



Neurotoxic impact of organophosphate pesticide phosphomedon on the albino rat

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Abstract

Organophosphate pesticide phosphomedon was exposed to albino rat at a concentration of 35 ppm in the time interval of 30, 45 and 60 days. During the exposure period neurobehavioral symptoms such as reduced food intake, weight loss, increased water intake, low defecation frequency, increased locomotion frequency at high dose were observed. Locomotion frequency was less initially but higher in increasing dose concentration. The result was also different in both the sexes. A decrease in social interaction and increase in force swimming with increasing dose concentration, which was not significant as compared to control. A significant histopathological change was observed in three dose concentrations. In 30 days phosphomedon exposure the nuclear shape changes to oval, pear shaped along with fibrosis, lipidosis, 45 days exposure showed the increase in number of nucleus, chromatolysis, inflammatory nucleus, pyknosis. In 60 days test group histopathological picture showed the swelling of cell body, lipidosis, demyelination, necrosis in brain cells. Overall result indicated that due to the impact of O. P. pesticide phosphomedon a severe histopathological change occurs which was distinctly observed in neurobehavioral changes.

Key words

Brain tissue, Histopathological, Necrosis-demyelization, Neurobehavioral changes, Phosphomedon

Introduction

Phosphomedon is a systemic organophosphate pesticide used extensively in the vast agricultural field especially for perennial crops. It is also known as dimethyl phosphate ester with 2-chloro-N,N-diethyl 3-hydroxycrotonamide. It is used to control sucking, chewing, mirming insects. Effect of phosphomedon on different systems of the body of mammals and other animals has been worked out (Prativa Rohankar et al., 2012, Akbarha and Srivastava 1998, Suke, et al 2006, Kuwar et al., 2008).

Organochlorine and organophosphorus insecticides cause pathological and biochemical changes of the organs of different species including mammals. (Anand kumar and Tripathy 2001; Soni, 2005; Ferh Sayrm et al., 2005). No such information is available on the effect of phosphomedon the organophosphate pesticide which is a popular insecticide among the Indian farmers. In view of above, the present investigation was undertaken to evaluate the neurotoxic and behavioral effect of

phosphomedon on white albino rat.

Materials and Methods

Albino rats weighing 180-200g were obtained from Zoology Department, Gauhati University, Assam and bred and housed in polypropylene cages (50×40×17cm) in the air cool room with natural day-light for 12-16 hr. They were fed regularly with standard food received from Hindustan Lever Limited, New Delhi. Tap water was provided *ad libitum*. Rats were selected from the colony and divided into four groups—three experimental groups and one control group. The animals of three experimental groups were treated with 35 ppm of phosphomedon for 30, 45 and 60 days, respectively. Water intake, food intake and weight gain were recorded weekly. The control animal received plain tap water. Food intake (gm per day), weight gain (gm per interval), water ingestion (ml per day) were recorded in this period according to standard method of Schwarz et al 2006. After treatment, the animals of respective groups were subject to neurobehavioral study following standard procedure at an

interval of 30,45 and 60 days of experimental period.

Neurobehavioral study : Animals were observed daily from 11 a.m. to 4 p.m. and water ingestion, food consumption and weight gain were recorded. For each group 5 male and 5 female were selected, which were not handled before any experiment. Locomotion frequency was determined by the number of floor units entered with both feet. For force swimming study, at least one male and one female were taken from the litter was used. Treated and controlled native rats were individually forced to swim inside vertical glass tube. (length 40cm ,diameter 18cm, filled with water). After 15 min, the animal were removed from water, dried and returned to their cages. For social interaction test, open field test same number of animals were used per group. Open field study was conducted to examine defecation (frequency/days), locomotion frequency (unit entered with both feet) to exposed and control group. Social interaction were conducted to measure the direct observation of pair of rats

stranger to each other . Defecation frequency was conducted counting the number of facial pellets.

After neurobehavioral study animals of the respective groups were sacrificed by cerebral dislocation after and their brains were removed and fixed in Carney fixatives and sections were cut at 6 μ m and stained with eosin and haematoxiline for histopathological study.

Results and Discussion

Food intake in rats treated with 35 ppm of phosphomedon at the time interval of 30, 45, 60 days were reduced as compared to control. Weight of exposed rat at the different time intervals of 30days, 45days, 60days were reducing. On the other hand water intake capacity increased with the increasing time intervals. Locomotion frequency was effected in both the sexes due to phosphomedon treatment . Reduce locomotion frequency was



Fig. 1 : Histology of mid brain tissue of albino rat ($\times 400$).

