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Effect of Exposure to Quinalphos at No Observed Effect Level Dose on Rat Liver

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Abstract

The present investigation was carried out to determine its toxic effects of quinalphos at low dose concentration on liver in the female albino rats. Technical quinalphos (2 mg/kg body weight) was administered orally to female albino rats for 15 and 30 days respectively. There was no significant change in weight of liver in treated and control rats of both the groups. The histologic observations of the liver revealed the presence of cytoplasmic vacuolization, sinusoidal congestion, multifoci and mono nuclear cell infiltration along with necrosis in the liver. The liver enzymes ie ALT and AST were significantly decreased in treated rats of both the groups as compared to their respective control rats. Hence quinalphos even at dose concentration of 2mg/kg body weight affects liver function.

Keywords: Quinalphos, Organophosphate, Pesticide, Female albino rats, liver, ALT, AST.

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Introduction

Agriculture in developing countries has become strongly dependent on the use of chemical substances [1]. Pesticides in agriculture are posing a serious threat to the people who are directly engaged with its handling. There is growing concern about the safety of pesticides and how their exposure, either through occupation, food residual or contamination of air and water, may affect human health in general[2]. Organophosphate compounds are among the pesticides that are widely used in agriculture and are also one of the environmental contaminants. Their application and usage have increased astronomically in the last decade and will likely increase further in future [3,4].

QP is highly toxic organophosphate to which mammals may be more vulnerable in the presence of sunlight because of its potential in generate $^1O_2$ and O_2^- [5]. Liver plays a central role in detoxification process and faces the threat of maximum exposure to xenobiotics and their metabolic by-products. The susceptibility of liver tissues to this stress due to exposure to pesticides is a function of overall balance between the degree of oxidative stress and antioxidant capability [6]. Microscopic examination of liver tissue from dams receiving high doses (3 or 4.5 mg/kg/day) of quinalphos revealed mild fatty degeneration of parenchyma. The hepatocytes were slightly vacuolated with increased sinusoidal space [7]. Intra peritoneal injection of QP (15 mg/kg b.wt) given to indian desert gerbil and hepatic cells revealed cytoplasmolysis, vacuolation and necrosis. The nuclei of the hepatocytes showed karyolysis[8].

A significant increase in serum AST (aspartate aminotransferase), ALT (alanine amino transferase) and LDH (lactate dehydogenase), ALP (alkaline phosphatase) activities was observed in dams (rats) exposed to a high dose of quinalphos (i.e. 3 or 4.5 mg/kg/day) showing significant changes in activities of serum enzymes and mild hepatic damage [7]. In our earlier studies we have found significant alterations in total proteins, lipids and cholesterol profiles in the rats after administration of quinalphos at dose level of 2 mg kg-1 body weight [9]. Hence the present study was designed to observe the effect of quinalphos on liver tissue at NOEL (2mg/kg b.wt/ day).

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Materials and Methods

Procurement and maintenance of animals

Three months old female albino rats (*Rattus norvegicus*) from Department of Livestock Production and Management, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana weighing 150-200 g were maintained under conditions of controlled temperature (75±2°F) and humidity (40-60%) with 12-hr light/dark cycle. The animals were housed in groups of three rats per cage. The rats were acclimatized for 15 days before using them for experimentation. They were provided with standard diet containing pelleted feed and water *ad libitum*. The cyclicity of rats was confirmed by examining their vaginal smears daily and the ones showing two or more regular 4-5 days estrous cycle were selected for the experiment. The rats were divided into 2 groups of 8 animals each for 15 and 30 day treatment with their respective control groups. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. This experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Exposure of quinalphos to rats

Commercial formulation of quinalphos (Ekalux) having 25% EC was used for the present study. Adequate dilutions were made with peanut oil to achieve the test concentration of 2 mg/kg b.wt/day. The test concentration of quinalphos was calculated from the percentage of active ingredient of commercial formulation of quinalphos. Rats were tube fed orally with quinalphos for two and four weeks. Simultaneously same amount of vehicle i.e. peanut oil was also administered orally to control group of rats.

