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Research Article

ACUTE ORAL TOXICITY STUDY OF ANILOFOS IN SWISS ALBINO MALE MICE

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ABSTRACT

The maximum tolerated dose (MTD) of anilofos was determined to be 235 mg/kg body weight in pilot dose range finding study following the standard method. Swiss albino male mice were administered with single oral dose of anilofos at MTD and observed for toxic signs and symptoms at frequent interval up to 24 hours and then daily for 14 days. The signs of acute toxicity included dullness, depression, respiratory dyspnoea, tremor, ataxia, rigidity, fasciculation of muscles, head drop and protrusion of eye ball. Study concluded that MTD of anilofos is 235 mg/kg body weight through oral administration with no delayed toxic effects up to 14 days following a single oral administration of anilofos at MTD in mice based on body weight, relative organs weight (heart, liver, spleen, kidney, testis and epididymis) and hematological parameters (Hb, TEC, TLC and DLC).

Keywords: Acute toxicity, Anilofos, Delayed toxicity, Maximum tolerated dose, Swiss albino mice

Now days, pesticides are used extensively in agriculture in order to increase crop yield. OP (organophosphorous) poisoning, a major health problem, even a small amount causes toxicity in both target species and non-target species due to its high concentration in environment and death due to respiratory failure (Fleischli *et al.*, 2004; Jokanovic, 2009; Mondal *et al.*, 2012). OPs act as acetyl cholinesterase (AChE) inhibitors (Eddleston, 2000) which results in the accumulation of acetylcholine at cholinergic receptor sites, producing continuous stimulation of cholinergic fibres throughout the central and peripheral nervous systems (Jokanovic, 2009).

Anilofos, an OP used on large scale as pre-emergence and early post-emergence herbicide for the control of annual grasses, sedges, and some broad-leaved weeds in transplanted and direct-seeded rice crops at the rate of 300–450 g active ingredient per hectare in agricultural fields of northern states of India (Singh *et al.*, 2012). The oral maximum tolerated dose and toxic effect of anilofos on haematological parameter in Swiss albino mice is not known. Thus, in the present study, maximum tolerated dose of anilofos in Swiss albino male mice was determined and its acute and delayed toxic effects were investigated.

MATERIALS AND METHODS

Chemicals and experimental animals: Anilofos (formulation Aniloguard 30EC) obtained from Gharda Chemicals Ltd., Mumbai, India was used in the present investigation. Swiss albino mice weighing between 17-27g were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary & Animal Sciences (LUVAS), Hisar. Experimental animals were acclimatized to laboratory conditions for 2-3 days before the experiments were conducted. Temperature of animal house was maintained between 22–27 °C throughout the experiment. The prior approval of Institutional Animal Ethical Committee was obtained for use of the animals in this study.

Determination of MTD: Various doses of anilofos were screened for determination of MTD in pilot dose range finding study following standard method (Bagri *et al.*, 2013). These doses are presented in Table 1. Briefly, the study was conducted in small groups of mice (n=2/3/5) using several doses including few lethal doses. Animals in each group were administered single oral dose through gavage and thereafter several iterations were conducted. The MTD was selected that produced clear observable signs of toxicity without resulting in lethality. The MTD was then verified in a larger group of animals (n=10) which were observed for 14 days for grossly observable behavioural effects.

Acute toxicity study: The acute toxicity study for testing of chemicals was performed as described by earlier workers (Bagri *et al.*, 2013). Following administration of anilofos at MTD level on first day, the body weights of all animals in all groups were recorded on alternate day for 14 days. The animals were sacrificed on 14th day and blood was taken directly from heart using EDTA coated vial. Haemoglobin, total erythrocytes count (TEC) and total leukocytes count (TLC) were estimated as previously described (Weiss and Wardrop, 2010). The blood smears were fixed with methanol for five min, stained with Giemsa's stain (1:10 dilution) for 25 min and observed under oil immersion lens for differential leukocytes count (DLC).

Necropsies were performed on sacrificed animals and weight of various body organs viz. heart, liver, kidney, spleen, epididymis and testis were recorded. The relative organ weights were calculated using formula- (organ

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weight (g)/body weight (g) × 100).

Statistical analysis: The student's t-test using SPSS statistics 17.0 software (IBM Corporation, New York, USA) was applied to statistically analyze the results obtained with different study groups.