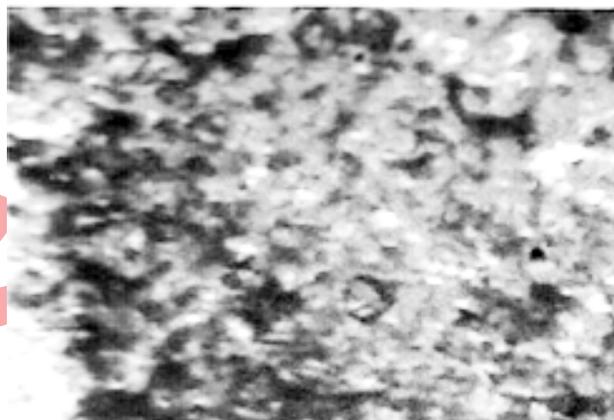


Fig. 2 : Histological changes of brain tissues of albino rat exposed to 35 ppm phosphomedon at 30hrs marked with rupture and vacuolation of cell structure and a centric nuclei ($\times 400$)

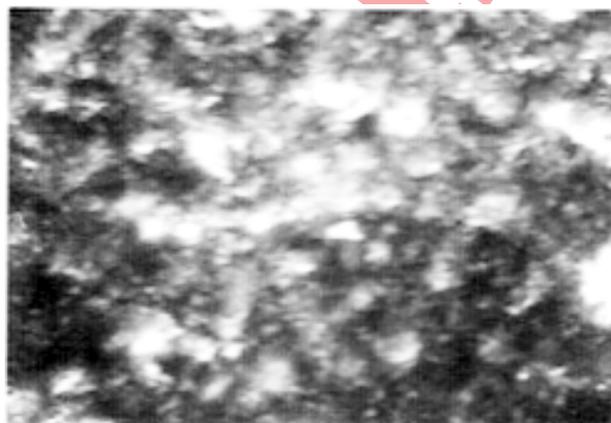


Fig. 3 : Histological changes in the brain tissues of albino rat exposed 45 hrs of 35ppm of phosphomedon marked with partial dissolution of cell membrane, vacuolation and necrotic changes ($\times 400$)

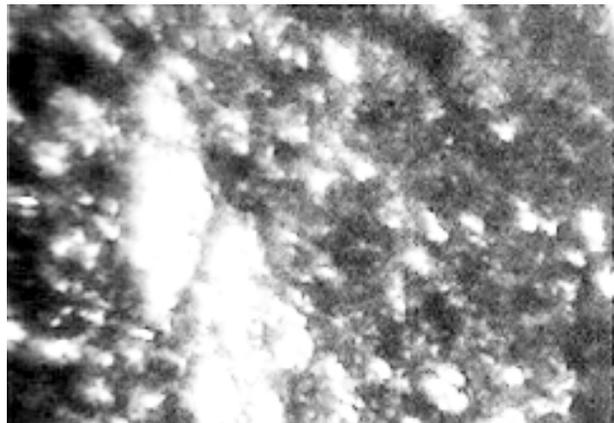


Fig. 4 : Histological changes in the brain tissue of albino rat exposed to 60 hrs. of 35 ppm concentration of marked with total loss of cellularity, profuse vacuolation, demyelization, swelling of cell body and fatty degeneration ($\times 400$).

observed at first in the experimental period but increasing with increasing time interval. The ANOVA test were applied to compare the mean control and exposed groups and between male and female (Schwarz *et al.*, 2006). From the experiment it is clear that the organophosphorus pesticide effect the reduce locomotion at first and increase later. On the other hand female showing higher locomotor activity than male. The result of social interaction, force swimming test, behavior showed that there was no significant alteration of result of the test groups as compared to control group. Social interaction was decreasing as compared to control. Different rate of force swimming was observed as compared to control. It was also different again in male and female. Defecation frequency is increasing up to 45 days in female, whereas in male it is decreasing up to sixty days.

Histopathological study showed that after 30 days of phosphomedon treatment alternation of cytomorphological pattern of neurons were observed, showing, elongated, oval and pear shaped from that of the control group (Fig. 1). Karyolysis was evident due to nuclear fragmentation. Cytoplasmic degeneration was associated with lipidosis which disrupted the cellular membrane along with vacuolation. Lipidosis was also apparent as the vacuole were filled with lipids throughout grey matter of the brain indicating abnormal distribution of lipids in the brain tissue. The nuclei became hyper chromatic associated with karyolysis (Fig. 2). After 45 days more degenerative changes were observed in this group. Large vacuolar nucleus were observed whereas some cells appeared to be shrunken with profuse vacuolation. Infiltration of macrophage, polymorph lymphocyte indicated a serious inflammatory changes (Fig. 3). Appearance of necrotic cells were also marked. After in 60 days of treatment severe

degenerative changes were observed, which indicated extreme neurotoxic phenomenon, known as demyelization. Extreme loss of cellularity with appearance of glial cells and vacuolation which affected blood supply was marked (Fig. 4).

