



## Selected blood parameters altered by different doses of pesticide Malathion in albino rat (*Rattus-norvegicus*)

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### Abstract

Organophosphate pesticides Malathion used as pest control agent in agriculture, household, kitchen and other places. Malathion is found in market with different trade names. It is used with its action properties against various insects and pest. It is used with the largest group of poisonous substances that are widely broadcast today. Present study is based on the effects of different doses of Malathion on routine hematological parameters like RBCs, WBCs, Hb%, CT, PT, ESR, PCV, MCV, MCH, and MCHC. Four doses were selected according to the body weight of albino rats. The doses were selected as four types on the basis of per kilogram body weight of albino rat. They were 25, 50, 75, 100 mg per Kg body weight for 7 and 15 days of treatment. The findings indicate that the above parameter fluctuates significantly as amount of dose increases. Hematological parameter indicates that the total body of the animals gets affected by the Malathion. It causes various other toxicological effects on the body of albino rats. There was a combined study of male and female albino rats.

**Key Words:** Dose, Erythrocyte, ESR, Hemoglobin, Leucocytes, Malathion Pesticides

### Introduction

Pesticides are the basic need of agriculture practice all over the world because it fulfills the aim of protection of plants from the harmful pests. Peoples are not aware about harmful action of pesticides on the human body. Workers used pesticides directly in his cropland without any precaution and prescription of experts. The use of pesticides in our country has been increasing considerably to boost the agricultural output. The beneficial effect also is obtained by pest killing effects on crops as reported by Hayes and Laws (1990). However, human beings, spray man, farmers and population in general are exposed to them and run the risk of pesticide toxicity. Cases of organophosphate toxicity caused by different pesticide have been reported in large numbers from different parts of the globe. According to WHO someone in the developing world is poisoned by pesticides every minute. Conservative estimates indicate that about fifty thousand and seven hundred people suffer from pesticide poisoning every year and about fourteen thousands of these

prove fatal. There are estimated Seventy thousand and three hundred cases of human poisoning by pesticides in developing countries every year with near about ten thousand deaths. The insecticidal property of Malathion was discovered in 1950 by American Cyanamid Company. Damalas and Koutroubas (2016) also noticed such type of problems in farmers. Since then it is extensively used as pesticides and insecticide. India's annual requirement is more than 10,000 metric tons. Such an extensive use of organophosphorus insecticide in our agriculture and public health practice may involve a good deal of risk. In order to take a realistic view of the hazardous consequent by use of pesticides by this group, the need for a critical study of nature in the extent of the likely outcome under the existing conditions in our country has become a matter of paramount importance. The study is therefore conducted to determine the Malathion induced hematological inconsistency on albino rats exposed to this pesticide.

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### Material and Methods

#### Selection of Pesticide

For present study Malathion pesticide was selected because of their maximum use in the agriculture field in Indian scenario. It is an organophosphorus

insecticide. It is sold in the trade name Cythion or Malathion. It is brownish liquid. Malathion is common pesticide accepted in different forms by the local farmers. It is widely used for pest control in vegetable and field crops.

### Experimental Animal

Sexually mature albino rats (*Rattus-norvegicus*) of controlled breed were procured from Humdard University Delhi and kept under controlled condition in Animal house of Zoology Department D.N. (PG) College Meerut U.P (India). Rats are selected for experiment as 60 to 200 gm. of their body weight. Before commencement of experiment the animals were acclimatized under laboratory conditions for a week according to the guideline of animal ethical society India. During acclimatization the normal water and food was given to animals. Then 40 rats for experiment are divided into five groups, 8 rats in each group, according to the dose of Malathion, were decided. After that animals group A was consider as control and remaining four groups were treated as experimental groups. Different doses of Malathion administered up to 7 and 15 days by the help of syringe. The dose of Malathion given to different experimental groups as Group A-Control (No dose administered)

Group B-25 mg per kg body weight per day.

Group C-50 mg per kg body weight per day.

Group D-75 mg per kg body weight per day.

Group E-100 mg per kg body weight per day.

