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ORIGINAL RESEARCH ARTICLE

Hepatotoxic Potentials of Profenofos on Wistar Albino Rats: A Histopathological Study

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ABSTRACT

Profenofos is a most common toxic organophosphorous insecticide extensively used in agriculture for the control of pests. The present study was carried out to evaluate histopathological effects of profenofos on the liver of wistar albino rats, upon its oral administration. The observations suggested the disparaging effects of profenofos at a sublethal concentration of $1/10^{th}$ of LD_{50} (330mg/Kg body Wt.). The findings recorded were, blood congestion in central vein, damaged blood sinusoids, degenerated hepatocytes, periportal inflammation, pyknotic nuclei and binucleation of cells for durations of 1 day and 15 days. However, a critical feature of focal necrosis was observed at 30 days of exposure indicating the high degree of damage due to profenofos intoxication. The recovery studies in the rats exposed to profenofos for 30 days indicate the positive probabilities of recovering ability at 30 and 60 day. The observations from the present study, it may be quoted that profenofos possesses hepatotoxic potentials against the liver of wistar albino rats upon its oral administration. Further, it can be even said that the sublethal concentration of $1/10^{th}$ of LD_{50} has the prevalence of its impact on liver tissue for up to 60 days of post recovery when the rats were exposed to the profenofos for a period of 30 days.

Key words: Hepatotoxicity, Histopathology, Profenofos, Recovery studies, Rat liver and Sublethal studies.

INTRODUCTION

Pesticides are one of the most harmful chemicals which have posed serious threat to animal's health worldwide (Chantelli-Forti *et al.*, 1993, Chaudhuri *et al.*, 1999, El-Shenawy *et al.*, 2009., Magar and Bais 2013). Based on the report of Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are pesticides (Stockholm Convention 2004). Application of pesticides in farms and organic insecticide poisoning remains one of the major health issues in both developing and developed countries (Peter and Cherian 2000). Organophosphorus (OP's) pesticides are one of the most importantly used pesticides in the field of agriculture and household for the control of insect pests (Sajjad *et al.*, 2009). They are the major source of environmental poisoning (WHO; 2003). Several investigators have suggested the possible catastrophic effects of OP's on animal's overall physiology (Abdou and ElMazoudy, 2010; Shah and Iqbal, 2010). It has also been reported that contact with OP's insecticides can result in health problems in agricultural workers (Hurtig *et al.*, 2003). This is because organophosphates are well reabsorbed after uptake via the oral, dermal or inhalation route (Fisher *et al.*, 1985; Feldmann and Maibach, 1974). Since there is a haphazard use of different pesticides in agriculture, it has resulted in inevitable demand for concern in developing countries to counter the problem of pesticide pollution (Santhakumar and Balaji, 2000). Profenofos is a broad spectrum organophosphorus which is one of the most extensively used pesticide for protecting agricultural crops from pests (Amer *et al.*, 2000). It is classified as a moderately hazardous (Class-II) insecticide from World Health Organization (Prabhavathy *et al.*, 2006). It is important to the study the impact of profenofos as the insecticide has a limited literature in terms of exposure and recovery studies histologically. Liver is an important organ which is capable of detoxifying OP's. (Kappers *et al.*, 2001; Subash Vijaya kumar *et al.*, 2010). However, the range to toxicity relies

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upon the concentration of dose and duration of exposure (Frank and Sielkenzr, 1991). Histology is an important tool for determining the damage caused by pesticidal intoxication. Thus in the present study, an attempt is made by employing a histological approach to understand the tissue level damage caused by profenofos in the liver of wistar albino rats upon its oral administration. Further, the present study is also concerned with the studies on recovering ability of the liver upon post exposure period as the literature on this kind of study is very limited.

MATERIALS AND METHODS

Profenofos [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] with 50% of emulsified concentration was procured from the local market under the trade name Curacron. The necessary concentrations were made just prior to the exposure studies. Freshly prepared profenofos was administered orally. The median lethal dose (LD_{50}) of profenofos was determined according to Weil (1952) and its value was obtained to be 330 mg/kg body weight.

Experimental Animals:

Adult Wistar albino rats, each weighing about 170-180 g was used in the present study. The rats were allowed to acclimate under laboratory conditions for two weeks prior to initiation of the sublethal studies. All the animals were housed in polypropylene cages, fed on standard laboratory ration, watered ad-libitum and exposed to a 12 h light/dark cycle (12h: D and 12h: N), and maintained at a laboratory temperature of 25 ± 2º C. The animals were handled in accordance with the standard guidelines for the care and use of laboratory animals.

