

Original Research Article

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Subacute Toxicity Assessment of Acephate and it's Amelioration by *Picrorhiza kurroa* in Female Wistar Rats

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ABSTRACT

Keywords

Acephate, Wistar rats, *Picrorhiza kurroa*, and Histopathological studies

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The experimental study was conducted on 30 female wistar rats through five groups for 28 days. The group I and III served as control groups. The rats of group IV and V were treated with Acephate and *Picrorhiza kurroa* and group II was treated with only Acephate. Amongst those, group II and IV showed non-significant reduction in mean body weight and group II, IV and V did not showed any behavioural changes, except, dullness and lethargy. Group II and IV Non-significant variation in mean relative organ weights of liver, kidneys, spleen and Adrenal gland were observed. Grossly, liver and brain showed mild congestion with minimal and focal haemorrhages on kidneys. On histopathology, liver showed focal to multifocal congestion, dilatation of sinusoidal spaces, dilatation of central vein, fatty changes, cytoplasmic rarefaction and MNC infiltration. Kidneys revealed mild to moderate congestion, vacuolar, hydropic, cystic degeneration, haemorrhagic cystic degeneration, coagulative necrotic changes, hyaline cast in lumen of exposed tubules and MNC infiltration. There was lymphocyte depletion, minimal to mild congestion in spleen. Neurotoxicity was characterized by vacuolar degeneration and congestion in brain. Intestines revealed acute catarrhal inflammatory changes. Heart showed congestion, minimal to mild haemorrhages and fragmentation. Adrenal glands showed congestion.

Introduction

At present, India is the largest producer of pesticides in Asia, and ranks 12th in the world for the use of pesticides, with an annual production of 90,000 tons⁵. A vast majority of the population in India (56.7%) is engaged in agriculture and is therefore exposed to the pesticides used in agriculture^{7,8}. Pesticides being used in agricultural tracts are released into the environment and come into human

contact directly or indirectly⁵. Organophosphorus (OP) insecticide self-poisoning is a major health problem, with approximately 200,000 deaths each year¹.

Acephate is an organophosphate foliar spray insecticide of moderate persistence with residual systemic activity of about 10-15 days. Acephate and its primary metabolite, methamidophos, are toxic to *Heliothis spp.* that are considered resistant to other

organophosphate insecticides⁶. National Pesticide Information Centre described that people exposed to acephate had nausea, diarrhoea, abdominal cramps, shaking, sweating, rapid heart rate, dizziness and confusion. Symptoms usually begin within minutes or hours after exposure.

Picrorhiza kurroa is an important medicinal plant used in traditional as well as modern medicines. It is used in treatment of liver disorder, fever, asthma, jaundice caused by environmental pollution, industrial toxicants, food adulteration, malnutrition, excessive consumption of alcohol and certain infections². Considering the present status of use of Acephate by the agrerians and the trend of herbal medication in treating many disorders, the present trial was conducted with following objective.

Objective

To assess toxicopathological effect of Acephate in female wistar rats and it's amelioration by *Picrorhiza kurroa* through body weight, symptomatology, relative organ weights and pathomorphological studies.

Materials and Methods

Female Wistar Rats

Thirty female Wistar rats of 6-8 weeks age having 140-160 gm body weight. The female Wistar rats were procured from M/S Worckdht Research center, MIDC Aurangabad, (MS) 431006.

Collection of Acephate Pesticide

Acephate (O,S dimethyl acetyl phosphoramidothioate) was procured from Department of Pesticide, College of Agriculture, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani (MS).

Feeding

Rats were provided with standard pellet feed (M/S L.R. Pharmaceutical Company, Parbhani, Maharashtra, India).

Preparation of Acephate doses

Acephate pesticide dose was calculated as 1/20th of LD₅₀ (1127mg/kg²²) of female wistar rats. Acephate is a powder form pesticide which was mixed with vehicle propylene glycol and fed daily through oral gavage to the experimental rats.