For haematological study animal are slaughtered after 7 and 15 days of treatment. The blood of slaughtered rat was collected by direct hart punctured by syringe. The blood sample was collected into 2 sample tubes: one was with EDTA (1-2 IU/ml) and another was without EDTA.

### Parameters of Hematological studies:

- RBCs were counted by neumbour's haemometer using Hayem's solution.
- Hemoglobin (Hb%) was estimated following Sahli, (1962) method.
- WBCs was counted by neumbour's haemometer using Turk's solution.
- Packed Cell Volume (PCV) by Wintrobe's method (3000 rpm for 1 hour).
- MCH, MCV, MCHC by standard formula as
- 

$$MCH = \frac{Hb \text{ in gm. per } 100 \text{ ml of blood}}{RBCs \text{ in million mm}^3} \times 10 \text{ (Pg)}$$

$$MCV = \frac{PCV \text{ per } 100 \text{ ml of blood}}{RBCs \text{ in million mm}^3} \times 10 (Fl)$$

$$MCHC = \frac{Hbin \text{ gm per } 100 \text{ ml of blood}}{PCV \text{ per } 100 \text{ ml of blood}} \times 100 \text{ (g/l)}$$

- Clotting Time (CT), Prothrombin Time (PT) by Lee and White method.

**Statistical study:** Data is collected and calculated by Standard deviation to calculate the variation of parameter. For statistical significance we applied student T test. T test is defined as the quantity representing the difference between the sample mean and true mean expressed as

$$t = \frac{\text{difference between sample mean}}{\text{standard error of the difference between mean}}$$

Calculate the t value i.e., the ratio between the observed difference and its standard error by the formula.

### Results and Discussion

#### RBCs value and Haemoglobin (Hb %)

RBCs play a vital role to gaseous exchange in between lungs and cells of the body. Deficiency of Erythrocytes is diagnosed in the anemia condition. Result present in table 1 clearly indicates that the number of RBCs suddenly decline with the increasing dose of pesticide. It was observed that dose of 25mg per kg body weight per day of Malathion, the RBCs (erythrocytes) decreased gradually with 3.31% at 7 days and 4.31% after 15 days as shown in (Fig.1) in comparison with the control group. Above results are found in agreement with the previous findings of Naik *et al.* (2018) who on their study observed that the chlorpyrifos cause reduction in RBCs, Hb, PCV whereas WBC count shows increased level in single, double and multiple doses respectively. Bhai *et al.* (1971) also found the similar results. But with a dose of 50mg, 75mg, 100 mg per kg body weight per day number of RBCs decreased gradually after 7 days by 28.70% and after 15 days of treatment the RBCs decreases by 38.7% in comparison with the control group. Our findings were found similar to the findings of Rahman *et al.* (1990) who concluded that after treatment of Chlorpyrifos, anemic condition was reported due to decrease in RBCs and hemoglobin amount.



Selected blood parameters altered by different doses of pesticide Malathion

Table 1. Selected parameter of blood of albino rats fluctuate with different dose of Malathion

