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Toxic effects of insecticides malathion and cypermethrin on hematological parameters in blood of male rabbits (*Oryctolagus cuniculus*)

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Abstract

The present study was undertaken to know the hematological alterations in the rabbits following oral intoxication with malathion and cypermethrin at the dose of 75 mg/Kg body weight for seven days by using gavage. Rabbits were divided into three groups A, B and C comprised of three animals each. Group A was considered as the control group and was kept as such without any intoxication. Group B was the malathion intoxicated group while C was the cypermethrin intoxicated group. Results demonstrated the negative effects of both insecticides. Malathion produced 95% increase in WBCs, 4.9% in RBCs, 3.34% in HCT, 3.2% in MCHC, while -1.1% decrease in Hb, -1.3% in MCV, -3.7% in MCH and -16.12% in PLT was also recorded. Cypermethrin caused an increase of 142% in WBCs, 17.45% in RBCs, 4.21% in MCHC, 1.24% in MCH, and 5.76% in PLT, and a decrease of -5.8% in Hb, -0.5% in MCV and -5.5% in HCT level. In conclusion both the insecticides induced alterations in hematological indices and are suggested to be neurotoxic with leukemic tendency for rabbits and other vertebrates including human.

Keywords: Rabbits, malathion, cypermethrin, intoxication, hematology

1. Introduction

Insecticides have a vital role in agriculture productivity, however due to their toxicity they also pose some health concerns to humans and other animals ^[1]. Organophosphorus insecticides are extensively used for a diverse agricultural and public health practices ^[2], producing toxicity in mammals by hindering acetylcholinesterase activity resulting in accumulation of the acetylcholine neurotransmitter in synaptic junctions and leading to cholinergic toxicity by extreme stimulation of the postsynaptic cells ^[3, 4]. The pyrethroids are very stable compounds and systemic toxicity arises by subsequent ingestion. Through skin the pyrethroids penetrate to the peripheral sensory nerves. Among pyrethroids, class II pyrethroids including malathion and cypermethrin are extensively used. They are the fourth generation synthetic chemicals ^[5]. Many pyrethroids causes biochemical and physiological changes and also induces hematological modulations ^[6]. Animals especially mammals received great attention in current years by exposing to pyrethroid insecticides. Exposed animals display changes in their biological activities as well as in neurotic features ^[7].

Malathion is considered as a prominent organophosphate insecticide which is extensively used for controlling pests ^[8]. Malathion has been proved to be mutagenic and contain extremely toxic impurities, damaging non-target organisms ^[9]. Malathion induced various hematological, biochemical, histological, physiological and immunological changes in experimental animals ^[10]. Cypermethrin effect the sperm concentration and motility, hormones and weights of testes as well as causing itching, restlessness and head shaking and other nervous disorders ^[11]. Cypermethrin effect the nervous system of the animals and is considered as a stomach poison ^[12]. Newborn animals were found to be more sensitive to cypermethrin comparatively to adult animals because liver enzymes which are responsible for the breakdown of cypermethrin in the body are not completely formed in the newborns ^[13].

Hematological studies are useful in ecological and physiological interest to understand the relationship of blood characteristics to the environment ^[14] and is considered as the main tool for the selection of genetically resistant animals to certain diseases and environmental conditions ^[15]. Changes in hematological parameters are often used to determine health status of the body and to determine stress due to environmental, nutritional and pathological factors ^[16]. The present study was aimed to evaluate the toxic effects of insecticides malathion and

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cypermethrin on hematological parameters in the blood of male rabbits (*Oryctolagus cuniculus*).

