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To study the effect on Haemopoiesis of *Rattus norvegicus* under the stress of some pesticides

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ABSTRACT

The present study includes toxicological effect on haemopoiesis of male ***Rattus norvegicus*** due to different pesticides as investigated. The experiment has been evaluated in male ***Rattus norvegicus*** using various parameters like TLC , DLC and erythropoiesis. This is the comparative study to investigate the haemopoiesis of normal and understressed condition to three groups of pesticides (organochlorne-endosulfan, carbamate-carbaryl and Organophosphorous-malathion). The study has drawn few possible correlations and conclusions to understand the effects of above pesticides with relation to their developmental days in laboratory rats. The present study includes the percentage changes in blood cells with relation to days (age) under the stressfull effect of above three categories of hazardous Chemicals.

Figure : 00 References : 16 Tables : 04
KEY WORDS : Carbaryl, DLC, Endosulfan, Erythropoiesis, Malathion, *Rattus norvegicus* (Berken), TLC

Introduction

The blood is the index of the state of health of an animal. Any change in the composition of blood reflects the change in tissue metabolism. Therefore, it is very essential to study the blood picture of model (various species of wild and domesticated) animals for the diagnosis of normal and diseased condition, of an animal[1,16].

In modern world pesticides vary greatly in toxicity[2]. Toxicity depends on the chemical and physical properties of a substance and may be defined as the quality of being poisonous or harmful to animals or plants. Pesticides have many different modes of action, but in general it causes bio-chemical[3,15] changes which interfere with normal cell functions as well as in serum homeostasis[1].

It is an undisputed fact that pesticides have come to stay and have bombarded the entire environment[16]as much they are used in agriculture. They have invaded every corner of globe. An increasing awareness has been seen among nations of the world about pollution caused by these pesticide. New stricter regulations[4] and legislation have been framed to check environmental contamination by various pesticides, specially in india to minimize the losses and slow poisoning effect which increases the suicidal trends among the animals.

Aim/Purpose

1. To calculate the blood cells changes under the stress of various pesticide in various concentrations.
2. To study the changes in blood cell number during the specific gap of days.
3. To calculate the changes in shape and size of blood cells as the age advances in various groups of rats.
4. To study the percentage increase or decrease in blood cells level at various stress parameters.
5. To calculate the complete blood chemistry changes during the stressfull condition of various pesticides.

Materials and Methods

1. Pesticides

(A) Endosulfan is an organochlorine biocide used for controlling pests and mites by generating neurotoxic effects (*i.e.,* hypersti-mulation) . Upon application, receiving soils act as primary reservoir of endosulfan in the environment; because of its hydrophobic properties. Endosulfan has shown high mobility across environmental compartments and in living system, it alters the serum analysis[5]. Classified as a semi-volatile compound, endosulfan is prone to evaporation. Subsequent atmospheric transportation may occur, resulting in wide

dispersion and remote deposition from application sites. Endosulfan is transfered to water bodies through runoff and favors accumulation onto sediments once in the water column. Both microbial and abiotic processes transform endosulfan in the environment: bacteria oxidize endosulfan to the respective sulfate whereas endosulfan diol is formed after alkaline hydrolysis. These intermediates exert similar toxic effects on the blood parameters[11]. Chronic exposure to endosulfan leads to bioaccumulation in fish; acute exposure results in neurotoxicity (hyperactivity and convulsions) in animals[11] and humans[2,12]; severe poisoning can lead to organ failure[7,14] and death.

(B) Carbaryl-(chemical name 1-naphthyl methyl-carbamate) is sold under many trade names, the most common being Sevin. It is widely used in agriculture, in horticulture and in residential settings. The primary mechanism of action is reversible inhibition of acetylcho linesterase and it is generally regarded as being safe with respect to human health.