Autopsy of animals and liver/ bodyweight ratio

One day after the last dose i.e. after two and four weeks, the animals were mildly anaesthetized using chloroform and were dissected. After the rats were sacrificed, liver from the control and quinalphos treated rats were cleaned off the adhering tissues and were weighed. Then the liver body weight ratio was calculated.

Enzymatic analysis

Liver enzymes were studied in plasma of control and treated rats. One day after the last dose i.e. after 15 days and 30 days, the animals were anaesthetized using chloroform and the blood was collected directly from heart in heparinized vials. Plasma was separated from blood by centrifuging the blood at 3000 rpm (rotations per minute) for 15 minutes. The plasma was used for biochemical estimation of liver enzymes i.e. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). Activities were assayed by colorimetric method of Reitman and Frankel as described by Bergmeyer[10] .

Histopathological studies

Liver tissues of dissected animals after cleaning were fixed in Bouin's fixative for 24 hours. After processing, paraffin sections were cut at 6µm thickness and stained with haematoxylin eosin for microscopic examination. Slides of control and treated groups of rats were studied under an optical light microscope. The damage to the liver tissue was detected by observing liver necrosis i.e. the absence of liver tissue in the section of slide, multi foci (presence of numerous veins), cytoplasmic vacuolization, sinusoidal congestion, mono nuclear cell infiltration etc.

Statistical analysis

Statistical analysis between experimental and control values for biomolecules were calculated according to Fishers' student *t*-test [11].

Results

Effect on liver weight

Weights of liver was determined on the day of sacrifice and expressed as g/100g body weight of rat. There was no significant difference observed in weight of liver in treated rats as compared to control rats after 15 and 30 days of treatment however there was slight decrease in the weight after 30 day treatment with quinalphos (fig 1).

Effects on Morphology of Liver

In light microscopic examinations, histopathological changes were observed in liver of treated animals of both durations as compared to their respective control groups. As shown in plate 1 (A,B), the liver sections of control female. With respect to the hepatic histoarchitecture of the quinalphos treated groups, necrosis in liver tissue (C, D), vacuolization of liver tissue, degenarion of hepatocytes, integration of nuclei (Plate II A), dilation and congestion

in central veins sinusoids (Plate II B), multi foci (Plate II C) and mononuclear cell infiltration was observed as compared to the control group rats.

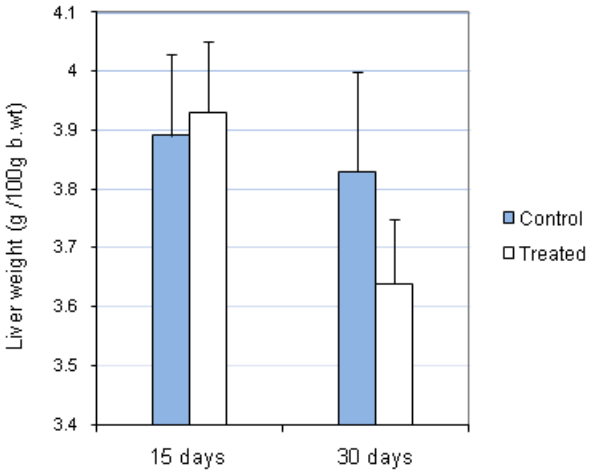


Figure 1. Effect of quinalphos at dose concentration of 2 mg/kg b.wt on weight (g/100g b.wt) of liver in treated and control rats.

