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TOXICITY OF ACEPHATE TO LIVER AND KIDNEY OF FEMALE WISTAR RATS

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ABSTRACT

Acephate is a broad spectrum insecticide used against pests of vegetables, cotton and ornamental plants. In the present study, acephate was orally administered to female wistar rats to examine its toxic effects, if any, at dose level of 1/50th, 1/25th and 1/10th of LD_{50} value along with a control group for 45 days. Results revealed a remarkable decrease in the feed intake of 1/10th acephate treated rats during 5th and 6th week of treatment. The net body weights and liver weight decreased non-significantly to a small extent over 45 days of treatment. The weight of kidney and content of total soluble protein decreased significantly in a dose dependent manner in treated rats. The significant alterations in the activity of antioxidative enzymes i.e. glutathione peroxidase, glutathione-S-transferase, glutathione reductase, superoxide dismutase, catalase and lipid peroxidation levels were observed. The appearance of comet in 1/10th dosed rats indicated DNA damage. Further, no formation of concentric rings in treated rats indicated the absence or low concentration of antibodies in the serum.

Key words: Acephate, antibodies, antioxidative enzymes, DNA damage, genotoxic, immunological, lipid peroxidation, LD_{50}, organophosphates, oxidative stress, *Rattus norvegicus*

Organophosphate (OP) pesticides have been used worldwide during past few decades in agricultural and household practices for crop protection and pest control. As a result of their widespread use these chemicals ultimately enter into the environment and affect the life there in. World Health Organization has reported approximately three million cases of organophosphate poisoning/ year accounting for about 300,000 deaths (Robb and Baker, 2019). The toxic effects of OPs on non target species include many neurotoxic, immunological, mutagenic, teratogenic, carcinogenic and reproductive effects (Mostafalou and Abdollahi, 2017). Though the main target of organophosphates is central and peripheral nervous system attributed to inactivation of enzyme acetylcholinesterase (AChE) (Ndonwi et al., 2019) but acute and subchronic toxicity of OPs is also due to disturbance in redox reactions resulting into oxidative stress. The disturbance in the balance between the generation and removal of free radicals within body by OPs leads to oxidative stress by production of free radicals such as reactive oxygen species (ROS). The major biomolecules such as proteins, lipids and nucleic acids are attacked by ROS, but lipids are targeted the most and hence lipid peroxidation of cell membranes takes place.

Acephate (O, S-dimethyl acetyl phosphoramid-othioate) is one of the top ten organophosphate insecticides used in agriculture (Ribeiro et al., 2016). It is used as foliar treatment in several vegetables, cotton and ornamental plants for the control of many biting and sucking insects. Acephate and its primary metabolite, methamidophos are toxic to both target as well as non target organisms (Lin et al., 2020). Studies conducted on effects of acephate on different organisms have pinpointed its potential cytotoxic, neurotoxic, mutagenic and carcinogenic effects (Bhadaniya et al., 2015) along with suppression of the immune system which results into more susceptibility to infectious diseases. Sankhala et al. (2012) reported suppression of humoral immune response in acephate treated rats. The administration of acephate resulted into genotoxicity and cytotoxicity and was also believed to affect sperm structure in rats (Dhanushka et al., 2017). Liver and kidney are the major organs responsible for drug metabolism and excretion, respectively. The assessment of oxidative stress biomarkers in these organs may give an account of the toxicity of acephate. The adverse effects of pesticides cannot be ignored and are a serious public health issue because a large portion of the population is indulged in agricultural and related activities which involve their high scale chronic exposure to the pesticides. The ability of acephate to produce ROS like other OPs (Dhanushka et al., 2017) highlighted the need to carry out present study to investigate the toxic effects of acephate in liver and kidney along with genotoxic and immunological effects in female wistar rats.

MATERIALS AND METHODS

All chemicals used were acquired from SD Fine-Chemical Ltd. and Sissco Research Laboratories Pvt. Ltd., Mumbai, India. Standard pelleted feed for rats was obtained from Ashirwad Industries, S A S Nagar, India. Acephate (Starthene 75 SP) was procured from Modern Pesticides, Ludhiana. The study was conducted on sexually mature female wistar rats, *Rattus norvegicus* (Berkenhout) weighing 130-200 gm procured from Central animal facility, NIPER, S A S Nagar. Before treatment, rats were acclimatized for 15 days and then divided into four groups (3 treatments and 1 control) containing six rats each. The rats were kept in standard laboratory conditions (22°C for 45 days) and were provided with standard pelleted diet and water ad libitum. The experiment was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) vide Memo no. IAEC/2019/63-97 and the protocol given by National guidelines on the proper care and use of animals in the laboratory research were followed. The exposure of commercial formulation of acephate (Starthene 75 SP) was given to female rats by mixing it in olive oil to increase its acceptability to the rats. The first group served as control and received olive oil only without pesticide. Acephate treatment was given to the other three groups of rats @ 1/50th, 1/25th and 1/10th of LD_{50} i.e. 866 mg/ kg of body weight (Gupta and Moretto, 2005) for 6 weeks by oral intubation. After pesticide treatment, they were examined for three to five hours for symptoms like, excessive salivation, hyperactivity and mortality. The feed intake of the acephate treated and control rats was measured and expressed as gm/100 gm b.wt at weekly intervals. Weighing of rats was done before starting the experiment and at weekly intervals for 6 weeks. Following formula was used to calculate the difference in body weight