(C) Malathion- Malathion is a broad-spectrum organo phosphorus insecticide for agricultural, industrial, and outdoor home uses, and for treating ectoparasites. It has low persistence in the environment. Agricultural workers[12] are exposed through inhalation, dermal contact, diet and water. Oxidative desulfuration converts malathion to malaoxon, which inhibits acetylcholinesterase in nervous tissues. Acetylcholine accumulation at synapses results in toxicity from cholinergic hyperstimulation. Alternate toxicity mechanisms are also possible. Detoxification through carboxylesterases converts malathion to carboxylic acids for further metabolism[8]. Reproductive, developmental and immunologic toxicity are plausible under some circumstances. Malathion exhibits moderate to high toxicity in nontarget organisms.

Test animals

The experiments were carried out using male albino rats (*Rattus norvegicus,* wistar strain). Two hundred eighty five (285) male albino rats (average body weight 145 g ± 10 g) were housed under uniform animal husbandry conditions in department of zoology D.A.V. Collage Kanpur.

Vehicle and route

Samples of insecticides name endosulfan, carbaryl and malathion were dissolved in refined peanut oil (postman brand) and administered orally to all the animals of both experiments in predetermined doses for a period of 56 and 90 days,which also shows subacute toxicity[9].

Doses

The following dose schedules in both the experiments were used for the treatment of animals

I. Endosulfan -Two dosage of endosulfan were selected for the study

(i) 0.011 g/kg/day *i.e.*, 1/10th of LD_{50} for 56 days

(ii) 0.0055 g/kg/day *i.e.*, 1/20th of LD_{50} for 90 days.

II. Carbaryl - Two dosage of Carbaryl were selected for the study

(i) 0.085 g/kg/day *i.e.,* 1/10th of LD_{50} for 56 days

(ii) 0.0425 g/kg/day *i.e.,* 1/20th of LD_{50} for 90 days.

III. Malathion - Two dosage of Malathion were selected for the study

(i) 0.280 g/kg/day *i.e.,* 1/10th of LD_{50} for 56 days

(ii) 0.140 g/kg/day *i.e.,* 1/20th of LD_{50} for 90 days.

5 Treatment schedule All the 285 animals were divided equally into 15 groups with 30 animals for 56d, 35 animals for 80d. 10 + 10 animals for haemopoiecis experiment[1] of 56d & 90d and 10 animals for control group *i.e.* 95 animals for one insecticide and total 285 animal for all the three insecticides. The treatment schedule of different groups are shown in (Tables 1 and 2).

I. Clinical signs of toxicity

II. Haematological and blood chemistry studies

III. Physiological studies

IV. Histopathological studies

V. Statistical analysis

Result and Discussion

BLOOD COUNT :

The blood is a fluid connective tissue consisting of cellular components[6]-erythrocytes, leucocytes and platelets and the fluid plasma containing organic and inorganic substances. It provides a means by which the constancy of the internal environment[7] is maintained and aids in regulating the body temperature. Its chief function is the transport of oxygen, food materials, salts, water, metabolic wastes and hormones. Besides this the blood plays a significant role in giving information about the adaptation of animals to their environmental stresses.

The composition of the blood was as follows

I. Cellular fractions (Volume : 45 percent) Erythrocytes Leucocytes, platelets.

II. Plasma Fractions (Volume 55 percent)

A. Non-diffusible constituents

Albumins, Globulins, Fibrinogen, Enzymes, Lipids *etc.*

B. Diffusible constituents :

Catabolic products , urea, creatinine, uric acid *etc.* Anabolic constituents : Glucose[13], Amino acid, creatine *etc.*

Electrolytes : Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, HCO_3^-, HPO_4^-, *etc.* The results of haematological changes in animals after different treatments are shown in Tables-1, 2, 3, 4

TABLE-1: Effect of Pesticides (1/10th of LD_{50}) on total leucocyte count (TLC × 10^4) of *Rattus norvegicus*

