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Evaluation of histopathological changes on acute exposure of profenofos in Swiss albino mice

SONU DEVI¹, VINOD KUMAR¹, PREETI BAGRI^{1*} and DEEPIKA LATHER²

¹Department of Veterinary Pharmacology and Toxicology, ²Department of Veterinary Pathology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004 (Haryana)

*Corresponding author's email id: preetibagri@luvas.edu.in

ABSTRACT: The acute toxic effects of profenofos were studied at a single dose of maximum tolerated dose (90 mg/kg bw) at 1 h 40 min (peak effect), 24 h and 14 days post-treatment in three treatment groups along with their respective control groups. There was no significant change in body weight and relative body weight gain in 1 h 40 min, 24 h and 14 days post-treatment groups as compared to control groups. A significant increase in relative organ weight of liver and right testis in 14 days post-treatment group was observed as compared to control group. Significant increase in relative organ weights of left testis and right epididymis was observed in 1 h 40 min post-treatment group as compared to control group. Histopathological lesions were observed in liver, kidney, brain and testes, suggesting its acute toxicity to these organs. No histopathological lesions were observed in bone marrow during acute toxicity study.

Key words: Histopathology, MTD, organ weight, profenofos, relative organ weight

Pesticides are the chemical substances frequently used for preventing, destroying, repelling or mitigating any pest ranging from insects to microorganisms (Alavanja, 2009). Pesticides are ubiquitous contaminants of the environment and have been found in samples from air, soil, water, and human and animal tissues all over the world. These cover a wide range of compounds used in pest control, such as fungicides, herbicides, molluscicides, insecticides, rodenticides and others (Bakry *et al.*, 2011; Nasri *et al.*, 2012).

Most commonly used agricultural insecticides include organochlorines, organophosphates (OP), carbamates, pyrethroids and neonicotinoids (Maienfisch, 2006). Among these, organophosphate compounds are the most toxic substances, used as broad-spectrum insecticides to control various pests and also act as chemical warfare agents. They are biodegradable, induce slow pest resistance and have lower persistence in the environment so used judiciously (Balali-Mood and Saber, 2012).

Profenofos [(O-4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is a widely used OP insecticide to kill pests that damage crops and thus, affect overall agricultural productivity. Globally increasing population and reducing arable land have resulted in higher demand for food crops resulting

in an urgent need to increase productivity. This factor is expected to drive the demand for profenofos (Das *et al.*, 2006).

In the United States, it is used exclusively on cotton and is primarily used against lepidopteran insects. The United States Environmental Protection Agency report identified profenofos as toxic to birds, small mammals, bees, fish, and aquatic invertebrates, noting several fish kill incidents in which profenofos exposure, primarily due to runoff, was implicated as a probable cause (USEPA, 2015).

In spite of the significant toxicity, profenofos is widely used all over the world and systematic toxicity studies are lacking. Hence, it is imperative to evaluate the profenofos induced acute toxicity in Swiss albino mice.

MATERIALS AND METHODS

Experimental animals

Healthy male and female Swiss albino mice weighing between 20-24 g were purchased from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana. In this study, 50 male mice were used for different parameters. Mice were housed in polypropylene cages in groups

of six per cage with free access to water and freshly prepared feed. They were acclimatized for a period of one week in the animal house of the department before the start of the experiment. The bedding material (rice husk) of cages was changed on alternate days. The temperature of animal house of the department was maintained between 22°C to 27°C throughout the experiment. Before the sacrifice, the animals were not fed for 12 h but water was provided *ad lib*. The present study was carried out after approval from the Institutional Animal Ethics Committee (IAEC) (Approval No. VCC/IAEC/630-51 dated 25-03-2021).

Chemicals and solutions used

The formulation product of organophosphate (OP) insecticide profenofos, Curacron® was purchased from Syngenta India Ltd. Absolute alcohol and hematoxylin and eosin (H&E) were procured from Sigma Ltd. Paraffin wax and 37% formaldehyde were procured from Qualigens Pharma Pvt. Ltd.