***Picrorhiza kurroa* Powder**

Dried powder of a plant of *Picrorhiza kurroa* (kutki) was procured from the local market of Parbhani.

Preparation of plant aqueous extract

Extraction of Plant with Hot water¹¹

250 gm dried plant powder of *Picrorhiza kurroa* mixed with 500 ml distilled water.

Prepared mixture was boiled for 30 min. cool it and filtered with whatman filter paper no. 42.

Extract was kept into conical flask and plugged with cotton swab and then liquid extract was kept at 2-4°C till its use

Experimental design

Body weights

Weekly body weights of experimental rats were taken and computed.

Symptomatology

All the experimental rats were observed twice daily throughout experimental period.

Relative organ weights

At 28th day of study period, Liver, kidney, spleen and Adrenal gland were excised, weighed and expressed.

Gross and Histopathological study

At the time of organ weighing, gross changes, if any, were recorded. Representative tissues were collected in 10% formal saline from organs and processed for histopathological studies.

Statistical analysis

The data generated in respect of body weight and relative organ weights were statistically analysed by Completely Randomized Design (CRD)¹⁹ to know the statistical differences between means at different intervals in each group.

Results and Discussion

Average body weight

Table 2 depicts mean body weights of experimental rats at weekly intervals. In female rats of Acephate toxicated groups (II and IV), the average body weight were reduced non-significantly than the control group rats. However, female rats of group V showed slight improvement in mean body weights as compared to Acephate toxicated rats, whereas, the female rats of group III did not show any significant variation at studied intervals. At 28th day of trial, the percent body weight gain in female rats of group I, II, III, IV and V when assessed, found to be 7.53%, 2.97%, 7.27%, 4.21% and 5.37% respectively. The percent body weight gain in female rats of group II and IV indicated reduction than the respective control group female rats body weight gain. However, the percent body weight gain in female rats of

group V showed improvement. The body weight gain in female rats of plant control group remained comparable with that of respective control group.

The decrease in body weight gain in Acephate toxicities could be preliminary attributed to the effect of insecticide on gastrointestinal tract resulting in decreased appetite and absorption of nutrients from gut or might be an indication of direct toxicity or stressogenic activity of insecticide compound^{3,9,10}. The reduction in body weight gain also could be attributed to anorectic properties of insecticides¹⁴.

Increase in body weight gain in *Picrorhiza kurroa* treated groups could be due to its beneficial properties like appetizer and immunomodulent¹⁵.

Symptomatology

The experimental rats of all groups did not reveal any apparent behavioural changes, except, hypersensitivity just after Acephate administration, dullness and lethargy in female rats of group II and IV. The signs observed in present study are in close approximation with earlier reports^{3,13}.

Relative organ weight

At 28th day of trial, the mean relative weights of liver, kidneys, spleen and adrenal gland of group I, II, III, IV and V remained comparable amongst themselves and their mean values obtained are shown in Table 3. The use of Acephate at specified dose level in present study might have not been so toxic to affect relative organ weight significantly. The observations in present study are in at par with the reports of earlier authors¹⁷. Increase in mean liver weights of experimental rats in toxicity studies have been reported by earlier researchers^{21,24}.

Table.1 Details of Experimental groups of female Wistar rat

Groups	Treatment	No. of Animals	Route
I	Control group with vehicle- <i>ad libitum</i> feed and water daily for 28 days	06	Normal feeding and watering
II	Acephate @ 56.35 mg/kg with vehicle	06	By oral gavage
III	<i>Picrorhiza kurroa</i> aqueous extract @ 50 mg/kg body weight	06	By oral gavage
IV	Acephate @ 56.35 mg/kg + <i>Picrorhiza kurroa</i> aqueous extract@ 25 mg/kg body weight	06	By oral gavage
V	Acephate @ 56.35 mg/kg + <i>Picrorhiza kurroa</i> aqueous extract@ 50 mg/kg body weight	06	By oral gavage
	Total	30	