S.NO.	*Toxicants*	Days										
		0	**7**	**% + Incr. - Decr.**	**15**	**% + Incr. - Decr.**	**21**	**% + Incr. - Decr.**	**35**	**% + Incr. - Decr.**	**56 - Decr.**	**% + Incr.**
1	***Endosulfan (0.011 g/kg/day)***	6.58 ± 0.18	7.90 ± 0.20[a]	+ 20.60	8.00 ± 0.25[a]	+ 21.58	8.50 ± 0.25[b]	+ 29.17	7.00 ± 0.19[a]	+ 6.32	7.5 ± 0.20[a]	+ 1.35
2	***Carbaryl (0.085 g/kg/day)***	6.58 ± 0.18	6.90 ± 0.19[c]	+ 4.84	7.50 ± 0.20[c]	+ 1.39	7.0 ± 0.19[c]	+ 6.32	7.50 ± 0.20[c]	+ 1.39	8.0 ± 0.25[a]	+ 2.31
3	***Malathion (0.28 g/kg/day)***	6.58 ± 0.18	6.80 ± 0.17[c]	+ 4.74	7.0 ± 0.19[a]	+ 6.32	7.50 ± 0.20[a]	+ 1.35	7.0 ± 0.19[a]	+ 6.32	7.50 ± 0.20[a]	+ 1.35

TABLE-2: Effect of Pesticides (1/20th of LD_{50}) on total leucocyte count (TLC × 10^4) of *Rattus norvegicus*

S. No.	*Toxicants*	Days											
		0	**15**	**% + Incr. - Decr.**	**30**	**% + Incr. - Decr.**	**45**	**% + Incr. - Decr.**	**60**	**% + Incr. - Decr.**	**75**	**% + Incr. - Decr.**	**90**	**% + Incr. - Decr.**
1	***Endosulfan (0.0055g/kg/day)***	6.58 ± 0.18	2.00 ± 0.13[b]	- 69.6	8.00 ± 0.21[a]	+ 21.56	6.00 ± 0.16[c]	- 8.85	8.50 ± 0.24[c]	+ 29.17	8.00 ± 0.21[c]	+ 21.56	7.50 ± 0.20[a]	+ 1.35
2	***Carbaryl (0.0425 g/kg/day)***	6.58 ± 0.18	8.00 ± 0.21[a]	+ 21.56	6.00 ± 0.16[c]	- 8.85	8.50 ± 0.24[a]	+ 29.17	8.00 ± 0.21[a]	+ 21.58	7.50 ± 0.20[c]	+ 1.35	8.00 ± 0.21[c]	+ 21.58
3	***Malathion (0.140 g/kg/day)***	6.58 ± 0.18	6.00 ± 0.16[c]	- 8.85	8.0 ± 0.21[a]	+ 21.50	7.50 ± 0.20[a]	+ 1.35	7.00 ± 0.19[a]	+ 6.32	8.00 ± 0.21[a]	+ 21.56	8.50 ± 0.24[a]	+ 29.17

respectively. The animals of different groups treated with endosulfan (1/10th of LD_{50} i.,e, 0.011 g/kg/day). The TLC was observed to be highest after 20 days which is highly significant ($P < 0.001$) while it is significant ($P < 0.05$) after 35 days (Table-1). When we treated the animals with endosulfan (1/20th of LD_{50} *i.e.,* 0.0055 g /kg/day). The TLC was observed to be highest after 60 days which is highly significant ($P < 0.05$) while it is more significant ($P < 0.001$) in 15 days and significant ($P < 0.05$) in 30 and 90 days (Table-2).

The animals of a group treated with carbaryl (1/10th of LD_{50} i.,e, 0.085 g/kg/day). The TLC was observed highest after 21, 35 and 56 days respectively which is significant ($P < 0.05$) while it is not significant ($P < 0.01$) after 7 days (Table-1). When we treated the animals with carbaryl (1/20th of LD_{50} i.,e, 0.425 g/kg/day). The TLC was observed to be increased after 15, 30, 45, 60, 70 and 90 days. The TLC was observed to be highest after 42 days. which is significant ($P < 0.05$) while it is not significant ($P < 0.01$) in 30 (Table-2).