2. Materials and Methods

This study was carried out in July 2014 for seven days. Nine male rabbits of about the same age and free from any clinical ailment procured from the local market. Rabbits were divided into three groups A, B and C having 3 rabbits in each group. Rabbits were housed with normal temperature and proper housing conditions. Food and water were provided at ad libitum. Malathion and cypermethrin were orally given to New Zealand rabbits through gavage. Group A was served as the untreated control group while group B and C were the treated groups intoxicated with malathion and cypermethrin respectively at the dose of 75mg/kg body weight. Rabbits received the dose for seven days. At the end of intoxication period the blood samples were collected and stored in the ethylene diamine tetra acetic acid (EDTA) tubes to prevent blood clotting. The amount of EDTA used was 2mg/ml blood, mixed with the blood by gentle rotation of the tube so that the blood cells may not be damaged. Blood samples were shifted to ice box and were transported to the laboratory for hematological analysis. The samples were stored in the refrigerator at 4 °C. Anticoagulant (EDTA) preserved blood was used for the estimation of various hematological parameters. Hematological analyzer (Model Symex kx-21) was used for analysis of various hematological indices. The collected data were analyzed using analysis of variance (ANOVA) and mean values were compared by using the SPSS version (16). The significance level was $P < 0.05$.

3. Results

A dose of 75 mg/kg body weight of both malathion and cypermethrin was fed to male New Zealand rabbits for 7 days. Five to 10 minutes later, after the intoxication of malathion and cypermethrin the rabbits displayed some clinical signs like itching, restlessness, muscular tremors, irregular and jerky movements, skin scratching, salivation and increased urination. Nervous signs comprising incoordination, ataxia and dizziness persisted for about 30 to 90 minutes. These signs appeared in both intoxicated groups but the itching and jerky movements were more common in cypermethrin intoxicated group. After the stipulated time period changes in hematological parameters were recorded.

Table, 1, shows Mean \pm S.E of white blood cells (WBC) in control group was $2.7E3 \pm 100$ μ L, while in malathion and cypermethrin fed groups it was 5266.67 ± 1623.097 μ L and $6.533E3 \pm 1407.52$ μ L, respectively. The results from ANOVA following t-test confirmed that malathion in comparison with control group presented significant increase ($p < 0.01$) while cypermethrin treated group displayed more significant increase ($p < 0.001$) in WBCs level (Fig.1).

Red blood cells (RBCs) in control group was $6.1000E6 \pm 1.527E5$ μ L, while in malathion and cypermethrin intoxicated group it was $6.49E6 \pm 55000$ μ L and $7.167E6 \pm 1.073E6$ μ L respectively (Table, 1). Malathion and cypermethrin both intoxicated groups exhibited an increase in RBCs level (Fig. 1).

Hemoglobin (Hb) in control group was 12.133 ± 0.4666 g/dl, while in malathion and cypermethrin administered groups it was 12 ± 0.4000 g/dl and 11.433 ± 0.2027 g/dl respectively (Table, 1). Malathion and cypermethrin both in comparison with control group exhibited decrease in Hb level (Fig. 1).

Hematocrit (HCT) in control group was $38.933 \pm 0.4701\%$,

while in malathion and cypermethrin intoxicated groups it was $40.2333 \pm 0.1333\%$ and $36.8 \pm 1.42\%$ respectively (Table, 1). Malathion intoxicated group displayed a significant increase in hematocrit level but a decrease in cypermethrin intoxicated group was observed (Fig. 1).

Mean corpuscular volume (MCV) in control group was 62.033 ± 0.6009 fL, while in malathion and cypermethrin fed groups it was 61.2333 ± 0.6935 fL and 61.97 ± 0.393 fL respectively. Malathion and cypermethrin both indicated a decrease in MCV level (Fig. 1).

Mean Corpuscular Hemoglobin (MCH) in control group was 18.8 ± 0.5 PS, while in malathion and cypermethrin intoxicated groups it was 18.1 ± 0.611 PS and 19.033 ± 0.18 PS respectively (Table, 1). Malathion fed group exhibited a decrease while cypermethrin served group displayed an increase in MCH level (Fig. 1).