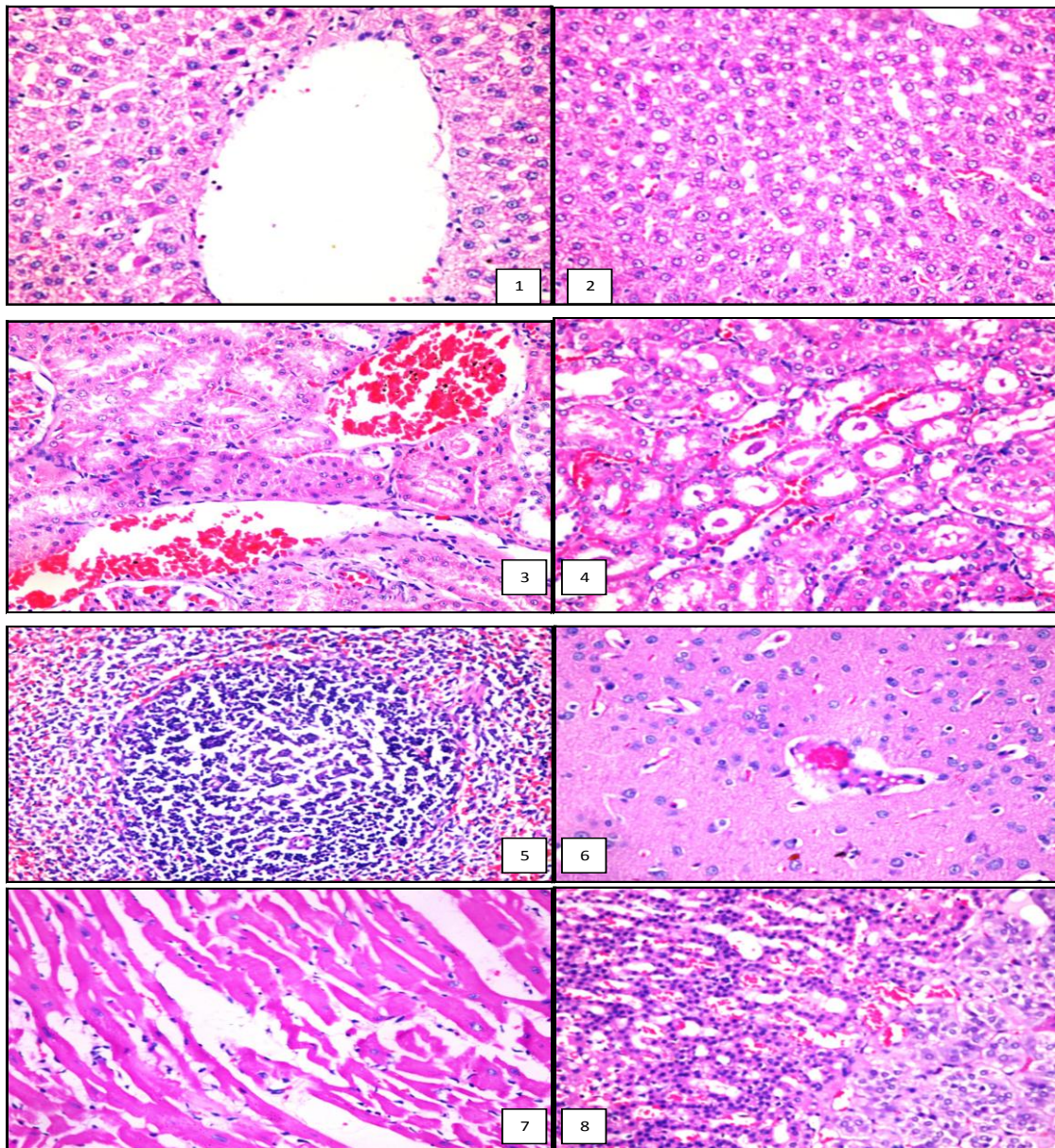
Table.2 (Mean \pm S.E.) Average weekly body weight (gm/week) in experimental rats at different intervals of study