$$\text{Difference in body weight (g/100 g b.wt/day)} = \frac{\text{Current body weight (g)- Previous body weight (g)}}{\text{Previous body weight (g) x number of days}}$$

After six weeks of treatment, rats were fasted overnight and sacrificed by cervical dislocation. After dissection, blood sample from each rat was collected directly from heart in EDTA coated vials and centrifuged at 2300 r.p.m. for 15 min. Supernatant was collected as plasma and stored for antioxidant activity. The liver and kidney were excised out, cleaned in saline and weighed. After weighing of organs, 0.5 gm each of kidney and liver were homogenized in 2 ml of 0.1 M phosphate buffer saline (PBS, pH 7.4). The homogenate formed was centrifuged at 3000 rpm for 10 min and supernatant collected was stored for estimation of oxidative stress biomarkers. The antioxidative activity (AOA) in blood plasma was estimated by the method of Koracevic and Koracevic (2001). The liver and kidney supernatant was used for estimation of total soluble proteins, Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) and lipid peroxidase (LPO) by the standardized methods- Lowry et al. (1951), Marklund and Marklund (1974), Aebi (1983), Hafeman et al. (1984), Habig et al. (1974), Carlberg and Mannervik (1985) and Stocks and Dormandy (1971), respectively. To assess genotoxicity bone marrow was extracted from femur of the rat with syringe and needle. The comet assay was performed as described by Singh et al. (1988). Slides were viewed under a fluorescent microscope Nikon Eclipse 80i for analyzing the presence of comet tail (DNA strand breaks) within 20 min of staining. Blood was collected from orbital socket by retro-orbital bleeding of rats before the treatment and after six weeks of treatment. Serum was obtained from blood and quantitative determination of serum immunoglobulins was done by observing the antibody-antigen reaction on the agarose coated slides through radial immunodiffusion as per Fahey and Mackelvey (1965). The data were subjected to ANOVA (p=0.05).

RESULTS AND DISCUSSION

No mortality was seen in acephate treated rats but other symptoms such as increase in sleep duration, cloudy eyes, red spots on thoracic region, itching were seen along with loss of body hair. There was no significant change in food consumption in the low dosed rats however a remarkable decrease was observed in the feed intake of 1/10th acephate treated rats during 5th and 6th week of treatment. Excessive salivation and decrease in feed intake was recorded in high dose acephate treated rats (Bhadaniya et al., 2015). The decrease in food intake may be due to disturbance in hormone level or direct cytogenetic effect of pesticide. The net body weights decreased to a small extent over 45 days of treatment while no such effects were observed in the control rats. The decrease in body weight of the organophosphate treated rats was reported by many workers and reduction in the food consumption can be the reason behind the reduced body weight of the treated rats which is a direct effect of application of organophosphate (Mokhtar et al., 2013). The liver of

treated rats showed non-significant decrease in weight (3.12± 0.01 gm/100 gm b.wt.) as compared to that of the control rats (3.25± 0.01 gm/100 gm b.wt.) however, the kidney weight decreased significantly in the treated rats (0.22± 0.006 gm/ 100 gm b.wt.) in dose dependent manner as compared to the control rats (0.37± 0.006 gm/ 100 gm b.wt.).

The AOA of plasma in control rats was determined to be 1.70± 0.10 mmol/ ml. The activity showed a significantly decreasing trend in plasma of 1/50th, 1/25th and1/10th of LD_{50} acephate treated rats with the values 1.43± 0.08, 1.19± 0.04 and 0.86± 0.02 mmol/ ml respectively (Fig. 1). The amount of total soluble proteins significantly decreased to half of its original value in the liver of acephate treated rats and to a significant extent in kidneys as well (Table 1). The disintegration of the structural proteins could be the reason for protein exhaustion. Protein content showed a declining trend in chlorpyrifos treated albino rats (Ubaidur Rahman et al., 2021). There was a significant reduction in SOD and CAT activity in kidney and liver of 1/10th acephate treated rats as compared to control. Non significant change was seen in the glutathione peroxidase activity in both the organs of treated rats as compared to control rats. The present study showed a significant increase in the GST activity in liver and kidney of the treated rats. There was significant decrease in the GR activity in liver but non-significant change in kidney of treated rats. In a study conducted by Arab et al. (2018), malathion increased MDA level and reduced GSH content compared with the control group in rat ovarian tissue. Muhammad et al. (2019) revealed that GPx levels decreased in fipronil treated rats. The biochemical studies in the rat liver demonstrated significant perturbations in the levels of glutathione-S-transferase (Gupta et al., 2019).