Mean corpuscular hemoglobin concentration (MCHC) value in control group was 29.3 ± 0.3511 g/dl, while in malathion and cypermethrin fed groups it was 30.23 ± 0.666 g/dl and 30.533 ± 0.233 g/dl respectively (Table, 1). Malathion intoxicated group revealed an increase while cypermethrin fed group exhibited a significant increase in MCHC level (Fig. 1). Platelets (PLT) value in control group was $3.76E5 \pm 2185.813$ μ L while in malathion and cypermethrin intoxicated groups it was $3.157E5 \pm 95293.12$ μ L and $3.98E5 \pm 12662.28$ μ L respectively (Table, 1). Malathion in comparison with control group presented a decrease while cypermethrin fed group showed an elevation in PLT concentration (Fig. 1).

4. Discussion

White blood cells (WBCs) count was increased significantly in both intoxicated groups in the present study. Significant increase in white blood cells was also found in the rabbits treated with cypermethrin [17], while in another study the rabbits treated with cypermethrin (24 mg/kg body weight) also presented an increase in total leucocyte count [18]. Higher white blood cells count in the present study indicating that the pesticides have toxic effect on the blood and in response the body immune system tried to overcome the toxicants and hence the number of WBCs were increased. Elevated WBCs count might be due to the prevalence of disease condition due to malathion and cypermethrin intoxication as increase in WBCs count occurred as a pathological response.

Present study revealed an elevation in RBCs level in both intoxicated groups. [19], also documented increased in RBCs count in rabbits treated with pesticide lambda cyhalothrin at different doses. Similarly karate intoxication in rabbits also caused increase in RBCs level [20]. This higher level of RBCs may suggest renal cell carcinoma or kidney cancer or it may occurred due to pulmonary fibrosis or due to polycythemia caused by the intoxication of malathion and cypermethrin.

In the present study, malathion and cypermethrin increased hemoglobin level. Increased hemoglobin concentration was also found in rabbits intoxicated with lambda cyhalothrin at doses, 1.0, 4.0 and 8.0 mg/kg body weight when injected intraperitoneally [19]. Rabbits treated with cypermethrin (24 mg/kg b.wt) for 12 weeks also presented increase in hemoglobin contents [18]. On contrary rabbits treated with cypermethrin exhibited reduced hemoglobin concentration [21]. Elevated level of hemoglobin in the present study could be due to the impaired biosynthesis of heme in bone marrow, increased rate of destruction or reduction in rate of formation of RBCs [20].