Experimental design and animal treatment schedule

For acute toxicity study, the animals were randomly divided into three treatment groups five animals each and their respective control groups (n=5) were also maintained. Single dose of profenofos at the rate of 90 mg/kg b.wt. maximum tolerated dose (MTD) was administered orally to each group and distill water was administered to their respective control groups (Devi *et al.*, 2022). Peak effect was determined by McDaniel and Moser, 1993. Animals were critically observed and mice of group I were sacrificed at 1 h 40 min (peak effect), group II animals were sacrificed at 24 h and group III were sacrificed after 14 days of treatment along with their respective control groups and various organs *viz* liver, kidney, brain and testes, were collected to study various acute toxicity parameters.

Body weight and Relative body weight gain

The body weight of various groups of animals was recorded before and after 1 h 40 min (peak effect), 24 h and on alternate days for 14 days after single MTD administration of profenofos. Relative body weight gain of each male mouse was determined on

alternate day for 14 days of treatment period by the following formula and was expressed as g/100 g b.wt.

$$\text{Relative body weight gain} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

Pathomorphological studies

Treated male mice were sacrificed under thiopentone anaesthesia and necropsy was performed. Blood was collected through cardiac puncture in heparinized test tubes. Vital organs were examined for gross toxicopathological changes, if any. Heart, liver, kidneys, spleen, brain, testes and epididymis were excised and weighed individually. Small pieces of liver, testes, brain, kidney and intact femur bone of mice from all groups were fixed in 10% buffered formalin for histopathological evaluation.

Relative organ weight

The relative organ weights of heart, liver, brain, kidneys, spleen, testes and epididymis were expressed as g/100 g body weight of male mice (Preeti *et al.*, 2014).

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

Histopathological examination

At the time of sacrifice, small pieces of liver, testes, brain, kidneys and intact femur bone of mice from all the groups were fixed in 10% buffered formalin, then dehydrated in ascending alcohol grades, cleared in benzene and embedded in paraffin wax. Approximately 5 µm thick sections were prepared and stained with hematoxylin and eosin (H&E) for assessment of patho-morphological changes under light microscopy (200X) (Luna, 1968).

RESULTS AND DISCUSSION

Effect of single oral administration of MTD in mice on body weight and relative body weight gain

The effects of single dose of MTD of profenofos on the body weight, relative body weight gain at 1 h 40 min, 24 h and 14 days post-treatment are presented in Tables 1, 2 and 3. There was no significant change in body weight and relative body weight gain at 1 h 40 min, 24 h and 14 days post-treatment.

Table 1: Effect of oral exposure of mice to single MTD (90 mg/kg b.wt.) of profenofos on the body weight (g) at 1 h 40 min and 24 h post-treatment

Treatment (p.o.)	Body weight (g)		Body weight (g)	
	0 th h	1 h 40 min	0 th h	24 h
Control (1 ml DW/100 g)	29.00 ± 0.85	29.00 ± 0.85	27.60 ± 1.60	27.10 ± 1.77
Profenofos(90 mg/kg b.wt.)	28.50 ± 1.39	28.50 ± 1.39	27.20 ± 2.01	24.30 ± 0.87

Data are presented as mean ± SEM (n= 5 mice/group). The values were compared using unpaired Student's t-test.

Table: 2 Effect of oral exposure of mice to single MTD (90 mg/kg b.wt.) of profenofos on the body weight (g) during 14 days post-treatment

Treatment (p.o.)	Body weight (g)							
	0 th	2 nd	4 th	6 th	8 th	10 th	12 th	14 th
Control (1ml DW/100g)	27.50±1.53	28.60±1.21	27.90±1.32	28.00±1.07	28.60±1.05	28.40±1.19	29.20±1.22	28.90±1.25
Profenofos(90 mg/kg b.wt.)	27.80±0.64	25.50±2.10	26.10±2.24	26.20±2.36	26.30±2.30	26.60±2.15	26.30±2.13	27.60±1.84

Data are presented as mean ± SEM (n= 5 mice/group). The values were compared using unpaired Student's t-test.

Table 3: Effect of oral exposure of mice to single MTD (90 mg/kg b.wt.) of profenofos on relative body weight gain (g/100 g b.wt.)