		Hb gm/dl	RBCs Mill/mm <sup>3</sup>	WBCs 10 <sup>3</sup> / mm <sup>3</sup>	PCV%	ESR mm/hr	PT sec.	CT minute	MCH pg	MCV fL	MCHC g/dl
<b>Control</b>	mean	14.8	17.8	8.9	46.10	11.00	22.5	2.62	7.52	25.85	29.37
	±SD	±0.08	± 0.62	±0.68	± 2.5	±1.06	±2.67	±0.91	±0.78	±2.67	±4.12
<b>Dose 25 mg/kg.b.wt/day</b>	7d	12 ± 1.51	17.21±	9.5	43.5	12.00	25.00	3.12	7.00	25.59	27.72
	mg		1.74	±1.41	± 3.85	±1.51	±0.75	±0.64	±0.95	±4.12	±3.85
	15 d	11.37	17.03	9.9	46.12	11.75	24.37	3.75	6.73	27.29	24.83
		±1.18	±1.44	±1.68	±3.87	±1.66	±0.51	±1.16	±1.03	±3.60	±3.56
<b>Dose 50 mg mg/kg.b.wt/day</b>	7 d	10.12	16.59	9.9	42.75	12.25	25 ±0.75	3.12	6.10	25.87	23.76
		±1.55	±1.10	±1.68	±1.90	±1.66		±1.24	±0.92	±2.23	±4.22
	15d	9.87±	15.77	10.5**	41.75	13.87	25.87	3.5 ±1.30	6.31	26.65	23.56
		1.72	±1.22	±1.03	± 2.25	±1.35	±1.88		±1.37	±2.99	±3.45
<b>Dose 75 mg mg/kg.b.wt/day</b>	7d	9.5	15.42	10.6	39.5	15.12	27.25	4.5***	6.14	25.74	24.11
		±1.41	±0.98	±1.05	±1.77	±1.35	±1.90	±0.72	±0.76	±2.72	±4.11
	15d	9.5**	14.93**	12.5**	36.25*	16.87**	27.15	5.37***	6.35	24.40	26.14***
		±1.77	±1.14	±1.21	±2.25	±1.24	±3.01	±0.51	±1.02	±2.29	±4.32
<b>Dose 100 mg mg/kg.b.wt/day</b>	7d	8.1***	12.69**	14.4***	31.5**	18.25***	32.25	5.75***	6.39	25.08	25.29***
		±.51	±1.47	±1.03	±1.77	±1.66	±1.18	±0.46	±1.47	±2.89	±3.98
	15d	7.75***	10.90***	16.9***	30***	21.25***	36.12	11***	7.22	27.61	26.15***
		± 1.66	±1.33	±1.25	±2.82	±3.19	±3.60	±1.06	±1.06	±1.84	±6.52
* p<0.05, **p<0.01, ***p<0.001											



Similar findings were reported by Kumar (1990) Taylor *et al.* (1968) who also reported the reduction in RBCs and Hb%. Prabhavati and Tombi (1988) also reported inhibition of RBCs and Hb% due to toxicity of pesticides.

### Hb Value

Hb findings of the study are represented in table 1. Study shows that haemoglobin concentration was found directly correlated to the RBCs count. Findings of the experiment indicates that when rats were treated with dose of 25mg per kg body weight per day the haemoglobin decreases insignificantly after 7 days of treatment as shown in fig. 2. The decrease was observed as 18.91% and after 15 days of treatment the Hb% decreased significantly by 23.17%, but with 50mg and 75mg per kg body weights of animal the value of Hb% decrease randomly after 7 days by 31.62 % and after 15 days it was decreased by 33.31%. After treatment with dose of 100mg per kg body weight of animal, the value of haemoglobin was drastically reduced by 45.9% and after 15 days it was reduced by 47.62 % as compared with control group. Similar findings were observed by Kartheek and David (2017), according to them an insecticide Phenylpyrazole causes significant changes of haematological parameter like RBCs Hb% etc. Similar findings were also given by Zartizian *et al.* (1961) as per their study infected Malathion 1/P in rats @ 500 mg/kg there was 70, 53 and 61% inhibition of brain RBCs and plasma ChE respectively after two hours. Sharma and Saxena (1983) analyses that haemorrhage conditions are found and decrease in RBCs and Hb%, they reported that the size of cell also reduces. Previously Jung- Hoon *et al.* (2005) reported the decline number of RBCs and haemolysis and due to either an increase in the rate at which haemoglobin concentration may be destroyed or a decrease in the haemoglobin synthesis. Conflicting findings with our result was found by Yekeen *et al.* (2007). They analyse in mice that pyrethroid deltamethrin effects on RBCs and other parameters were not noticeable. Akinrotimi *et al.* (2009) and Ambali *et al.* (2010) on *Tilapia guineensis*, Solanke and Singh (2000) observe the similar effects of thiodon in rats and find similar result.

