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Effect of acetamiprid on malathion induced haematological alterations in wistar rats

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Abstract

The present investigation was carried out on "Effect of acetamiprid on malathion induced clinical signs, general performance and hematological alterations in Wistar rats". The study was conducted on 36 Wistar rats which were divided into five groups viz., group I (control), group II (malathion 30 mg/kg BW), group III (acetamiprid 27 mg/kg BW), group IV (malathion 30 mg/kg + acetamiprid 13.5 mg/kg) and group V (malathion 30 mg/kg + acetamiprid 27 mg/kg BW) received treatment for 28 consecutive days. In results of the study, group III and V rats showed depression, pasty faeces, reduced feed consumption and mild salivation. From first week onwards significantly ($P < 0.05$) lowered weekly body weights were observed in group II, III, IV and V rats as compared to control till the experimental period of 4 weeks. Hematological findings of group II, III and V revealed significant ($P < 0.05$) decrease in Hb, PCV, TEC, lymphocyte along with significant increase in TLC and neutrophil count as compared with control. In conclusion, acetamiprid alone and its high dose combination with malathion produces deleterious effect on haematological parameters. However, acetamiprid at low dose combination with malathion, antagonizes toxic effect of malathion on hematological parameters.

Keywords: Wistar rats, hematological alterations, acetamiprid, malathion

1. Introduction

In India, insecticides represented as the highest share in total pesticides used. Organophosphorus and neonicotinoids are frequently used insecticides in agriculture and in animal husbandary to manage crop pests and ectoparasites. Acetamiprid is extensively used synthetic chlorinated neonicotinoid insecticide frequently used against insects that have increased resistance to organophosphate, synthetic pyrethroid, and carbamate (Si *et al.*, 2005)^[15] in agricultural practices. It works as agonists on the nicotinic acetylcholine receptors. Acetamiprid induces oxidative stress due to production of ROS and also produces haematological changes in rats result in diverse tissue damage (Dwivedi *et al.*, 1998)^[6]. Malathion is one of the most extensively used organophosphate insecticide and acaricide in agricultural, veterinary, medical and public health practices. Malathion is principally a nerve toxin, worked by inhibiting the action of an enzyme acetylcholinesterase (AChE) at the synaptic junction (Cabello *et al.*, 2001)^[3]. Malathion aggravate a range of physiological, biochemical, immunological, and histological alteration in experimental animals. Recently, it has been postulated that OPs produce oxidative damage in distinctive tissues via formation of reactive oxygen species (Akhgari *et al.*, 2003)^[11].

In recent times combinations of insecticides are prepared by farmers on the suggestion of pesticide sellers in order to get better quality and quicker results. Though such combinations could be more lethal to pests but it could be very hazardous to farmers and farm workers or animals and human who exposed through fruits and vegetables (W.H.O. 2009)^[18]. There are several reports in newspapers regarding inadvertent mixing of insecticides from different states of India. Hence, the present was undertaken to evaluate the toxicological consequence due to interaction between acetamiprid and malathion in Wistar rats.

2. Materials and Methods

2.1 Test Chemicals

Technical grade acetamiprid and malathion were procured from Krishi Rasayan Export Pvt. Ltd., Samba, Jammu and used for inducing toxicity in Wistar rats.

2.2 Animals

Thirty six male Wistar rats of 9-10 weeks of age were procured from National Institute of Bioscience, Pune. Before to start experiment all experimental animals were acclimatized for a

week to the new environment under identical managerial and hygienic condition with ad-lib feed and water in laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS, Akola. The experimental protocol on laboratory animals was approved from Institutional Animal Ethics Committee (IAEC Reg. No. 312/CPCSEA) which follows the recommendations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Publication in 2010), New Delhi, India.

2.3 Experimental protocol

Thirty six adult male Wistar rats of 9-10 weeks of age weighing 150-200 g divided into five groups of six animals each except in group II and V where nine animals were taken in each group. Group I served as a normal saline control while group II treated with Malathion @ 30mg/kg body wt. p.o. once daily, Group III treated with Acetamiprid @ 27 mg/kg body wt. p.o. once daily, group IV treated with combination (low toxic dose) of Malathion @ 30mg/kg body wt. p.o. + Acetamiprid @ 13.5 mg/kg body wt. p.o. once daily, while group V treated with combination (high toxic dose) of Malathion @ 30 mg/kg body wt. p.o. + Acetamiprid @ 27 mg/kg body wt. p.o. once daily. In this study except control group animals, all other animals were exposed to pesticides orally for 28 consecutive days. Initial body weights of individual rats from all groups were recorded, before start of experiment. The following parameters were studied during experimental period of 28 days.