PLATE – I

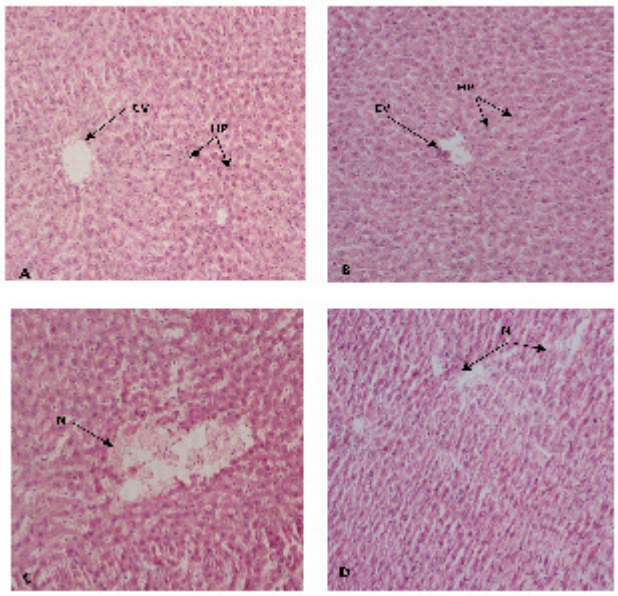


PLATE – I. **Fig. A** Portion of liver of 15 days control female albino rats, showing normal section of liver tissue with Central Vein (CV) and Hepatocytes (HP) (x20X).
Fig. B Portion of liver of 30 days control female albino rats, showing normal section of liver tissue with Central Vein (CV) and Hepatocytes (HP) (x20X).
Fig. C Section of liver of 15 days treated female albino rats, showing Necrotic Area (N) (x20X).
Fig. D Section of liver of 30 days treated female albino rats, showing increased Necrotic Area (N) (x20X).

PLATE –II

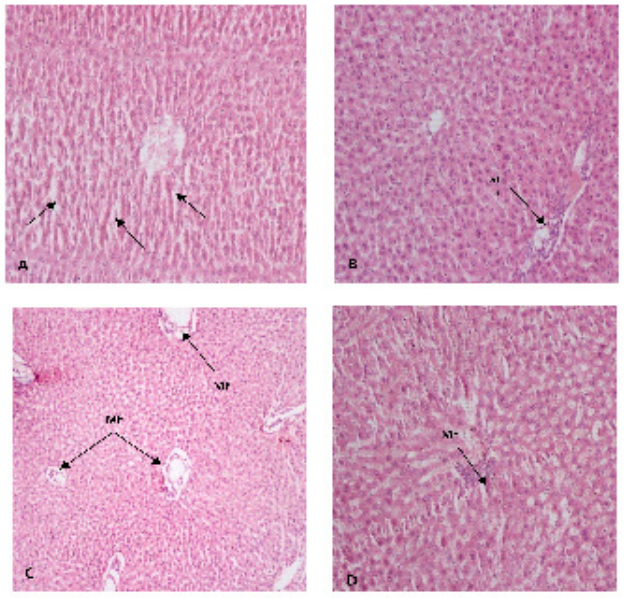


PLATE – II. **Fig. A** Section of liver of treated albino rats, showing Cytoplasmic Vacuolization ( ) of hepatocytes and integrated nuclei (x20X) after 15 days of treatment.
Fig. B Section of liver of treated albino rats, showing Sinusoidal Congestion (SC) (x20X) after 30 days of treatment.
Fig. C Section of liver of treated female albino rats, showing numerous veins (Multi Foci) (MC) (x10X) after 30 days of treatment.
Fig. D Section of liver of treated albino rats, showing Mononuclear Cell Infiltration (Mn I) (x20X) after 15 days of treatment.

Effects on Enzymes of Liver

To evaluate the pesticide toxicity at cellular level, liver enzymes (aminotransferases) were studied in plasma of rats after treatment with quinalphos. The activity of alanine aminotransferase (ALT) decreased significantly ($p< 0.01$) in rats treated with quinalphos which was 7.12±2.19 mg/100 ml of plasma at 15 days and 5.90±1.97 mg/100 ml of plasma at 30 days after treatment as compared to their levels in respective control rats in which the value was 17.61±0.40 mg/100 ml of plasma at 15 days and 17.17±0.24 mg/100 ml of plasma at 30 days after treatment. The activity of aspartate aminotransferase (AST) also decreased significantly in rats treated with quinalphos which was 42.41±5.40 mg/100 ml of plasma at 15 days and 27.92±3.64 mg/ 100 ml of plasma at 30 days as compared to their levels in respective control rats. The decrease was statistically significant ($P< 0.01$) at both 15 and 30 days of treatment (Table 1).