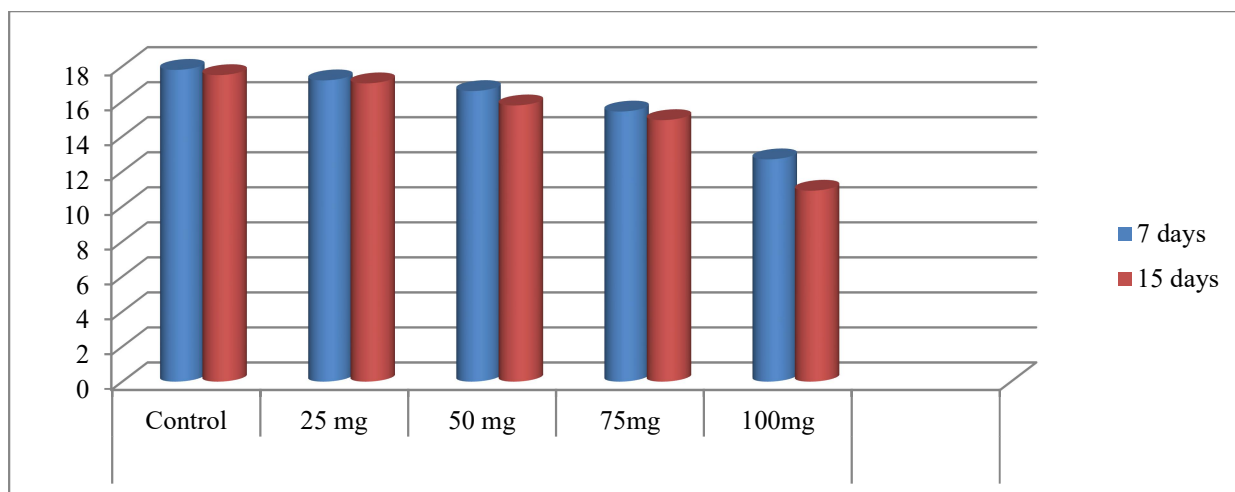
### PCV and WBCs

The value of mean packed cell volume are represented in table 1. Results of the findings of