The animals of a group were treated with malathion (1/10th of LD_{50} *i.e.*, 0.28 g/kg/day). The TLC was observed increased after 7, 15, 21, 35 and 56 days respectively. The TLC was observed to be highest after 21 and 56 days. Which is significant ($P < 0.05$) while it is not significant ($P < 0.01$) in 7 days (Table-1). When we treated the animals of group B with malathion (1/20th of LD_{50} *i.e.*, 0.140 g/kg/day). The TLC was observed to be increased after 15, 30, 45, 60, 75 and 90 days. Which is significant ($P < 0.05$) while it is not significant ($P < 0.01$) in 15 days (Table-2).

Diameter of red blood cells (RBCs) :

These are nonnucleated in rats and contain haemoglobin in cytoplasm. Usually they are by concave disc shape with and average diameter of 5 to 9 µm.

The diameter of RBC is 5.0 µm in normal *Rattus norvegicus*. In the present findings of endosulfan treated rats[5] (0.0011 g/kg/day *i.e.* 1/10th of LD_{50}) of a group, the diameter of RBC was found to be reduced to 20.0, 33.0, 29.0, 20.0, 23.0 percent after 7, 15, 21, 35 and 56 days which is more significant ($P < 0.001$) in 15 days and significant ($P < 0.05$) in 7 days(Table-3). The red blood cells showed poikilocytic, cresentric and tear shapes. Microcytic condition was observed after 56 days (Table-3).

In the next experiment in which the dose was 0.0055 g/kg/day *i.e.;* 1/20th of LD_{50} the diameter of RBC was again found to be much reduced to 19.6, 42.4, 33.0, 32.8, 28.6, 25.0 percent after 15, 30, 45, 60, 75, 90 days which is more significant ($P < 0.001$) after 30 days while significant ($P < 0.05$) after 15 days (Table-4).

In the animals treated with carbaryl (0.085g/ kg/ day) *i.e.,* 1/10th of LD_{50}) for 56 days. diameter of RBCs showed little reduction in 7, 15, 21 days to 4.1, 8.0, 8.0 percent respectively which is not significant ($P < 0.01$). But later on after 35 days of experiment, their diameter decreased much to 34.4, 23.0 percent in 35 and 56 days which is significant ($P < 0.05$). (Table-3)

The RBC showed varied shapes *e.g.,* target cells, skill cells and dumble cells . The cells showed Hypo chromic and Macrocytic condition after 56 days.

In the other experiment where the dose was 0.425 g/kg/day *i.e.,* 1/20th of LD_{50}), the diameter of RBC showed light decrease to 35.2, 9.0, 9.0 percent after 15, 30, 45 days; respectively (group-) which was not significant ($P < 0.01$) in 45 days but it decreased significantly ($P < 0.05$) to 44.6, 47.0, 47.60 percent after 60, 75, 90 days (Table-4).

In the third experiment of malathion (0.280 g/kg/day *i.e.,* 1/10th of LD_{5O}) for 56 days (group-), the diameter of RBC reduced[8, 9] insignificantly ($P < 0.05$) to 3.58, 20.0, 13.0 percent after 7, 15, 21 days while decreased a little more 28.8, 25.0 percent after 35 and 56 days, which is also significant ($P < 0.05$). The RB cells showed poikilocytic, skill, target, dumble and fragmented cells in different days of experiment. Microcytic conditions were observed after 15 days. Hematological changes and serum analysis[13] show chronic changes in hormonal level which severally damage the vital organs[14] like, liver, kidney and endocrine glands[7]. Repeated dose also causes nausea,vomiting,diarrhoeaand weakness which are common but potentially fatal causes pesticide poisoning[10].

In the next experiment of malathion where the dose was 0.14 g/ kg day i.e .1/20th LD_{50}) (group), the RBC diameter decreased to 37.0, 4.0, 34.8, 28.8, 30.6, 20.0 percent after 15, 30, 45, 60, 75 and 90 days. Which is not significant ($P < 0.01$) in 30 days while significant ($P < 0.05$) in 15 days(Table 4).