2.4 Clinical observation

During experimental period rats were thoroughly examined for clinical signs like agility, muscle tremors, diarrhoea, ataxia after dosing of insecticides.

2.5 Body weight

The body weight of experimental animals was taken at

weekly intervals throughout the experimental period of four weeks.

2.6 Haematological investigations

For haematological estimation blood sample from six rats of each group were collected from the inner canthus of the rats at the end of experiment. Haematological parameters viz. Hb, PCV, TEC, TLC, MCV, MCH, MCHC were estimated as per the standard method using hematoautoanalyser.

2.7 Statistical Analysis

The data obtained during present investigations was analyzed by applying Completely Randomized Design (CRD) as described by Snedecor and Cochran (1989)^[17].

3. Results and Discussion

The rats from group II and group IV did not exhibit any apparent signs of toxicity till the end of experiment. However, group III rats exhibited symptoms viz., dullness and pasty faeces after 20th days of treatment. Group V rats showed symptoms viz., reduced feed consumption, dullness, depression and mild salivation. No mortality was recorded during the entire period of experiment in any of the treatment groups. Our results about absence of toxic signs and symptoms with malathion appeared to be in corroborated with results of Hazarika *et al.* (2002)^[8] and Geng *et al.* (2015)^[7] reported similar symptoms at malathion subacute doses.

From first week onwards significantly ($P < 0.05$) decreased weekly body weights were observed in group II and group III rats till the experimental period when compared to control. The significant ($P < 0.05$) decrease in the weekly body weight was also observed in group IV (low toxic combination) and V (High dose combination) rats in a dose dependent manner throughout the experimental period of 4 week as compared to control (Table 1). Like in present study Mosbah *et al.* (2017)^[11] reported significant decrease in body weight gain in rats exposed to acetamiprid alone.

Table 1: Weekly body weight in control and acetamiprid or malathion alone on their combination groups of Wistar rats. (n = 6)

Groups	0 week	1 st week	2 nd week	3 rd week	4 th week
I	151.5±2.43	181.33±3.27 ^a	207.17±5.54 ^a	247.33±3.76 ^a	288.00±2.77 ^a
II	151.67±1.23	158.33±1.72 ^{bc}	173.17±1.58 ^c	206.83±6.42 ^{cd}	255.17±4.00 ^b
III	150.17±3.46	160.00±2.07 ^{bc}	177.17±3.50 ^{bc}	220.00±5.32 ^{bc}	252.33±4.53 ^b
IV	151.83±0.74	164.17±1.60 ^b	187.33±4.29 ^b	231.17±5.51 ^b	259.33±4.32 ^b
V	151.33±1.77	156.50±1.70 ^c	167.17±0.94 ^c	200.17±4.44 ^d	249.33±4.68 ^b
Significance/ NS	NS	*	*	*	*

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels * $P < 0.05$, NS- Non significant.

Haematological values (Hb, PCV, TEC, MCV, MCH and MCHC) related to erythrocytes were analysed from different group rats at the end of 28th days. Mean values of Hb, PCV and TEC were found to be decreased significantly ($P < 0.05$) in all treated groups as compared to control. Group IV treated rats showed least change in the Hb, PCV and TEC values as compare to other treatment groups (Table 2). Similar to the observations in present study, Doltade *et al.* (2012)^[5], Singh *et al.* (2012)^[16] and Preeti *et al.* (2015)^[12] found significant decrease in the values of Hb, TEC and PCV following acetamiprid administration at different doses and time intervals.