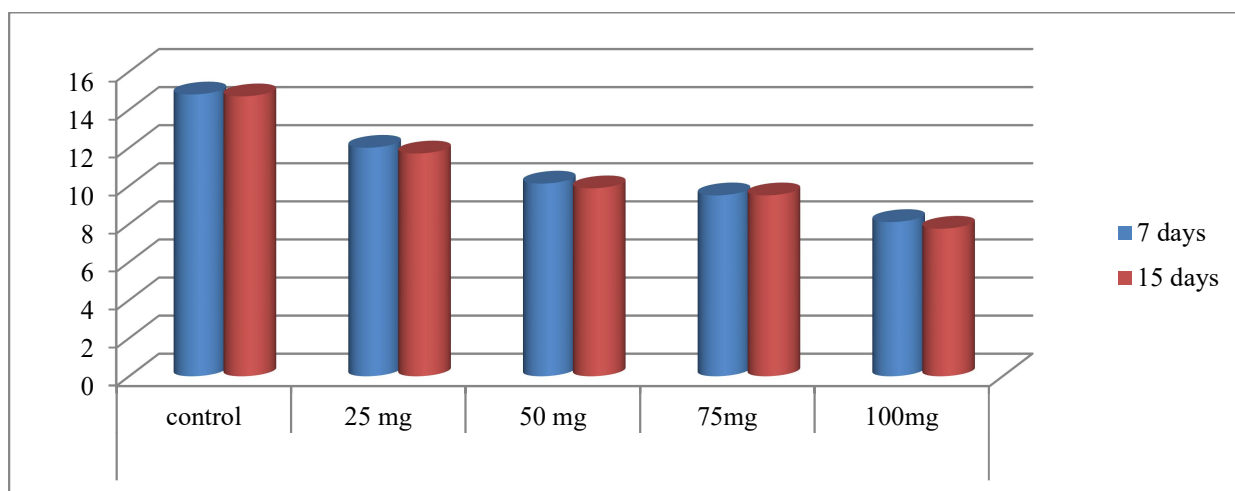
PCV when compared with control group were found insignificant decrease at dose of 25mg per kg body weight per day after 7 days decrease of 5.6% and after 15 days of treatment decrease of 0.04% was observed but with a dose of 50mg per kg body weight per day 7.2% decrease was observed after 7 days while after 15 days of treatment the PCV decreased to 9.4%. Our findings were in agreement with that of Madhavee (2006), who reported that the number of erythrocyte decreases due to ribosomal abnormalities in mice, which clearly indicates that the toxic agents directly affect the genetic material of the cell. The PCV decreased gradually after 7 days which was observed as 31.67% while after 15 days it was noted as 34.92% decrease as shown in Fig (3) and Table 1. Our findings countered to the previous observation of Prasad and Rai (1988) who reported increase in PCV in rats treated with 10% BHC. During the present study in the rats the PVC value decreases significantly in comparison with low dose to high dose of pesticide but increase in same pesticide dose in between 7 to 15 days in rats. WBCs values observed during experimental studies are presented in table 1 which indicates gradual increase as compared to control group this may be due to body response of animals against toxic exogenous substance i.e. Malathion. A gradual increase in the value of leukocyte (WBCs) was found due to the toxic effect of Malathion as represented in fig. 2. Table 1 indicates that the number of WBCs increases slightly at lower dose of 25mg per kg body weight per day but at the dose of 50mg per kg body weight per day the number of WBCs increases simultaneously. Graphical value indicates that during treatment with 75mg per kg body weight per day and 100mg per kg body weight per day dose the numbers are significantly increased. Our findings are in agreement with that of Singh *et al.*, (2016) who reported that the number of WBCs increase and decrease depends on the surroundings. Same observations were noticed by Das and Mukherjee (2003) who reported the number of WBCs increase due to stimulation of lymphopoiesis. Other works find the similar incensement of WBCs in experimental animal as effects of aldrin by Kumar *et al.* (1996). Choudhary and Joshi (2002) reported the effect of endosulfan pesticide. Rahman and Siddiqui, (2006) reported the effects of cypermethrin pesticide. Siddiqui, (2004) found that after exposure of imidacloprid and quinalphos did not effects the PCV and Hb level in White leghorn cockerels.



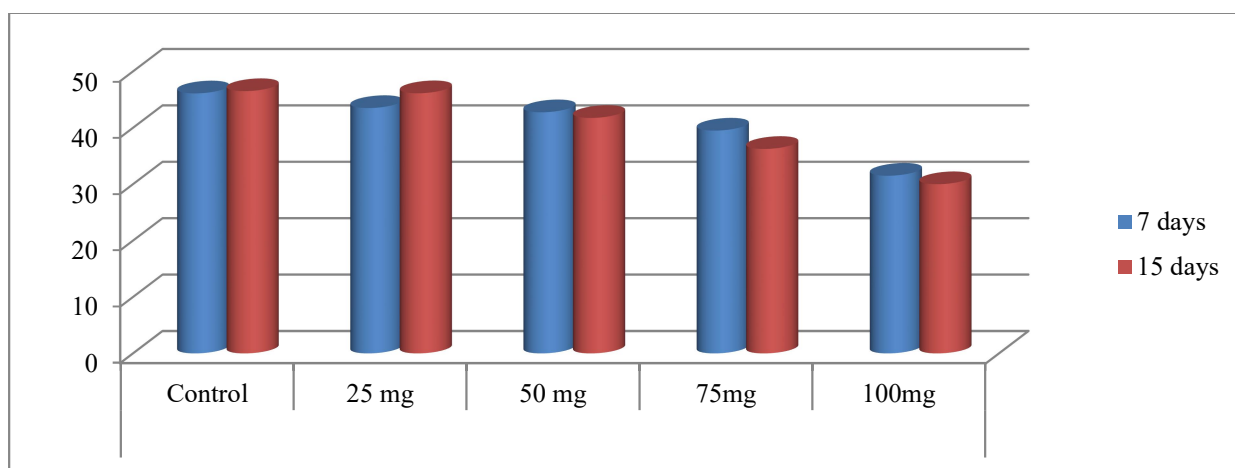
### Selected blood parameters altered by different doses of pesticide Malathion