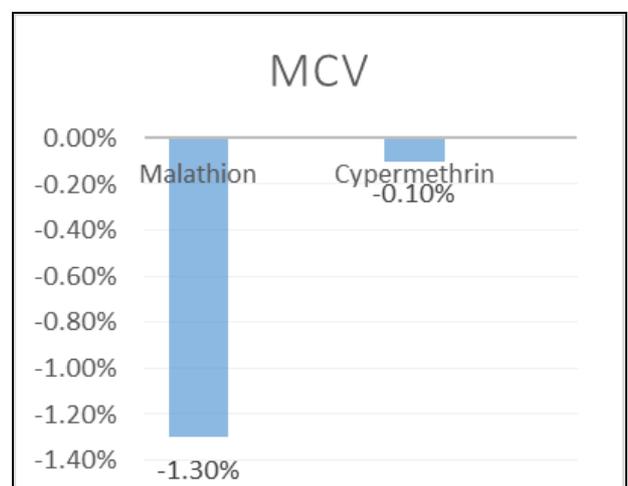
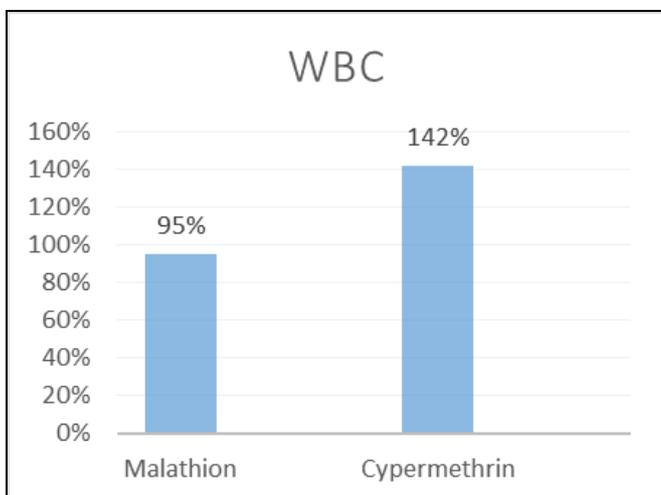
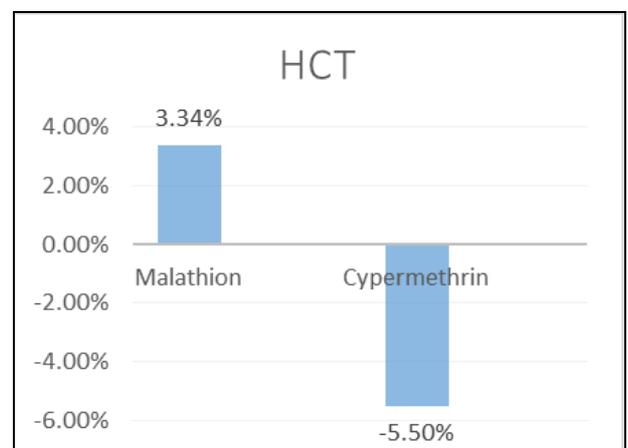
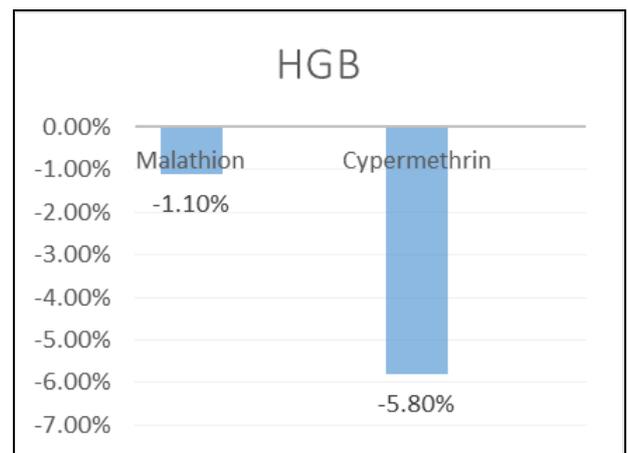
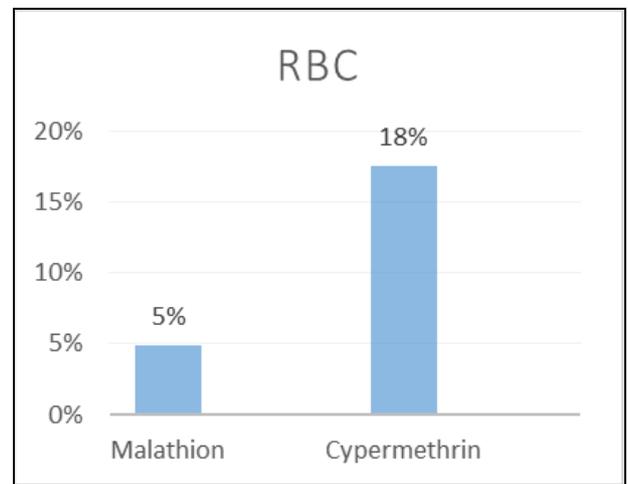
Malathion intoxicated group showed a significant increase in

hematocrit (HCT) concentration while cypermethrin fed group exhibited a fall in HCT level. This increase in HCT is directly in relation with increase in RBCs indicating erythropoiesis in the treated rabbits [22]. As cypermethrin intoxicated group displayed decrease in HCT level so this might be due to decrease in RBCs or their contraction [23]. In another study rabbits treated with cypermethrin displayed decrease in hematocrit values [18]. Similarly Karate administration in rabbits also caused increase in hematocrit level [20].