Groups	Weekly Body weight of rats (gm/week)				
	Intervals of study				
	0 Day	7 th Day	14 th Day	21 st Day	28 th Day
I	201.33 \pm 09.07	203.66 \pm 08.13	208.00 \pm 10.38	215.50 \pm 06.46	216.50 \pm 07.54
II	201.66 \pm 04.50	201.83 \pm 02.92	199.66 \pm 05.44	205.66 \pm 07.15	207.66 \pm 05.81
III	197.00 \pm 09.05	199.16 \pm 08.23	208.83 \pm 08.40	209.83 \pm 08.53	211.33 \pm 08.20
IV	205.50 \pm 07.88	203.83 \pm 08.50	201.33 \pm 08.39	211.16 \pm 07.84	214.16 \pm 07.87
V	204.50 \pm 09.35	204.00 \pm 9.36	208.00 \pm 10.47	215.16 \pm 09.51	215.50 \pm 10.17
Statistics	NS	NS	NS	NS	NS

Table.3 (Mean \pm S.E) values of Relative organ weights of Liver, kidneys, Spleen and Adrenals in experimental rats

Groups	Relative organ weights of female experimental rats			
	Liver	Kidney	Spleen	Adrenals
I	3.17 \pm 0.21	0.65 \pm 0.06	0.24 \pm 0.02	0.05 \pm 0.00
II	3.16 \pm 0.21	0.68 \pm 0.06	0.25 \pm 0.03	0.05 \pm 0.00
III	3.28 \pm 0.08	0.74 \pm 0.07	0.30 \pm 0.03	0.04 \pm 0.00
IV	3.22 \pm 0.16	0.61 \pm 0.02	0.18 \pm 0.01	0.03 \pm 0.00
V	3.48 \pm 0.07	0.64 \pm 0.02	0.23 \pm 0.02	0.05 \pm 0.00
Statistics	NS	NS	NS	NS

Fig.1 Section of liver with dilatation of central vein, sinusoidal spaces, focal fatty changes and degenerative changes in hepatic parenchyma (H&E 400X) Fig. 2 Note multifocal fatty changes, cytoplasmic rarefication of hepatocytes with focal congestion and MNC infiltration in liver (H&E 400X) Fig. 3 Section of a kidney with multifocal haemorrhagic cystic degeneration and MNC infiltration (H&E 400X) Fig. 4 Note hyaline casts in the lumen of proximal, distal convoluted tubules, multifocal degenerative changes, congestion and MNC infiltration in kidney (H&E 400X) Fig. 5 Note depletion of lymphocytes in a spleen (H&E 400X) Fig. 6 Microphotograph of a brain with vacuolation, neuronal degeneration and minimal congestion (H&E 400X) Fig. 7 Microphotograph of a heart with distortion of cardiac muscles and focal MNC infiltration (H&E 400X) Fig.8 Section of an adrenal gland with congestion (H&E 400X)



Gross and Histopathological studies

The gross pathological changes did not showed any appreciable changes in any of the organ studied except, mild congestion in liver, brain and minimal focal petechial haemorrhages in kidneys of few female rats of group II and IV.

The histoarchitectural changes noted in present trial are depicted through illustrations (Fig. 1 to 8).

Liver

On histopathological examination, liver of toxicated female rats of group II and IV showed minimal to moderate, focal to multifocal congestion, dilatation of sinusoidal spaces, dilatation of central vein, fatty changes mostly characterized by circumscribed vacuolation, cytoplasmic rarefication, MNC infiltration, degenerative changes and necrotic changes in hepatic parenchyma. Similar histopathological lesions in liver of female wistar rats of Acephate toxicated groups have been observed in earlier studies^{3, 12, 13}.

