serum AST, ALP, and ALT activities [15]. Malathion increases the levels of various serum marker enzymes AST, ALT, and ALP in male rats [16]. Exposure to fenitrothion in rats elevated the serum ALT, AST, ALP, and bilirubin but the reduction was noticed in liver glycogen and albumin [17]. Malathion induced hepatic and nephric toxicity and vit. C plays a protective role for Malathion toxicity in rats [18].

The serum alkaline phosphate activity was not changed at the 25 mg dose after 7 and 15 days from 33.5 ± 4.98 to 33.5 ± 5.45. show the normal function of the liver. After high dose of pesticide the value increased highly significant in rats after administration of ,50mg,75mg and 100 mg doses after 7days 34.62 ± 4.24, 39.62 ± 3.46 and 46.87 ± 4.18. there was a slight increase at 25mg dose after 15 days as 33.5 ± 4.98 to 34.87 ± 6.62. The value of parameter noticed highly significant at the high dose of Malathion up to 48.37 ± 4.65 as shown in (table 1 and Fig-4). An increase in serum alkaline phosphatase activity after the administration of pesticides studied indicates that they cause liver toxicity. similar findings were previously findings that chronic exposure to Malathion causes a significant increase in Alkaline phosphatase in Oreochromis niloticus [19]. In a review of four decay that cypermethrin causes liver damage and altered enzymatic activity [20]. Artesunate cause a significant increase in alkaline phosphatase, sGOT, and sGPT in the rat [21]. The contradictory suggestion about enzymatic activity was a mild dose of commercial Malathion the transaminases and ALP biomarker enzymes decline in a time-dependent manner [22].

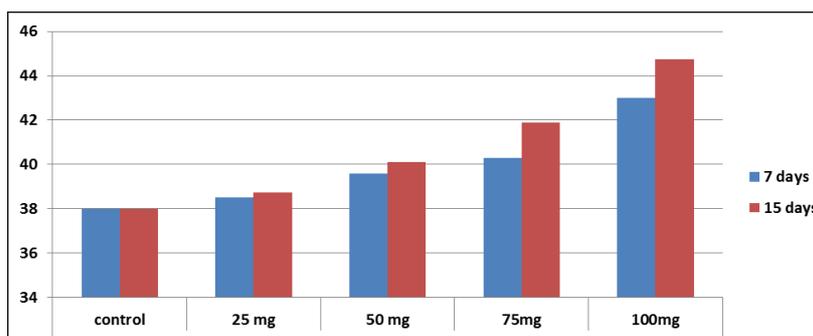


Fig 1: Different doses of malathion induced sGOT mg/dl value

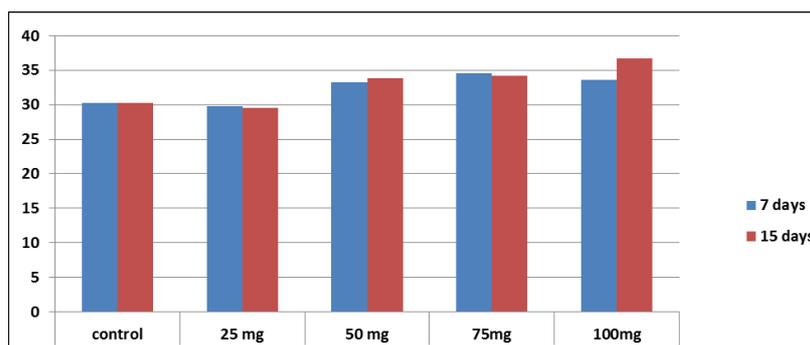


Fig 2: Different doses of malathion induced value of SGPT (IU/dl)

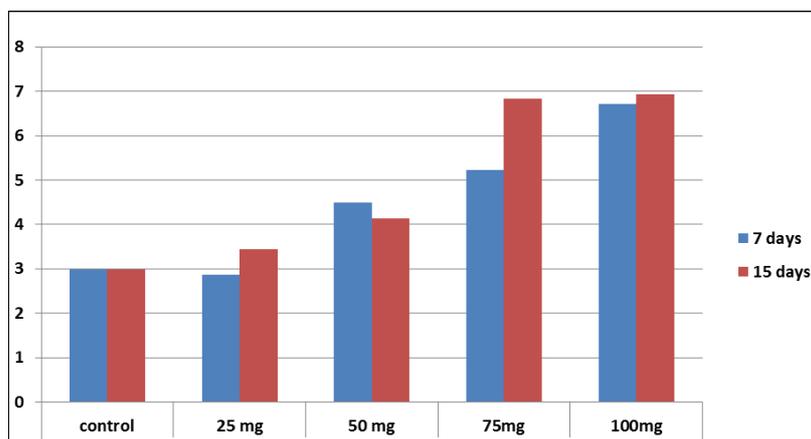


Fig 3: Different doses of malathion induced value of Acid phos.(mol/g/h)

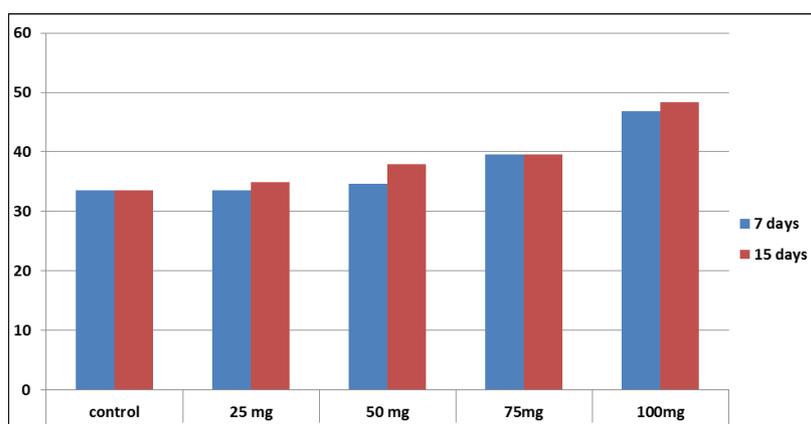


Fig 4: Different doses of malathion induced value of alkaline phos.(mol/g/h)

In the case of Acid phosphatase activity at 25 mg dose decreased moderately after 7 days from 2.99 ± 1.98 to 2.87 ± 1.62 . There was a highly significant increase was observed in high doses of Malathion i.e. at 100mg 6.72 ± 1.31 mol/g/h recorded as given in (table I and fig-3). The high dose of Malathion after 15 days shows significant increases in the value up to 6.94 ± 1.21 mol/g/h. findings were similar that organophosphorus pesticide Diptera reduces hepatic alkaline phosphatase activity in rats [23]. Similar findings were reported that pretreatment with susceptibility of mice to poisoning by Dimethoate and by Guthion [24]. These findings are contradictory to the suggestions that exposure of pesticide show decrease in the activity of the oxidative enzyme and amino acids contents [25]. Finally we say that organophosphate pesticides cause serious illness in man as supported as pesticides cause renal and hepatic dysfunction in spray man [26].

Conclusion

Our findings of enzymatically liver and kidney function test indicate that the different dose of Malathion cause damage at the cellular level so based on the complete study, it is suggested that occupational workers and spray men and farming population in India should take proper precautions during handling these chemicals. Because acute and chronic exposure to organophosphate Malathion pesticides causes serious illness in man. There is an acute requirement for awareness regarding the toxicity of chemicals.

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