RESULTS AND DISCUSSION

Estimation of MTD and clinical profile: Following the pilot dose range finding study, the MTD of anilofos in Swiss albino mice was found to be 235 mg/kg b.wt. by oral route as presented in Table 1. To the best of our knowledge, the MTD of anilofos in Swiss albino male mice through oral route was determined for the first time which is lesser as compared to the LD50 (660 mg/kg) (Ram Naraian, 1993).

Effects of various doses used for determination of MTD on gross observable behaviour were noted in which toxic symptoms started in 10-15 min after anilofos administration and were found to be dose dependent in onset and severity of effects. The acute toxic signs observed were dullness, depression, respiratory dyspnoea, tremors and altered gait. Dyspnoea and difficulty in maintaining the complete prostrate position were observed just before death. Animal showed ataxia, rigidity and fasciculation of muscles, head drop and protrusion of eye ball. Several workers related these toxic symptoms of organophosphate poisoning with inhibition of the enzyme AchE (Blain, 2011, Carey *et al.*, 2013). Acute OP exposure produces increased Ach levels at the post-junctional receptors resulting in a variety of central and peripheral symptoms, including centrally mediated respiratory dysfunction. The mechanisms of the peripheral effects of OPs include hypotension (via muscarinic (Kullmann *et al.*, 1982) and non-muscarinic mechanisms (Kojima *et al.*, 1992)), weakness and paralysis (via effects on the neuromuscular junction (Wadia *et al.*, 1987)), and muscarinic effects of bradycardia, vomiting, diarrhea, salivation, mydriasis, bronchoconstriction and muscle fasciculation.

The absence of toxic signs after 24 hours indicated no delayed toxic effect of anilofos.

Table 1

Maximum tolerated dose (MTD)* of anilofos administered orally in swiss albino mice

Dose (mg/kg b. wt.)	Number of mice died/administered	Percent Mortality
300	1/2	50
280	1/2	50
240	1/2	50
238	1/2	50
235*	0/10	0
230	0/2	0
220	0/2	0

Effect of anilofos treatment on body weights of mice: Following administration of pesticide at MTD dose level on first day, the body weights of mice were recorded on alternate day for 14-days and are presented in Table 2. The increase in body weight gain was more in control animals than in anilofos treated animals. No delayed toxicity was observed as the severity of symptoms decreased with time.

Acute toxic effects of anilofos on various hematological parameters of mice: No significant changes in the hematological parameters (Hb, TEC, TLC, differential neutrophilic and lymphocytic count) were found in anilofos treated animals when compared to control group. The mean values of Hb and TEC decreased non-significantly in anilofos treated animals. However, the mean values of TLC increased non-significantly which might be due to rebound effect of anilofos on haemopoietic tissues as suggested earlier (Frame and Mann, 2008).

Effect of anilofos treatment on relative organ weights: The mean values of relative organ weights of different organs viz. heart, liver, spleen, right kidney, left kidney, right testis, left testis, right epididymis and left epididymis did not differ significantly between the control and anilofos treated groups. The non significant changes in the values of body weight gain, haematological parameters and relative organ weights indicated that exposure of anilofos at MTD level has no delayed toxic effects on haematological system and weights of body organs. Further, delayed toxic effects were also absent in the experimental animal. The present study also revealed the high MTD of anilofos and non toxic effects on body growth and hematopoietic system. The substitution of anilofos for other organophosphorous compounds may be promoted after considering the relative benefits and hazards with other used pesticides; however, more research is required on anilofos with respect to its toxic potential on genomic, metabolic, physiologic and developmental processes.

CONCLUSION

To the best of our knowledge, the MTD of anilofos (235 mg/Kg b.wt.) in Swiss albino mice through oral route was determined for the first time. Present study also revealed that anilofos at MTD level have no adverse delayed toxic effects on haematological parameters and growth profile (weights) of various body organs.

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Table 2

Effect of acute toxicity study of anilofos on body weights (g) of mice (mean ± S.E.)

Treatment (mg/kg b.wt., p.o.)	Days post anilofos administration							
	0	2	4	6	8	10	12	14
Control	20.25±0.68	20.85±0.78	22.25±0.66	23.10±0.59	24.00±0.48	24.30±0.58	26.05±0.46	27.10±0.43
Anilofos (235)	21.20±0.35	20.80±0.75	21.70±0.52	22.45±0.48	24.25±0.68	24.10±0.66	24.20±0.62	25.85±0.60

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