Histomorphological alterations are mostly related to the metabolic capabilities of liver. As is fully established that OPs can affect the enzymatic pathways involved in the metabolism of lipids, carbohydrates and proteins. Additionally, OPs could also cause oxidative stress characterized by formation of reactive oxygen species and triggering apoptotic pathways²⁰.

The female experimental rats of group IV and V were subjected to toxicities and these were also treated with *Picrorhiza kurroa* plant aqueous extract daily for 28 days. The plant extract used in the trial might have helped in restoration of plasma membrane permeability including repairement of injured hepatic cells,

increasing protein and nucleic acid synthesis. Also, aqueous extract of *Picrorhiza kurroa* might have protected the liver by its membrane stabilizing, antioxidant properties and its capabilities of counteracting free radicals¹⁸.

Kidneys

The kidney sections of female rats of group II and IV revealed mild to moderate congestion, tubular degeneration, vacuolar degeneration, hydropic degeneration, cystic degeneration, haemorrhagic cystic degeneration, coagulative necrotic changes, hyaline/proteinous casts in the lumen of proximal, distal convoluted tubules and focal to multifocal mononuclear cell infiltration. As stated by earlier researchers^{3,12,13} and histopathological observations in present study as observed, Acephate could lead to renal injury consequencing nephrotoxicity²³.

The reduced extent and intensity of nephropathic lesions in female rats of group V could be probably due to multifaceted role being played by aqueous extract of *Picrorhiza kurroa*.

Spleen

The spleen of group II and IV on its histopathology revealed focal haemorrhages and lymphoid depletion. However, the sections of spleen of experimental rats of group I, III and V did not showed any appreciable changes.

Intestines

The histopathological studies of intestine of female rats of group II and IV appeared to be with mild congestion, inflammatory cell infiltration in mucosal lining, acute catarrhal inflammatory changes, minimal goblet cell hyperplasia, mild necrotic changes in the

lymphoid areas of intestine, desquamation of epithelial linings and its exfoliation in lumen probably could be due to enteropathic effect of Acephate toxicity. However, the sections of intestines from female rats of group I, III and V did not showed any considerable histomorphological alterations.

Heart

The sections of heart from the rats of group I, III and V did not showed any noticeable histomorphological changes. However, the tissue sections of heart from the rats of group II and IV were with congestion, minimal to mild haemorrhages, fragmentation, distortion of myocardial fibers, focal mononuclear cell infiltration and degenerative changes in cardiac muscles.

Lungs

The lungs of female rats of almost experimental groups on its histopathological assessment revealed mild congestion and inflammatory cell infiltration indicating incidental changes, however, the sections of lungs from rats of group II and IV were with comparatively more intense histopathological changes.

Ovary

The sections of ovary did not showed any appreciable histoarchitectural change in either of the rats of almost experimental groups, except, congestion in two sections of ovaries in female rats of group II.

Adrenal gland

The histopathological examination of adrenal glands from female rats of control as well treatment groups did not revealed noticeable changes, except, minimal focal congestion in three sections of adrenal glands of female rats of group II.

Skin

The skin of female rats on its histopathology did not showed any histopathological changes. These histopathological findings observed in spleen, brain, intestines, heart, lungs, ovary and adrenal glands in present study goes well with reports of earlier researchers^{3,13} who conducted studies on Acephate toxicity. The changes noted in brain and heart probably could have been resulted due to neurotoxic and cardiopathic effects of Acephate toxicity. The OP pesticides could inhibit AchE activity and elicits neurotoxicity²³. The considerable recovery in histoarchitecture of studied organs in female rats of group V might have obtained due to multifactorial role being played by *Picrorhiza kurroa* aqueous extract.

In conclusion, Acephate at scheduled dose induced toxicity as supported by findings in respect to body weight, behavioural changes and pathomorphological studies. Also, the experimentally induced toxicity during the present trial has shown amelioration by aqueous extract of *Picrorhiza kurroa* at specified dose level as evidenced by findings recorded because of the phytochemical action of plant.

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