Treatment (p.o.)	Relative body weight gain (g/100 g b.wt.)						
	2 nd	4 th	6 th	8 th	10 th	12 th	14 th
Control(1 ml DW/100g)	4.37±1.80	1.69±1.32	2.26±2.06	4.50±2.45	3.62±1.65	6.56±1.95	5.48±2.47
Profenofos(90 mg/kg b.wt.)	-8.40±6.63	-6.20±7.37	-5.85±7.77	-5.44±7.74	-4.31±7.35	-5.41±7.24	-0.65±6.50

Data are presented as mean±SEM (n= 5 mice/group). The values were compared using unpaired Student's t-test.

Table 4: Effect of oral exposure of mice to single MTD (90 mg/kg b.wt.) of profenofos on relative organ weight (g/100g b.wt.) of mice at 1 h 40 min, 24 h and 14 days post-treatment

Relative organ weight gain (g/100g b.wt.)	Treatment					
	1 h 40 min		24 h		14 days	
	Control (1 ml DW/ 100 g)	Profenofos (90 mg/kg b.wt.)	Control (1 ml DW/ 100 g)	Profenofos (90 mg/kg b.wt.)	Control (1 ml DW/ 100 g)	Profenofos (90 mg/kg b.wt.)
Heart	0.42±0.02	0.46±0.03	0.40±0.02	0.45±0.01	0.46±0.02	0.44±0.01
Liver	4.5 ±0.20	4.87±0.31	4.87±0.31	4.99±0.09	4.33±0.23	5.18*±0.19
Left kidney	0.76±0.03	0.72±0.07	0.63±0.03	0.67±0.04	0.75±0.05	0.63±0.02
Right kidney	0.82±0.06	0.71±0.06	0.61±0.02	0.69±0.06	0.72±0.04	0.67±0.03
Spleen	0.44±0.06	0.54±0.07	0.31±0.04	0.33±0.05	0.43±0.07	0.34±0.04
Left testis	0.36±0.01	0.40*±0.01	0.36±0.02	0.36±0.02	0.32±0.01	0.40±0.04
Right testis	0.39±0.01	0.40±0.01	0.34±0.02	0.38±0.03	0.32±0.02	0.38* ±0.01
Left epididymis	0.14±0.01	0.14±0.01	0.12±0.01	0.13±0.00	0.12±0.01	0.13±0.01
Right epididymis	0.14±0.00	0.15*±0.00	0.13±0.01	0.13±0.01	0.11±0.01	0.13±0.00
Brain	1.46±0.04	1.43±0.09	1.24±0.13	1.43±0.07	1.43±0.07	1.50±0.08

Data are presented as mean±SEM (n= 5 mice/group). The values were compared using unpaired Student's t-test. *P≤0.05 in comparison to control

Effect of single oral exposure of mice to MTD (90 mg/kg b.wt.) of profenofos on necropsy findings

The gross examination of heart, liver, kidneys, testes, epididymis and brain were done in acute toxicity study and no pathognomonic lesions were observed in these organs.

Effect of oral exposure of mice to single MTD (90 mg/kg b.wt.) of profenofos on relative organ weight

Effect of oral exposure to single MTD (90 mg/kg b.wt.) of profenofos on relative organ weight of mice at 1 h 40 min, 24 h and 14 days post-treatment are presented in Tables 4. A significant increase in

relative organ weight of liver and right testis in 14 days post-treatment group was observed as compared to respective control group. Significant increase in relative organ weights of left testis and right epididymis was observed in 1 h 40 min post-treatment group as compared to respective control group. No significant difference in relative organ weight was observed in rest of treatment groups.

Histopathological studies of liver, kidney, brain, bone marrow and testes of mice exposed to single dose of MTD (90 mg/kg b.wt.) of profenofos orally
Liver

Histopathological lesions in liver of control and MTD group of profenofos at a single dose of 90 mg/kg b.wt. at 1 h 40 min, 24 h and 14 days post-treatment time intervals are presented in Fig. 1. Control group mice revealed normal histological structure of liver. Histopathological investigations of profenofos treated group at 1 h 40 min revealed congested portal veins, focal areas of necrotic hepatocytes characterised by pyknotic nuclei and infiltration of leucocytes. Liver in 24 h post-treatment group showed diffuse congestion, cellular swelling and vacuolar degenerative changes in hepatocytes. Group of 14 days post-treatment showed moderately congested sinusoids, haemorrhages, disruption of hepatocytic chords because of necrosis, perivascular oedema and dilated portal vein.