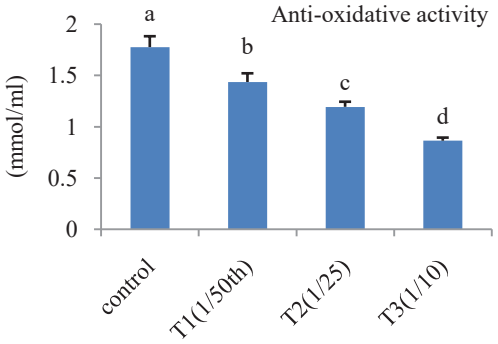

Fig. 1. Effect of acephate on antioxidant activity (mmol/ml) of plasma of wistar rats

A significant increase in level of LPO was observed in both the organs of treated rats. An increase in levels of reactive oxygen species causes oxidative stress and lipid peroxidation (Selmi et al., 2018). Lipid peroxidation and tissue damage is the result of induction of oxidative stress due to excessive generation of free radicals and ROS by the toxicity of pesticides. The mechanism of generation of oxidative stress is related to pesticide biotransformation as in the case of chlorpyrifos (Ndonwi et al., 2019). It further may induce developmental and behavioral abnormalities, hematological malignancies, histopathological alterations, oxidative stress, genotoxicity and immunotoxicity as evidenced by animal modeling (Ubaidur Rahman et al., 2021). According to recent studies conducted by Upadhyay et al. (2019), acephate exposure caused changes in biomarker responses like total protein and total cholesterol in female wistar rats. Liver and kidney collectively play an important role in metabolization process by transformation of thiono organophosphates accompanied by elimination process and act as main sites where maximum effects of pesticide by generation of oxidative stress can be seen. Rats treated with 200

Table 1. Effect of acephate on oxidative stress biomarkers in liver and kidney tissues of female wistar rats

Para-meters	Treatment							
	Liver				Kidney			
	Control	1/50th	1/25th	1/10th	Control	1/50th	1/25th	1/10th
Protein	7.29±0.10^{a}	6.61±0.10^{b}	5.91±0.10^{c}	4.11±0.20^{d}	9.55±0.10^{a}	9.01 0.02^{a}	8.63±0.20^{b}	8.39±0.03^{c}
SOD	0.68±0.20^{a}	0.57±0.006^{b}	0.52±0.009^{b}	0.41±0.11^{c}	0.70±0.00^{a}	0.55±0.007^{b}	0.40±0.009^{b}	0.30±0.00^{b}
CAT	0.82±0.01^{a}	0.75±0.02^{b}	0.69±0.006^{b}	0.57±0.004^{c}	1.87±0.004^{a}	1.65±0.03^{b}	1.44±0.6^{b}	1.03±0.007^{a}
GPx	0.57±0.02^{a}	0.57±0.03^{a}	0.42±0.01^{a}	0.30±0.004^{b}	0.54±0.007^{a}	0.45±0.02^{a}	0.44±0.02^{a}	0.43±0.008^{a}
GST	2.35±0.013^{a}	4.99±0.011^{b}	6.68±0.04^{c}	7.68±0.04^{d}	0.56±0.009^{a}	0.69±0.034^{b}	0.74±0.013^{c}	0.83±0.004^{d}
GR	4.77±0.008^{a}	3.47±0.026^{b}	2.48±0.04^{b}	1.97±0.08^{b}	4.76±0.03^{a}	4.58±0.02^{a}	4.37±0.011^{a}	3.95±0.017^{b}
LPO	0.57±0.004^{a}	0.69±0.006^{a}	0.75±0.02^{a}	0.82±0.01^{b}	1.03±0.007^{a}	1.44±0.6^{a}	1.65±0.03^{a}	1.87±0.004^{b}

Values expressed as mean± SE; ^{abcd} represents significant difference between treatments for different organs at p≤0.05 as compared to control. Units: Protein (mg/ gm wet weight of tissue), SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GPx(U/ mg protein), GST (µmoles of GSH-CDNB conjugate formed/min/mg protein), GR (µmoles of NADPH oxidized/min/mg protein), Lipid peroxidation (nmol MDA/100 mg tissue)

mg/ kg b.w. of malathion showed oxidative alterations and many histopathological lesions in the liver and kidney tissues (Selmi et al., 2018); severe damage was observed in hepatic tissue including prominent enlargement of sinusoids, infiltration of mononuclear cell, dilation, hemorrhage and necrosis, while it was degeneration of glomeruli, Bowman's capsules and associated tubules structure in kidney. Londhe et al. (2020) reported degeneration in liver and kidney of $1/20^{th}$ of LD_{50} of acephate treated female rats.