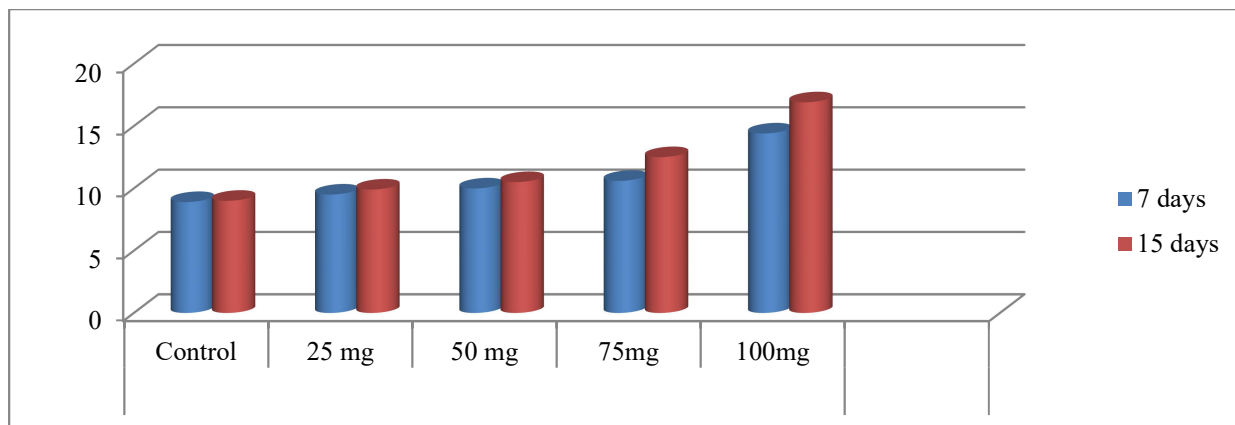
**Fig 1. Effect of different dose of Malathion on RBCs million/mm<sup>3</sup>**



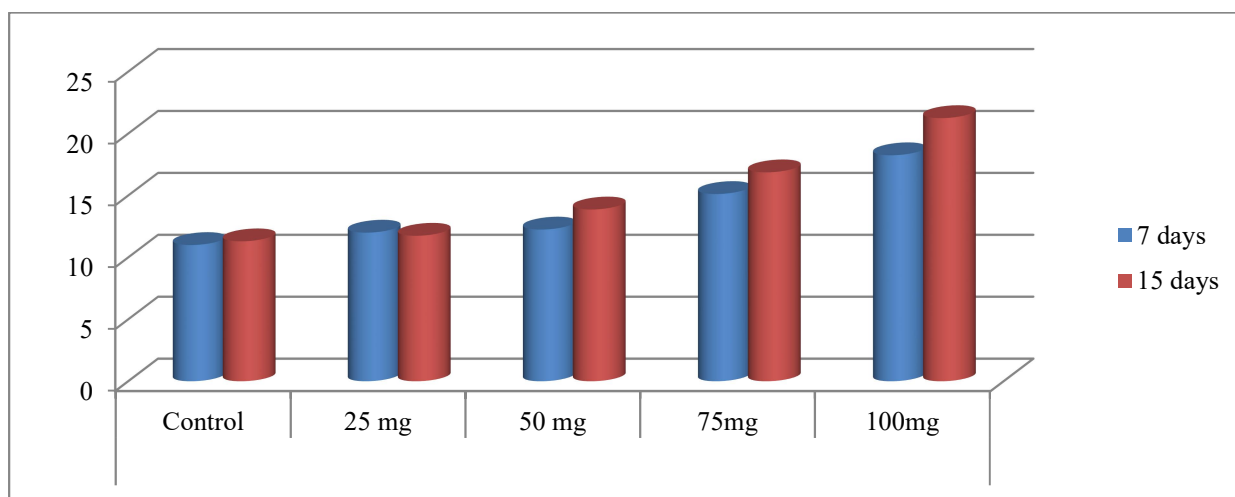
**Fig 2. Effect of different dose of Malathion on Hb gm/dl**