Dose fixation:

A sublethal concentration of $1/10^{th}$ was fixed on the basis of LD_{50} value (330 mg/kg of body weight).

Experimental Groups:

In the present study, the rats were divided in to two major groups namely, Group 'A' and Group 'B'. Here Group 'A' was maintained as control. Further, on the basis of the rats to be treated and those which were to be allowed for recovery were subdivided in to two groups namely (Group B1 and Group B2). The Group B1 was for exposure studies and Group B2 was for post exposure studies.

Exposure periods:

The duration of exposure periods chosen for the present study were, 1, 15 and 30 days, and duration for post exposure periods were of 30 (30 day recovery) and 60 days (60 day recovery).

Experimental protocol:

The rats were anesthetized by chloroform and sacrificed by performing cervical dislocation. Later the organs were immediately removed and kept in different vials containing Bouin's fluid for fixation.

Histopathological analysis:

For the histopathological examination, the liver was dissected. The sample was isolated immediately and was fixed in Bouin's fluid for 24 to 48 h. The tissue was processed in a series of graded alcohol and embedded in paraffin which was being filtered thrice earlier. The organs in paraffin sectioned into 5 µm thick ribbons by using semi-automated microtome (LeicaRM 2255) and sections were stained primarily with haematoxylin and counter stained with eosin (H & E) for light microscopic examination (Lille, 1969). The sections were observed under 200X and 400X magnifications respectively. The microscopic view was photographed by using an Olympus phase contrast microscope (Olympus BX51, Tokyo, Japan) with attached photography machinery (ProgResC3, Jenoptic-Germany). The photographed images were further observed for differences and the findings were recorded.

RESULTS

Control group or Group A

The sections of liver of control rats showed normal structures of central vein and blood sinusoids. Further, the number, size, shape and overall histo-architecture of hepatocytes and kupffer cells were found to be normal. The cytoplasmic distribution was even. The hepatocytes showed prominent nucleus with darkly stained nucleolus. The bile ducts were found perfectly intact and normal (**Fig A & B**).

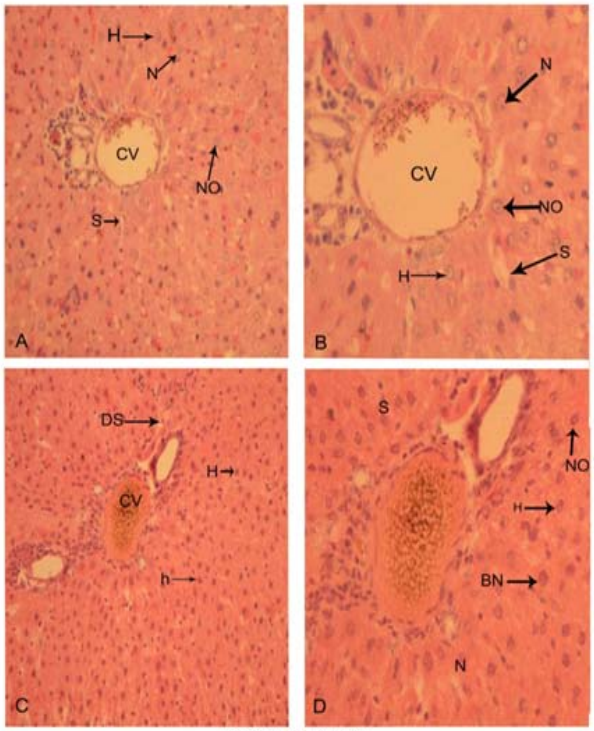
Results for exposure studies (Group B1):

The section of rat liver when exposed to a dose of $1/10^{th}$ of profenofos, the changes for Day 1 were, congestion of blood in central vein, hypertrophy of nucleus, mild dilation of blood sinusoids, slight changes in structural conformation of hepatocytes and binucleated cells (**Fig C & D**). For Day 15 the changes noted were, congestion and dilation of blood in central vein, damaged blood sinusoids, periportal inflammation consisting of collection of

lymphocytes around the bile duct (Fig: E and F). The results for 30 days of exposure showed congestion of blood in central vein, severely damaged blood sinusoids, periportal inflammation with collection of lymphocytes around the bile duct and focal necrosis. Here focal necrosis was observed as a prominent feature at the exposure duration of 30 days (**Fig G & H**).