Mean corpuscular volume was decreased in both malathion and cypermethrin intoxicated groups when compared with control group. The decreased level of MCV is the sign of anemia indicating that malathion and cypermethrin interfered with the normal physiology of the RBCs. On contrary an elevation in mean corpuscular volume was also reported in the male rabbits treated with cypermethrin [21].

In current study as mean corpuscular hemoglobin showed decrease in malathion administered group while an increase in cypermethrin administered group. The decrease in MCH might be as a result of breakdown of RBCs and dropping in the concentration of formation of hemoglobin exhibiting hypochromic microcytic anemia [24]. As mean corpuscular volume was decreased in both groups while mean corpuscular hemoglobin concentration was increased in both groups in our present study. This increase and decrease could be due to the increased activity of bone marrow and deficiency of some hemopoietic factors [25]. A reduction of 31% in mean corpuscular hemoglobin level was also reported in rabbits fed with 10mg/kg body weight of danitol [26].

In the present study platelets showed decrease in malathion intoxicated group while an increase was found in cypermethrin fed group in comparison with control group. Decrease platelets count was also found in the study [27]. Elevated platelet concentration exhibited thrombocytosis which may be congenital or due to abnormal platelets production while decreased platelet count can lead to thrombocytopenia. As blood platelets have a great role in blood clotting so the decrease in platelets count in malathion fed group suggests that the process of clot-formation will be prolonged in case of any injury, resulting into excessive loss of blood [28]. These results are consistent with the results reported in literature that organophosphates insecticides caused changes in hematological parameters in the experimental animals indicating that these insecticides are very toxic to rabbits as well as other non-targeted organisms.