Kidney

Histopathological lesions in kidney of control and

MTD group of profenofos at a single dose of 90 mg/kg b.wt. at 1 h 40 min, 24 h and 14 days post-treatment time intervals are presented in Fig. 2. Control group mice revealed normal histological structure of kidney parenchyma. Histopathological investigations of 1 h 40 min post-treatment group mice revealed glomerular congestion, proteinaceous material in bowman's space, focal interstitial nephritis, hyaline deposition in lumen of tubules indicating mild inflammation and hyperplasia of parietal epithelial cells lining the bowman's capsule. Group of 24 h post-treatment also revealed glomerular congestion, intertubular and glomerular haemorrhages, cellular swelling and vacuolar degenerative changes in few epithelial cells. Group of 14 days post-treatment revealed glomerular congestion, hyaline casts in tubules, perivascular infiltration of leucocytes and focal interstitial nephritis.

Brain

Histopathological lesions in brain of control and MTD group of profenofos at a single dose of 90 mg/kg b.wt. at 1 h 40 min, 24 h and 14 days post-treatment time intervals are presented in Fig. 3. Control group mice revealed normal histological structure of brain. Profenofos treated group at 1 h 40 min revealed mild congestion of cerebral blood vessels, perivascular oedema, vacuolation of neuropil, mild satellitosis and loss of purkinje cells layer in cerebellum. Histopathological investigations of 24 h post-treatment group mice showed meningeal

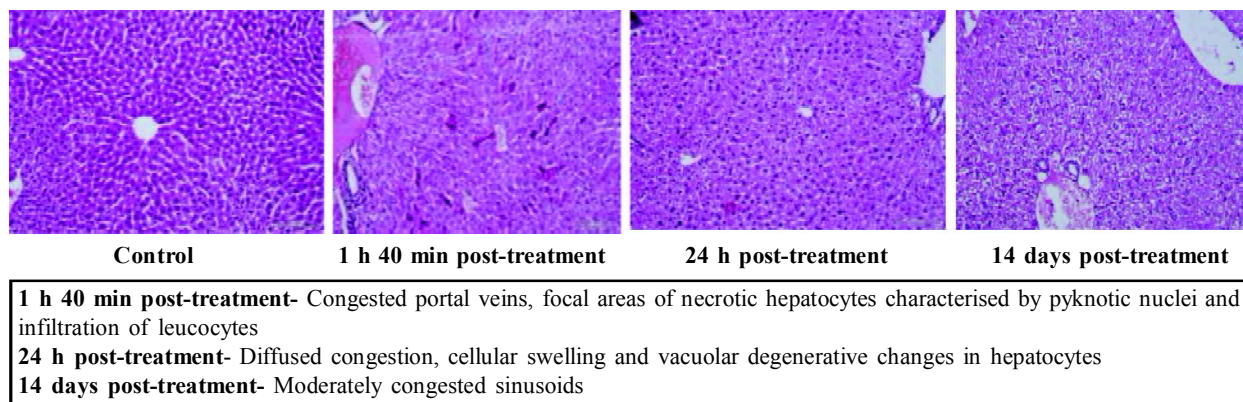


Fig. 1: Representative images (H & E stain 200x magnification) of liver of mice of control and treatment groups exposed to single dose of MTD (90 mg/kg b.wt.) of profenofos at 1 h 40 min, 24 h and 14 days post-treatment