The neurotoxicity of phosphomedon on brain tissue of albino rat clearly indicate severe dysfunction of central nervous system, which interfere with the normal behavior of rats. A higher dose of phosphomedon showed some physical and developmental alterations during exposure period. Administration of different doses of pesticide not only alter food and water intake habit, but the stress of pesticide alter the pathological picture such as cell vacuolation, necrosis, acentric nucleus, pyknosis, which disrupt the neural transmission of central nervous system of animal. (Calore *et al.*, 2000, Latuszynska *et al.*, 2001).

The behavioral abnormality observed in the present study was also reported due to the exposure of cypermethrine, pyrethroids pesticides in albino rats. Histopathological alteration in epididymis of male reproductive system of albino rat due to the effect of phosphomedon was reported by Sayrm Ferah *et al.* (2005).

Histopathological changes in the hippocampus and stratum granulosum tissues of the brain in rats treated with delta methrin. The focal pyknosis of the cytoplasm was observed in cerebral cortex and cerebellum. Various changes such as acentric nucleolus, fibrosis, lipidosis, demyelination and necrosis observed in the present experiment due to phosphomedon. Luty *et al.* (2000) also reported that changes concerning cells, the

Table 1 : Effect of 35 ppm phosphomedon on water ingestion, food intake and weight gain after 30, 45 and 60 days of exposure

Parameters	Control	30 days	45 days	60 days
Water intake (ml days ⁻¹)	40±2.1	40±1.2	38±1.5	42±.3
Food intake (gm day ⁻¹)	19.3±1.1	22.2±1	20.3±1	20.9±.9
Weight gain (gm intervals ⁻¹)	25.5±1.7	20.7±3	18.6±1.8	18.4±2.9*

Values are mean of replicates ±SE; P<.05 compared to control group

Table 2 : Effect of 35 ppm phosphomedon exposure on the rats after 30, 45 and 60 days

Parameter	Sex	Control	30 day	45 day	60 day
Locomotion frequency	M	5±.1	50±.2	40±.31	100±.25***
	F	56±.2	56±.25	40±.39	120±.23***
Force swimming/second	M	40±.1	30±.2	43±.3	48±.1
	F	45±.2	30±.1	40±.1	50±.2**
Social interaction/second	M	250±.2	220±.2	230±.1	220±.4
	F	230±.1	200±.1	210±.3	210±.2
Defecation frequency	M	1.5±.2	2±.3	1.2±.2	1±.18
	F	2.7±.2	5±.2	2.5±.2	1.7±.19*

Values are mean of replicates ±SE; ANOVA significant at *P<.05, **P<.01, ***P<.001 statically significant

Table 3 : Histopathological changes observed in brain tissues of albino rat treated with 35 ppm phosphomedon for a period of 35, 45 and 60 days

Exposure	Histopathological changes	Effect
30 days	1) Nuclear changes normal structure to dot like structure	+
	2) Vacuolation around the Nucleus and nucleus shifted to the side of the vacuole	+
	3) Fibrosis, Lipidosis.	++
45 days	1) Increased number of nucleus	+
	2) Chromatolysis	++
	3) Less effected peripheral cell in normal histopattern	++
	4) Large round and vacuolar nucleus	
	5) Inflammatory nucleus	
	(6) Pyknosis	+++
60 days	1) Swelling and hyalinization of cell body	+++
	2) Abanance of lipidosis	+++
	3) Demyelination	+++
	4) Necrosis.	+++

Perkinji cells in the cerebellum, concentration of cytoplasm of single pyramidal cells, hippocampus layer and a focal pyknosis of neurocytes of the nuclei lateralis hypothalami and cerebral cortex in the rat exposed to alpha cypermethrine. Ferah Sayrm *et al.* (2005), reported the parking cells in the cerebellum, concentrations of the cytoplasm of the single pyramidal cells of the hippocampus layer and the focal pyknosis of neurocytes of the nuclei lateralis hypothalami and cerebral cortex in the rats exposed to higher dose of pesticide.

The present study showed that phosphomedon induced a neurotoxic effect manifested by neural conductivity and peripheral nervous system, effecting the behavioral pattern in rats.

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