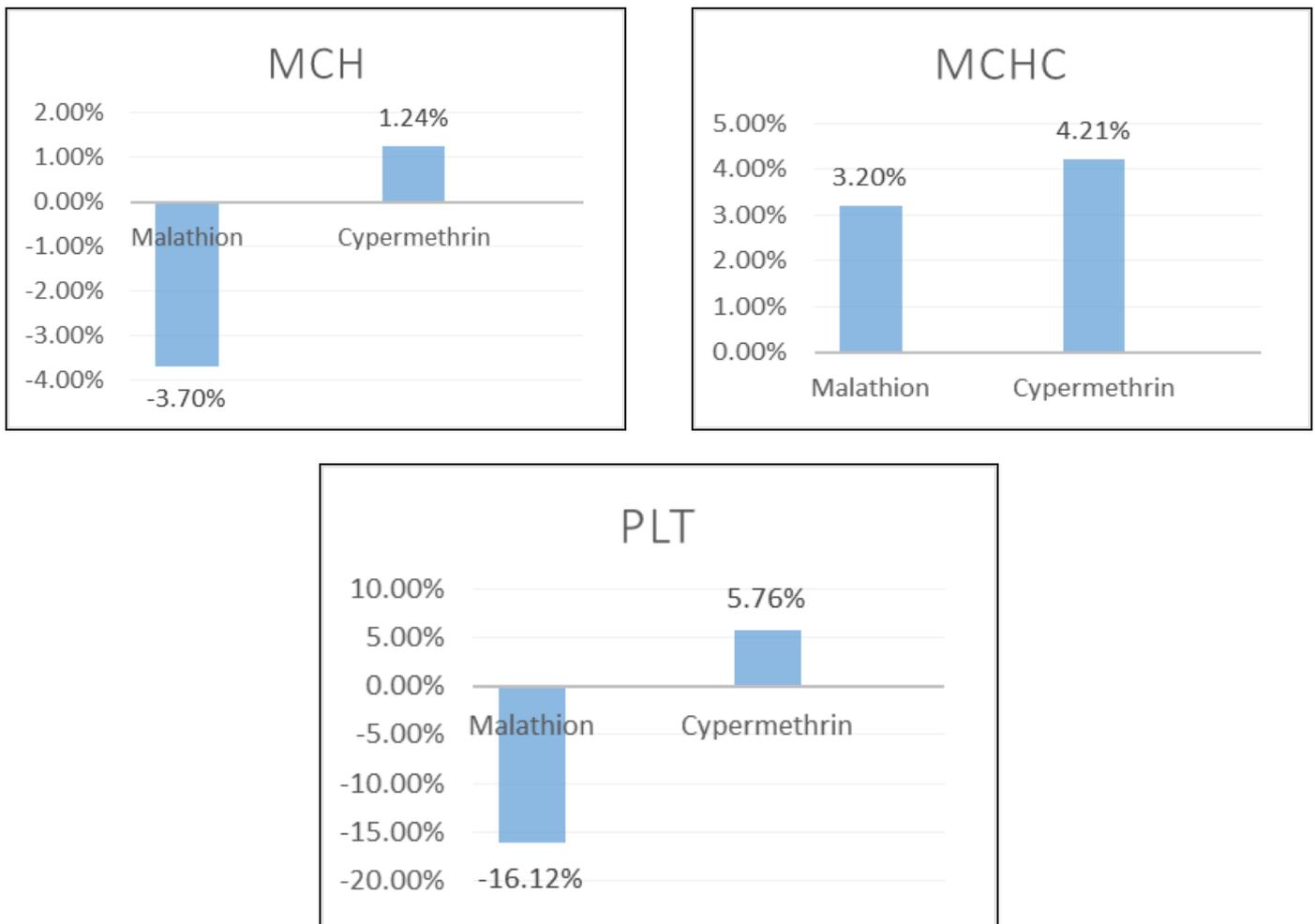


Fig 1: Blood hematological responses of male rabbits (*Oryctolagus cuniculus*) showing % increase (+) or decrease (-) due to seven days oral intoxication of malathion and cypermethrin

Abbreviations used: WBC, White blood cells; RBC, Red blood cells; HGB, Hemoglobin; HCT, Hematocrit ;MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PLT, Platelets.

Table 1: Hematological parameters after seven days of oral administration of malathion and cypermethrin at a dose of 75mg/Kg body weight to male New Zealand white rabbits

Parameters	Control	Malathion	Cypermethrin
WBC	2.7E3±100	5266.67±1623.097	6.533E3±1407.52*
RBC	6.1000E6±1.527E5	6.49E6±55000	7.167E6±1.073E6
HGB	12.133±0.4666	12±0.4000	11.433±0.2027
HCT	38.933±0.4701	40.2333±0.1333*	36.8±1.42
MCV	62.033±0.6009	61.2333±0.6935	61.97±0.393
MCH	18.8±0.5	18.1±0.611	19.033±0.18
MCHC	29.3±0.3511	30.23±0.666	30.533±0.233*
PLT	3.76E5±2185.813	3.157E5±95293.12	3.98E5±12662.28

Values are expressed as mean of three rabbits Mean± SEM, Student "t" test; *Significance (p<0.05), ** more Significance (p<0.01) *** High significance (p<0.001) vs. control.

Abbreviations used: WBC, White blood cells; RBC, Red blood cells; HGB, Hemoglobin; HCT, Hematocrit ;MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PLT, Platelets.

5. Conclusion

From the present study it is concluded that the organophosphorous insecticides malathion and cypermethrin greatly affect the hematological parameters as well as it effect the nervous system. Itching and irritation was more common in cypermethrin treated rabbits as compared to malathion intoxicated group showing that rabbits are probably more sensitive to cypermethrin as compared to malathion. As these pesticides adversely affect the non-targeted organisms. Percussion should be taken against these pesticides use as well as the concerned health authorities and law agencies should look out these agriculture practices.

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