Results for post exposure studies (Group B2):

In the section of liver of rat which were exposed to 1/10th of sublethal concentration of profenofos and further underwent a recovery period of 30 days, the findings recorded were; partial development of central vein, slight recovery in histo-architecture of hepatocytes indicating their tendency for restoration were observed, but, the damaged sinusoids were only slightly regenerated indicating the absence of complete recovery (**Fig I & J**). On 60th day recovery, the findings showed the regenerated central vein, most of the hepatocytes underwent restoration of their cellular structure but blood sinusoids however showed lack of complete recovery (**Fig K & L**).



Control Fig. (A & B)
Central vein (CV), in the center of the hepatic lobule which is intern surrounded by number of hepatocytes (H), sinusoids (S) are normal in their architecture. Hepatocytes show prominent nucleus (N) with nuclear membrane and nucleolus (NO)

Fig. (C & D)
Congestions of blood in central vein (CV), hypertrophy (h), mild dilation of blood sinusoids (S), mild changes in structural confirmation of hepatocytes (H), binucleated cells (BN)

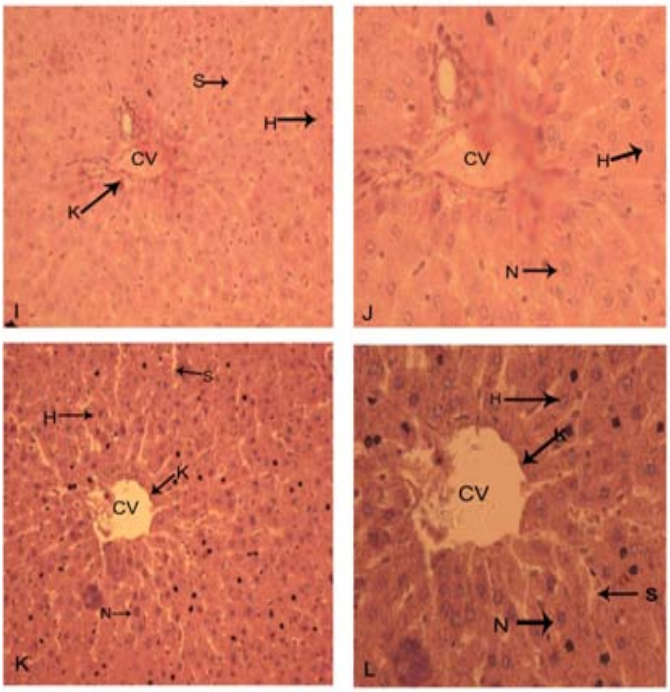


Fig. I & J
Partial Development of Central (CV) With Mild Congestion of of blood. Mild Regeneration of hepatic Sinusoids(S) and Hepatocytes (H)With nucleus(N)and Kupffer Cells(K)

Fig.K & L
Regenerated Central Vein(CV) With Minimum Congestion of blood ,Prominent nucleus (N), slightly developed sinusoids (S), Proper architecture of hepatocytes (H) and Kupffer cells (K) are Observed.

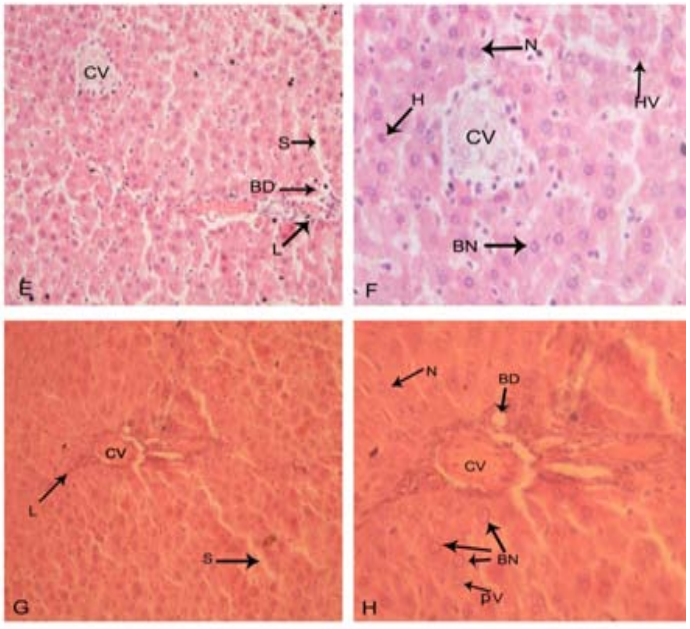


Fig. E & F
Congestion and dilation of blood in central vein (CV) damaged blood sinusoids (S), periphery of portal inflammation consisting of collection of lymphocytes (L) around the bile duct (BD), hypertrophy of Nuclei (H), Vacuolation of hepatocytes(HV),Mild degeneration of nucleus (N), Binucleated nucleus (BN).(H&E)