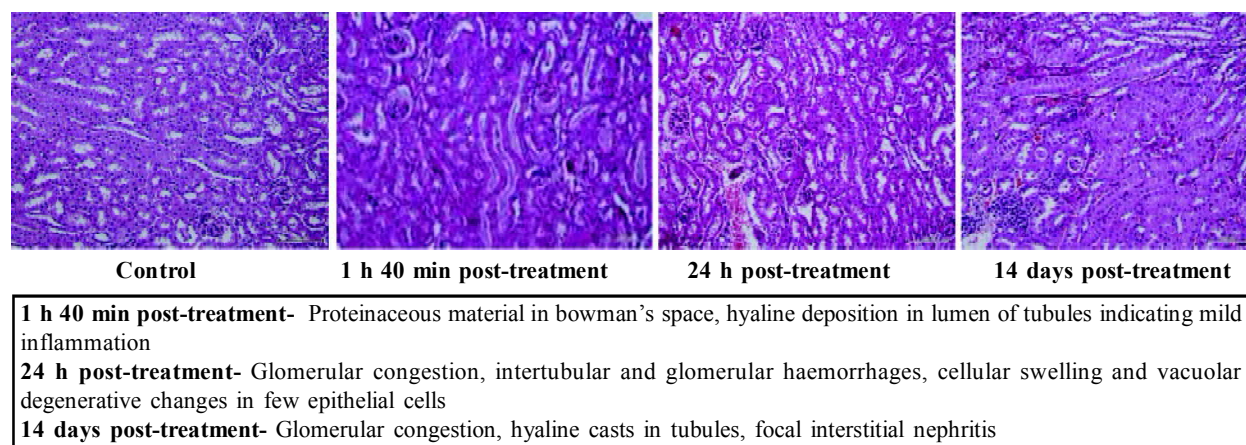


Fig. 2: Representative images (H & E stain 200x magnification) of kidney of mice of control and treatment groups exposed to single dose of MTD (90 mg/kg b.wt.) of profenofos at 1 h 40 min, 24 h and 14 days post-treatment

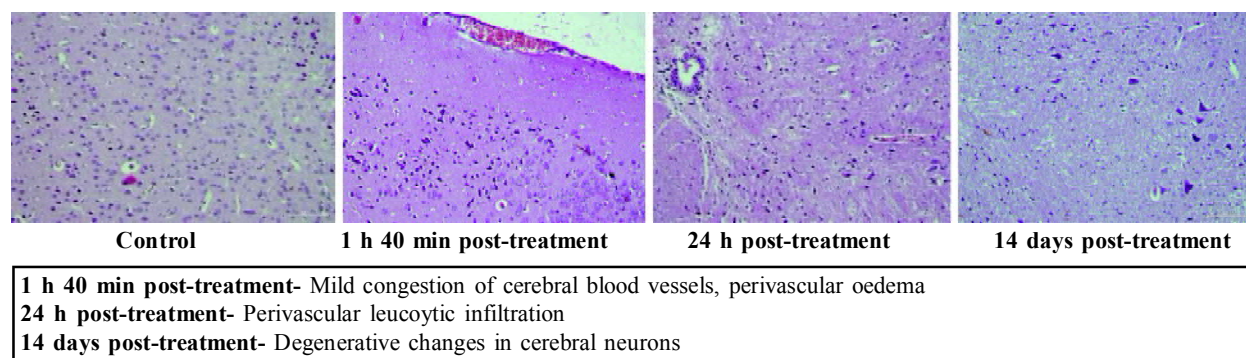


Fig. 3: Representative images (H & E stain 200x magnification) of brain of mice of control and treatment groups exposed to single dose of MTD (90 mg/kg b.wt.) of profenofos at 1 h 40 min, 24 h and 14 days post-treatment

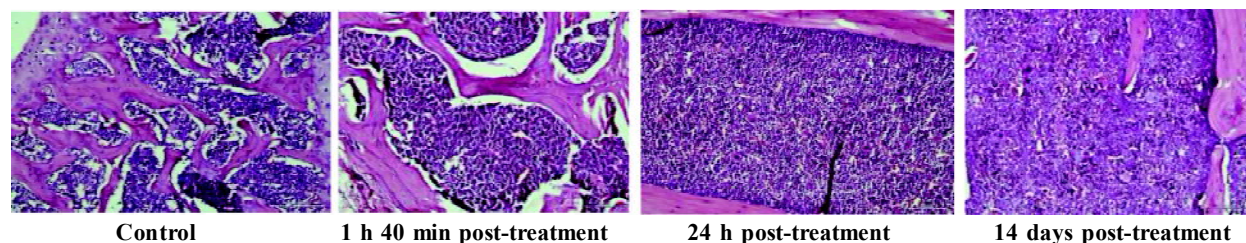


Fig. 4: Representative images (H & E stain 200x magnification) of bone marrow of mice of control and treatment groups exposed to single dose of MTD (90 mg/kg b.wt.) of profenofos at 1 h 40 min, 24 h and 14 days post-treatment

congestion, perivascular infiltration, spongiosis of neuropil, vacuolation and degenerative changes in pyramidal cells in hippocampus region, loss of purkinje cells layer in cerebellum. Brain in 14 days treatment group revealed congestion and haemorrhages in meningeal blood vessels, degenerative changes in neurons and pyramidal cells in cerebellum.