In the present study, significant decrease in TEC and PCV values may be attributed to impairment of biosynthesis of

heme in bone marrow which ultimately results into decrease in hemoglobin contents (Shakoori *et al.*, 1990)^[14]. In addition, the reduction in the blood parameters (Hb, PCV and TEC) which might be either due to a hyperactivity of bone marrow leading to production of red blood cells with impaired integrity which was readily destructed in the blood circulation by reticulo-endothelial system or due to the toxic effects of malathion and acetamiprid (Doltade *et al.*, 2012)^[5] on bone marrow, kidney and liver affecting haemopoiesis and erythropoietin production in these organs. However, literature scanned did not reveal research data on malathion and acetamiprid combined toxicity on haematological parameters in any of the species.

Table 2: Haematological values related to erythrocytes (Hb, TEC, PCV, MCV, MCH, MCHC) in different groups at the end of 4th week (n =6)

Groups	Hb (g/dL)	TEC (10 ⁶ /cumm)	PCV (%)	MCV (fL)	MCH (Pg)	MCHC (%)
I	13.18±0.49 ^a	6.97±0.06 ^a	37.80±.94 ^a	54.25±1.25	18.93±0.74	34.86±0.80
II	10.21±0.23 ^c	5.17±0.35 ^{bc}	28.70±1.03 ^c	56.33±2.48	20.34±1.70	35.92±1.90
III	9.67±0.24 ^c	4.97±0.14 ^c	26.82±1.34 ^c	54.30±3.47	19.57±0.87	36.41±1.62
IV	11.77±0.20 ^b	5.81±0.26 ^b	33.10±2.01 ^b	57.34±3.68	20.39±0.73	36.19±2.20
V	9.57±0.24 ^c	4.81±0.21 ^c	26.63±1.30 ^c	56.07±4.15	20.07±1.08	36.26±1.60
Significance/ NS	*	*	*	NS	NS	NS

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels * $P \leq 0.05$, NS= Non significant

At the end of 4th week, MCV, MCH, and MCHC % values were found to differ non significantly in malathion (II), acetamiprid (III) or combination administered groups (IV) and (V) rats as compared to control. While, significant ($P \leq 0.05$) increase in TLC and neutrophil count and decreased in lymphocyte count was observed in all treatment group

except group IV rats. TLC, neutrophil and lymphocyte values of group (IV) revealed slightest changes between treatment groups and found comparable to control group (Table 3). Kalender *et al.* (2010) [9] observed non significant changes in MCV, MCH and MCHC values in malathion intoxicated rats which are in support with the present observations.

Table 3: Haematological values related to leucocytes (TLC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils) in different groups at the end of 4th week (n =6)

Groups	TLC (10 ³ /cumm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
I	10.76±0.85 ^c	17.00±2.14 ^c	78.33±2.20 ^a	1.67±0.33	1.67±0.42	1.33±0.21
II	16.28±0.56 ^a	24.17±0.70 ^{ab}	72.67±0.89 ^{bc}	1.5±0.22	1.00±0.51	0.66±0.33
III	17.02±0.47 ^a	24.33±1.85 ^{ab}	71.83±1.53 ^{bc}	1.5±0.43	1.33±0.49	1.00±0.36
IV	13.60±0.52 ^b	20.00±2.27 ^{bc}	76.00±2.56 ^{ab}	1.33±0.33	1.50±0.56	1.17±0.17
V	17.98±0.64 ^a	28.83±1.40 ^a	69.17±1.10 ^c	0.67±0.21	0.83±0.40	0.50±0.22
Significance/ NS	*	*	*	NS	NS	NS

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels * $P \leq 0.05$, NS= Non significant

Similar to present findings, Chakroun *et al.* (2016) [4] observed increased TLC values following oral administration of acetamiprid in rats. Significant increase in TLC was observed in malathion, acetamiprid alone and combined treated groups might be due to activation of leucopoiesis which can act as immunosuppressive agent (Ravikanth *et al.*, 2017) [13] or due to rebound effect of neonicotinoids on haematopoietic tissue. Similarly, Awasthy, (2013) [2] and Mondal *et al.* (2015) [10] also reported significant increase in the neutrophils count and decrease in lymphocyte count in acetamiprid treated rats.

Conclusion

From the study it is concluded that acetamiprid alone and its high dose combination with malathion produces deleterious effect on haematological parameters. However, acetamiprid at low dose combination with malathion, antagonizes toxic effect of malathion on hematological parameters

Conflict of Interest: All authors declare no conflict of interest.

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