Slides of bone marrow cells showed orange-coloured cells of control rats which indicated healthy DNA. In $1/50^{th}$ and $1/25^{th}$ acephate treated cells, DNA strands were seen which were yellow in colour. It indicated incomplete damage of DNA i.e. only unwinding of the DNA strands whereas in $1/10^{th}$ acephate treated rats which was the highest dose, a proper comet tail was observed which showed DNA damage (Fig. 2,3). Exposure of rat lymphocytes to commonly used organophosphate pesticides i.e. chlorpyriphos, methyl parathion and malathion caused significantly marked increase in DNA damage (Ojha and Gupta, 2015). The DNA damage in acephate treated rats may be due to the production of ROS as well as electrophilic free-radical metabolites which interacts with DNA to induce DNA strand breaks. According to Goldoni et al. (2019), the appearance of tail in DNA strand is dose dependent. A significant increase in DNA damage was observed by Aranha et al. (2020) in peripubertal male rats exposed

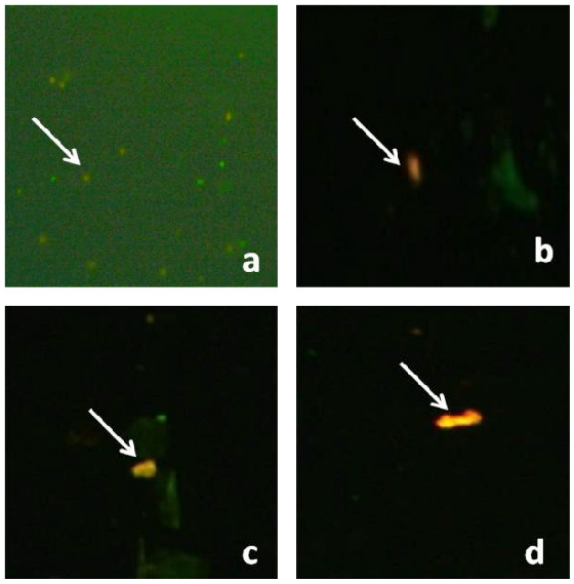

Fig. 2. Comet assay showing a) normal cells with no DNA damage in control rats, b) small comet tail in $1/50^{th}$ of LD50 of acephate treated rats, c) medium comet tail in $1/25^{th}$ of LD50 of acephate treated rats and d) large comet tail in $1/10^{th}$ of LD50 of acephate treated rats

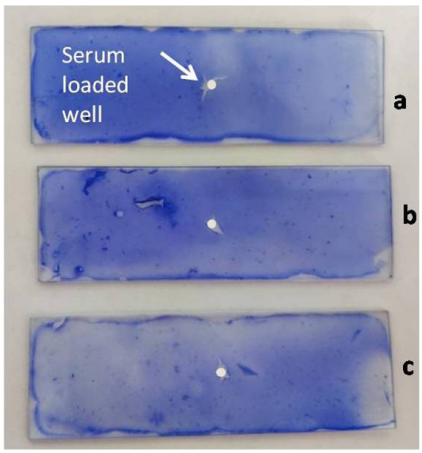

Fig. 3. Agar-coated slides a) $1/50^{th}$, b) $1/25^{th}$, c) $1/10^{th}$ of LD_{50} value of acephate treated rats showing no antibody-antigen reaction thus no concentric rings formed

to acephate in combination with other agrochemicals. In Radial immunodiffusion assay, there was no formation of concentric rings in the acephate treated slides which indicated the absence or low concentration of antibodies in the serum as compared to control. This may be due to lesser production of antibodies by the plasma cells. The chlorpyrifos treated rats showed a significant decrease in the IgG level in the 2^{nd} week of treatment (8368± 1192 mg/ l) when compared to control animals (10252± 555 mg/ l) (Elelaimy et al., 2012).

It can be concluded that acephate showed effects in female wistar rats at both the organ and cellular level. DNA strand breakage and absence of concentric rings in RIA highlighted the genotoxic and immunomodulatory potential of acephate in the exposed organisms. Liver and kidney both the organs were found to be affected in terms of acephate induced oxidative stress by elevation of pro-oxidants markers and depletion of antioxidant enzymes markers in female wistar rats in dose dependent manner.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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