Bone marrow

Histopathological lesions in bone marrow of control

and MTD group of profenofos at a single dose of 90 mg/kg b.wt. at 1 h 40 min, 24 h and 14 days post-treatment time intervals are presented in Fig. 4. All the groups did not reveal any significant change in histological structure of bone marrow.

Testes

Histopathological lesions in testis of control and MTD group of profenofos at a single dose of 90 mg/kg b.wt. at 1 h 40 min, 24 h and 14 days post-

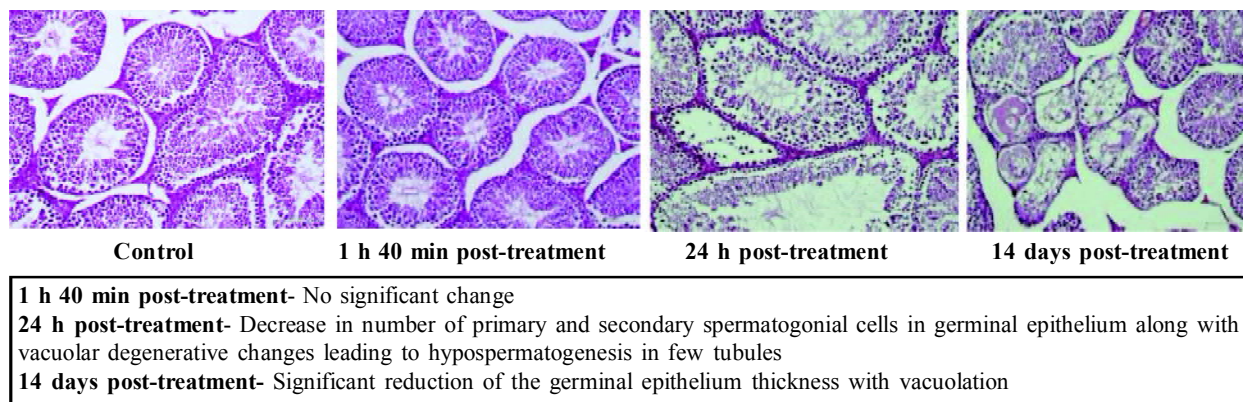


Fig. 5: Representative images (H & E stain 200x magnification) of testes of mice of control and treatment groups exposed to single dose of MTD (90 mg/kg b.wt.) of profenofos at 1 h 40 min, 24 h and 14 days post-treatment

treatment time intervals are presented in Fig. 5. Control group mice revealed normal histological structure of testis. Group of 1 h 40 min post-treatment showed no significant change. Group of 24 h post-treatment showed congestion, decrease in number of primary and secondary spermatogonial cells in germinal epithelium along with vacuolar degenerative changes and hypospermatogenesis. Group of 14 days post-treatment showed significant reduction of the germinal epithelium thickness with vacuolation, focal atrophy of seminiferous tubules resulting in architectural distortion. The lumen of such tubules contained necrotic cells.

DISCUSSION

Animal growth in terms of body weight, relative body weight gain, organ weight and relative organ weight are important for toxicological studies (Hoffman *et al.*, 2002). From many decades, several studies have revealed that pesticides adversely affect the body weight gain (Brkic *et al.*, 2008; Bhardwaj *et al.*, 2010; Bal *et al.*, 2012; Singh *et al.*, 2023). Body weights were recorded at 1 h 40 min, 24 h and 14 days of post-treatment with profenofos at MTD. No significant change in body weight and relative body weight gain was observed indicating profenofos neither enhances nor suppresses the metabolic processes in mice at this dose. The gross examination of vital organs viz. heart, liver, kidneys, spleen, testes and epididymis did not show any gross pathological change.