Fig G & H
Congestion of blood in central vein (CV), severely damaged sinusoids(S), Periphery portal inflammation with Collection of Lymphocytes (L) around the the bile duct (BD) pyknotic nuclei (pv) visible, binucleated nucleus(BN) cell necrosis is also observed (N)

DISCUSSION

Liver is one of the most important organs which play a crucial role in the mechanism of detoxification and elimination of toxic substances from the body. Liver is extensively involved in breakdown of toxic substances and aid in anabolism of complex molecules from simple substances absorbed from the gastro-intestinal tract. It neutralizes toxins and manufactures bile which aids fat digestion and removes toxins through the bowels (Buraimoh *et al*., 2011). Continuous exposure and intoxication of liver to different types of exogenous compounds on a daily basis may lead to hepatic dysfunction (Nithya *et al*., 2012). The results from the present study, suggest that the histopathological changes were caused in liver of rats upon the oral administration of profenofos. In the present study, histopathological methods were employed to evaluate the toxicity of profenofos on wistar albino rats. The important histopathological findings observed in the rat liver exposed to $1/10^{th}$ sublethal dose of profenofos were mild congestion and dilatation of central vein, vacuolation of hepatocytes, hepertrophy of hepatocytic nucleus and necrosis in few number of hepatocytes. However, there were no altercations visible in sections of control rats. The findings like blood congestion in central vein coincide with the studies of Luty *et al*., (2003); Aldana *et al*., (2001), Mani *et al*., 2004; Manna *et al*., 2004, Nashwa *et al*., (2012) have reported the abnormalities in the section of rat liver exposed to different types of organophosphorous insecticides. A short duration of exposure of 1 day showed minimum symptoms indicating less amount of damage to the liver tissue. In the present investigation, the liver blood vessels became dilated under the effect of profenofos which is in agreement with the studies reported by El-Shenawy *et al*., (2007). Farrag and Shalby (2007). The other findings like hypertrophy of nuclei and binucleation of hepatocytes in addition to the above mentioned findings match with the studies of Sadaf *et al*., 2009 and Abdel *et al*., 2012 who reported the pesticidal impact on the liver of Wistar albino rats. Our study showed the signs of damaged blood sinusoids, lymphocytic infiltrations, vacuolization, degeneration of hepatocytes and their binucleation. This confirms the results reported by El Elaimy *et al*., (1995) and Abdel Hadi & Abedin (1997).

The necrosis in liver of profenofos exposed rats derives support from the previous observations of Sata *et al*., (2004). The reduction of blood flow in the liver causes hypoxia of hepatocytes, and eventually induces their necrosis. This suggests that impact of pesticide might have caused the reduction in blood flow thereby resulting in hepatic necrosis or ischemic reperfusion. Hypertrophy of nuclei in addition to present study was also noticed by Mostafa *et al*., (2009) after chloroform administration to rats. It is to be noted that the signs of damage increased with the increase in duration is in agreement with the studies reported by Abdel Hadi and Abedin (1997). These findings however, reduced when the rats were allowed for the recovery period of 30 and 60 days suggesting the possible recovering tendency of the rats with respect to their liver organ.

The results obtained suggested that the intensities of these changes were dependent on the dose and its respective durations. This is in agreement with Abdel Hadi and Abedin (1997). The liver of profenofos exposed rats underwent cytoplasmic vacuolation and degeneration. These results are in agreement with the findings reported in mammals by Sata *et al*., (2004), Latuszynska, (1999); Hatipoglu *et al*., (2008) after they were exposed to different toxic compounds. Filtration and sinusoidal blood congestion after treatment with profenofos were evidence for liver damage. Based on the above observations, it can be said that the liver of rats exposed to profenofos for a sublethal concentration $1/10^{th}$ of LD_{50} suffered from serious deleterious effects at all the three durations of exposure studies in terms of histopathological levels and when the same were allowed to recover after a duration of 60 day recovery, certain findings were still persistent indicating the rats required further more duration for their complete recovery. This kind of work is being presented for the first time to the best of our knowledge, hence underlining the genuine importance of our work.

CONCLUSION

The overall findings in the present study showed the potential threat and possibility of histopathological alterations in liver of rats upon the oral administration of an organophosphorous insecticide, profenofos. From the observations, it is evident that the profenofos is potentially a harmful substance which can cause serious damages to the liver of the rats suggesting the compromising the overall functioning of liver and hence its toxicity towards mammalian proximity. It is therefore suggested that the care is to be taken during its use and disposal in order to avoid its

impact on non target organisms at mammalian levels including human beings.

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