Table 1. Effect of quinalphos treatment on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma of control and treated rats after two and four weeks of treatment

Plasma Enzymes	After 2 Weeks		After 4 Weeks	
	Control	Treated	Control	Treated
ALT (U/.1ml of plasma)	17.61±0.11	7.12±1.70[a]	17.17±0.04	5.90±1.37[a]
AST (U/.1ml of plasma)	88.05±1.10	42.41±10.3[a]	89.94±3.14	27.92±5.38[a]

All the values are Mean ± SE values of 8 animals in each group.[a] statistically significant (P<0.01)

Discussion

In accordance with the present study Srivastava and Raizada [8] also found no decrease in liver weight up to dose concentration of 2 mg/kg/day of quinalphos. Surana et al. [12] fed male rats with quinalphos at dose concentration of 14mg/kg body weight found significant reduction in liver weight. Organophosphates like methyl parathion, dimethoate and monocrotophos given to female albino rats have also resulted in significant decrease in weights of liver of treated rats as compared to control rats [13].

Mixture of four organophosphates (chlorpyrifos, malathion, diazinonand, profenofos) at NOEL induced to male albino rats resulted in dilation and congestion of central vein sinusoids, degenation of hepatocytes, kupffer cells proliferation and inflammatory cells infiltration in between hepatocytes [14]. Histopathological changes found in liver tissue of rats treated with methidathion were infiltration with mononuclear cells in all portal areas, sinusoidal dilation, focal microvesicular steatosis and hydropic degenerations in parenchymal tissues [15]. Gibel et al. [16] reported liver damage including necrosis, steatosis and cirrhosis after oral treatment of rats with trichlorfon. However quinalphos at a dose concentration of 14 mg/kg body weight in male wistar rats produced a reversible type of liver necrosis [12].

The ALT and AST levels decreased significantly in the present study both at 15 days and 30 days exposure. The altered levels of enzyme activity of ALT represented the functional status of different vital organs and overall status of animals. Change in enzyme activity in general may be related to intensity of cellular damage [17]. The alteration of enzymes suggests that serum enzyme activities are associated with hepatic degeneration [18,19,8]. Transaminases are the metabolic enzymes which collect the amino groups from many different amino acids in the form of only one C-glutamate. The glutamate channels amino groups either into biosynthetic pathways or onto a final sequence of reactions by which nitrogenous waste products are formed and then excreted [13]. Lu and Li [20] reported a decrease in ALT by concurrent administration of aflatoxin and bicyclol. Diminished activity of ALT has been found in the brain, kidney and testis of rats exposed to low doses of cypermethrin [17]. A decrease in lever of ALT and AST was observed by Hussain *et al* [21] when rats exposed to aflatoxin and cypermethrin. Srivastava and Raizada [8] observed no change in these enzymes at lower doses of quinalphos (0.5, 1.5, 2 mg/kg/day) treated rats. Ambali et al. [22] also had observed a significant decrease in the values of ALT and AST, when rats were treated with chlorpyrifos at dose concentration of 23.1 mg/kg b.wt. In our present study the decline in level of AST/ALT may also be attributed to the heat stress during the period of exposure.

Conclusions

The present study has revealed altered liver functioning as evidenced by changes in the structure of hepatocytes and decreased levels of AST and ALT at No Observed Effect Level (NOEL) as compared to control animals. The long term implications of quinalphos exposure even at low doses need to be worked out further.

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Conflict of Interest

None Declared

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