In toxicity studies, organ and relative organ weights are important criteria for evaluation of organ toxicity (Heikal *et al.*, 2013; Singh *et al.*, 2023). No significant change was observed in organ weight in comparison to their respective control in all groups at 1 h 40 min, 24 h and 14 days post-treatment with profenofos at MTD. A significant increase in relative organ weight of left testes and right epididymis at peak effect and significant increase in relative organ weight of liver and right testes at 14 d post treatment were observed. A non-significant decrease in body weight was observed in these groups which may be the cause of such findings.

In acute toxicity study, histopathological examination of liver, kidneys, brain, testes and bone marrow were conducted at 1 h 40 min, 24 h and 14 days post-treatment of profenofos at MTD. In control groups, no histopathological lesions were observed in testes. The group of 24 h post-treatment with profenofos showed congestion, decrease in number of primary and secondary spermatogonial cells in germinal epithelium along with vacuolar degenerative changes and hypospermatogenesis. Group of 14 days post-treatment showed significant reduction of the germinal epithelium thickness with vacuolation, focal atrophy of seminiferous tubules resulting in architectural distortion. The lumen of such tubules contained necrotic cells. Aita *et al.* (2012) also found that testis of profenofos (23 mg/kg b.wt.) administered rat showed degeneration and

intraluminal accumulation of necrosed germ cells as well as interstitial oedema.

Group of 1 h 40 min post-treatment with profenofos revealed glomerular congestion, proteinaceous material in bowman's space, focal interstitial nephritis, hyaline deposition in lumen of tubules indicating mild inflammation and hyperplasia of parietal epithelial cells lining the bowman's capsule in kidney. Group of 24 h post-treatment with profenofos also revealed glomerular congestion, intertubular and glomerular haemorrhages, cellular swelling and vacuolar degenerative changes in few epithelial cells. Group of 14 days post-treatment with profenofos revealed glomerular congestion, hyaline casts in tubules, perivascular infiltration of leucocytes and focal interstitial nephritis. Kidney of profenofos (23 mg/kg b.wt.) administered rat showed congestion of renal blood vessels, hypertrophy and vacuolation of glomerular tuft as well as renal tubular epithelium (Aita *et al.*, 2012). Group of 1 h 40 min post-treatment revealed congested portal veins, focal areas of necrotic hepatocytes characterised by pyknotic nuclei and infiltration of leucocytes in liver. Liver in 24 h post-treatment group showed diffuse congestion, cellular swelling and vacuolar degenerative changes in hepatocytes. Group of 14 days post-treatment showed moderately congested sinusoids, haemorrhages, disruption of hepatocytic chords because of necrosis, perivascular oedema, and dilated portal vein. Aita *et al.* (2012) found that liver of profenofos (23 mg/kg b.wt.) administered rat showed congestion of central veins and hepatic sinusoids as well as vacuolation and necrosis of hepatocytes and pyknosis of their nuclei.

Profenofos treated group at 1 h 40 min revealed mild congestion of cerebral blood vessels, perivascular oedema, vacuolation of neutrophils, mild satellitosis and loss of purkinje cells layer in cerebellum. Group of 24 h post-treatment showed meningeal congestion, perivascular infiltration, spongiosis of neuropil, vacuolation and degenerative changes in pyramidal cells in hippocampus region, loss of purkinje cells layer in cerebellum. Brain in 14 days treatment group revealed congestion and

haemorrhages in meningeal blood vessels, degenerative changes in neurons and pyramidal cells in cerebellum. Badawy *et al.* (2017) also observed degenerative changes in brain tissue in the form of congestion of cerebral blood vessels, edema vacuolations and severe cellular infiltration, which were evident after 24 h at a single dose of 20 mg/kg b.wt. in rats.

Histopathological lesions in bone marrow of control and MTD group of profenofos at a single dose of 90 mg/kg b.wt. at 1 h 40 min, 24 h and 14 days post-treatment time intervals did not reveal any significant change in histological structure of bone marrow.

CONCLUSION

The acute toxic effects of profenofos were studied at a single dose of MTD (90 mg/kg bw) at 1 h 40 min (peak effect), 24 h and 14 days post-treatment in three treatment groups along with their respective control groups. Histopathological lesions were observed in liver, kidney, brain and testes, suggesting its acute toxicity to these organs. Profenofos showed acute toxicity at MTD level to mice.

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