Conclusion

TLC increased significantly after 56 days and 90 days in both the doses of malathion which again shows leucocytosis which is a diseased condition. While In higher dose of endosulfan, lymphocytes initially decreased (after 15 days) and increased after 21 days while in lower dose initially increased and decreased thereafter. The Polymorphs in both the doses increased significantly. This reveals that the immunity system of animals was affected. While in the doses of carbaryl the lymphocytes increased which shows more production of antibodies due to stress full condition of both the doses. Polymorphs decreased in the higher dose and increased in the lower dose. Polymorphs in both 'the doses of malathion[13] increased which shows improved power to fight against

TABLE 3: Effect of Pesticides(1/10th of LD_{50}) on diameter of red blood cells(RBC)of *Rattus norvegicus*

S.NO.	*Toxicants*	Days										
		0	**7**	**% + Incr. - Decr.**	**15**	**% + Incr. - Decr.**	**21**	**% + Incr. - Decr.**	**35**	**% + Incr. - Decr.**	**56 - Decr.**	**% + Incr.**
1	***Endosulfan (0.011 g/kg/day)***	5.0 ± 0.14	4.05 ± 0.13[a]	- 20.0	3.35 ± 0.11[b]	- 33.0	3.55 ± 0.11[b]	-29	4.0 ± 0.13[b]	- 20	3.85 ± 0.11[b]	- 23
2	***Carbaryl (0.085 g/kg/day)***	5.0 ± 0.14	4.85 ± 0.12[c]	- 4.1	4.52 ± 0.11[c]	- 8.00	4.60 ± 0.10[c]	- 8.0	3.28 ± 0.09[a]	- 34.4	3.85 ± 0.09[a]	- 23
3	***Malathion (0.28 g/kg/day)***	5.0 ± 0.14	4.40 ± 0.13	- 3.58	4.0 ± 0.13[a]	-20.0	4.35 ± 0.12[a]	- 13.0	3.56 ± 0.10[a]	- 28.8	3.75 ± 0.11[a]	- 25

TABLE-4:Effect of Pesticides(1/20th of LD_{50}) on diameter of red blood cells (RBC) of *Rattus norvegicus*

S. No.	*Toxicants*	Days											
		0	**15**	**% + Incr. - Decr.**	**30**	**% + Incr. - Decr.**	**45**	**% + Incr. - Decr.**	**60**	**% + Incr. - Decr.**	**75**	**% + Incr. - Decr.**	**90**	**% + Incr. - Decr.**
1	***Endosulfan (0.0055g/kg/day)***	5.0 ± 0.14	4.02 ± 0.13[a]	- 19.6	2.88 ± 0.10[b]	- 42.4	3.35 ± 0.12[b]	- 33	3.36 ± 0.12[b]	- 32.8	3.57 ± 0.12[a]	- 28.6	3.75 ± 0.12[a]	- 25.0
2	***Carbaryl (0.0425 g/kg/day)***	5.0 ± 0.14	3.24 ± 0.10[a]	- 35.2	4.55 ± 0.13[c]	- 9.4	4.55 ± 0.13[c]	- 9.0	2.77 ± 0.08[b]	- 44.6	2.65 ± 0.8[b]	- 47.0	2.62 ± 0.08[a]	- 47.6
3	***Malathion (0.140 g/kg/day)***	5.0 ± 0.14	3.15 ± 0.10[a]	- 37.0	4.80 ± 0.13[c]	- 4.0	3.26 ± 0.11[a]	- 34.8	3.56 ± 0.11[a]	- 28.8	3.47 ± 0.11[a]	- 30.60	4.0 ± 0.13[a]	- 20.0

bacteria and foreign bodies. Haemoglobin percentage, in all the pesticides treated animals were observed to be much reduced more significantly P < 0.001) which shows less oxygen[10,] intake by the animals would have caused suffocation throughout the period of experiment which shows subacute toxicity[9],which was found in all the three pesticide experiment..

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