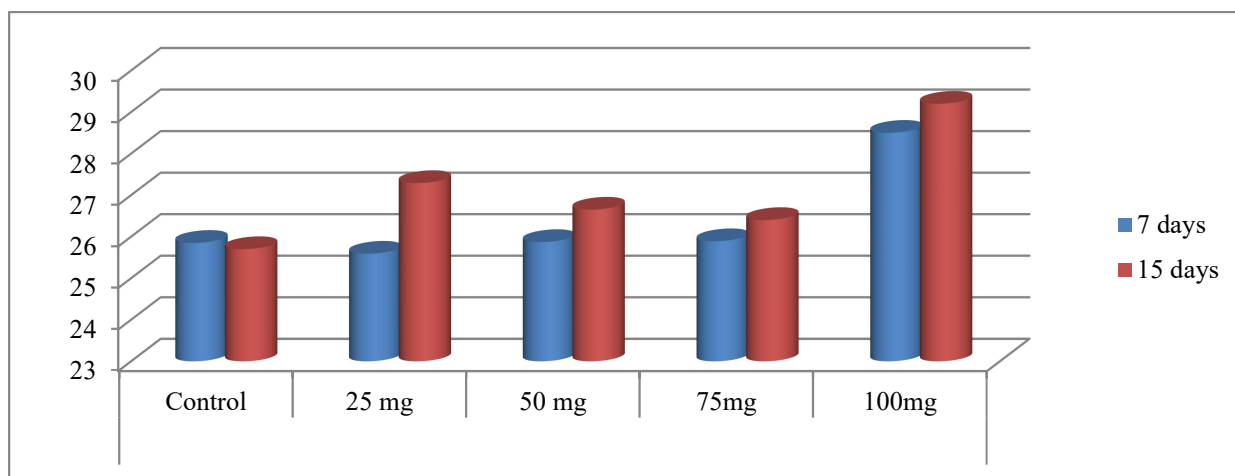
**Fig 3. Effect of different dose of Malathion on PCV %**



**Fig 4. Effect of different dose of Malathion on WBCs cubic/mm<sup>3</sup>**

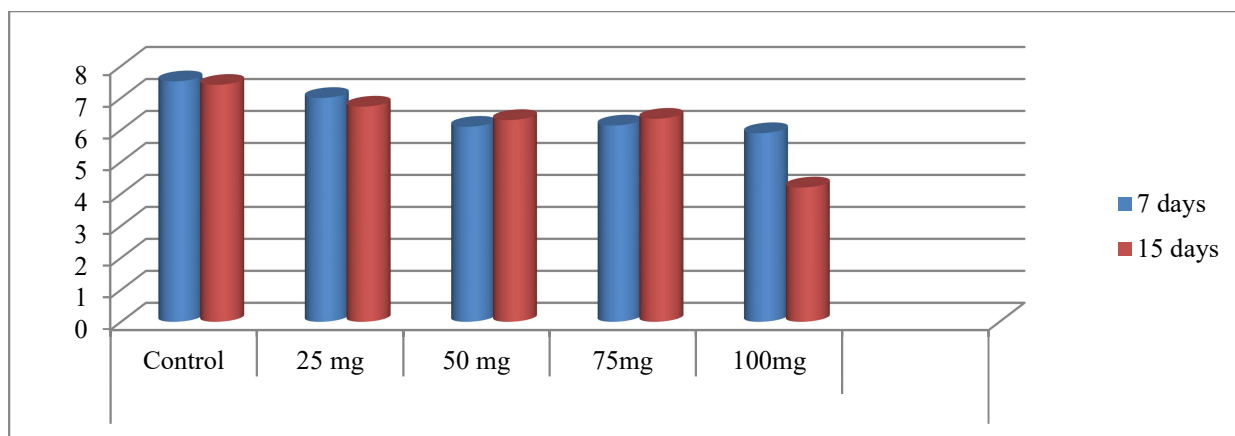


**Fig 5. Effect of different dose of Malathion on ESR mm/hr**

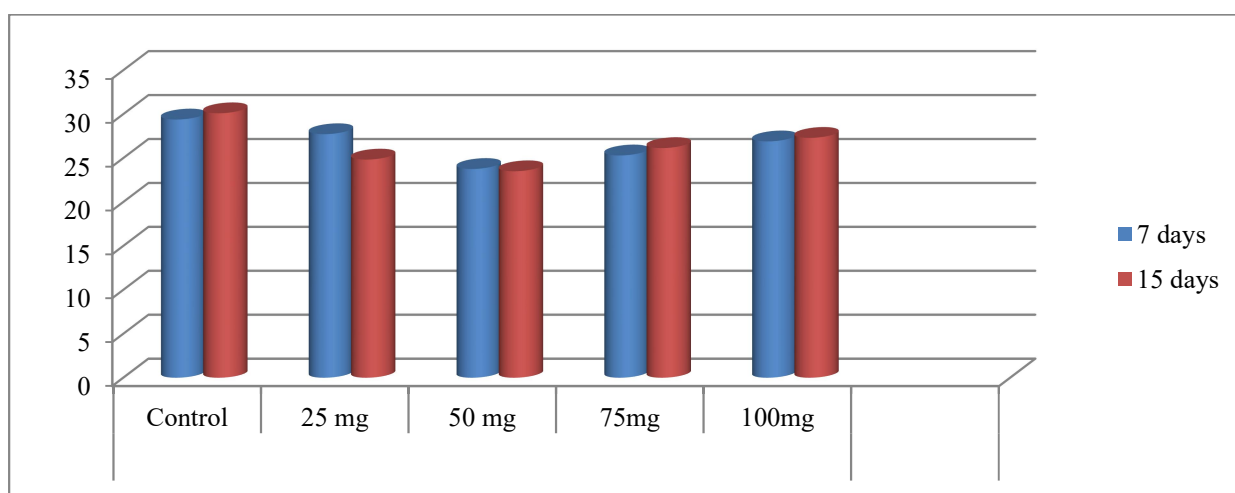


**Fig 6. Effect of different dose of Malathion on MCV (fl)**

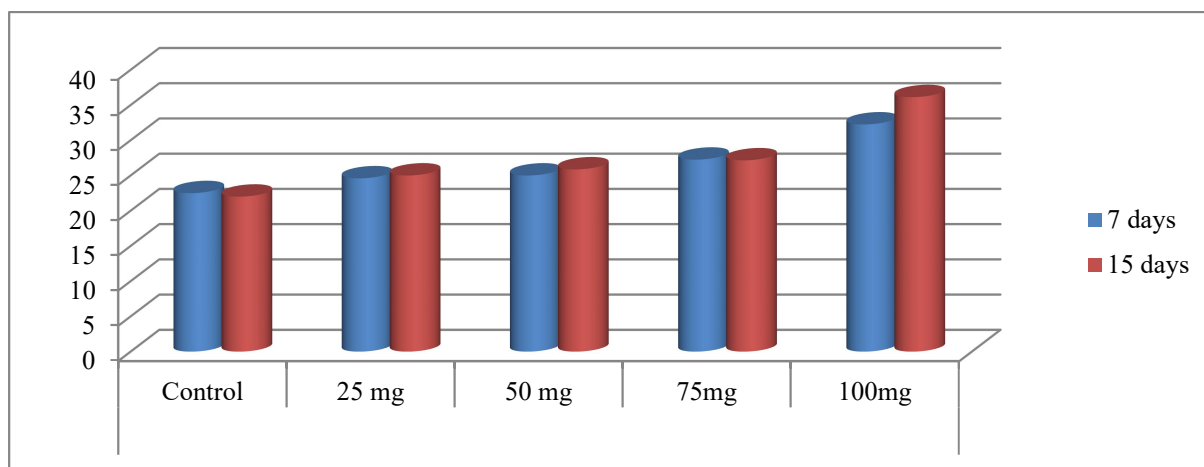
# Selected blood parameters altered by different doses of pesticide Malathion



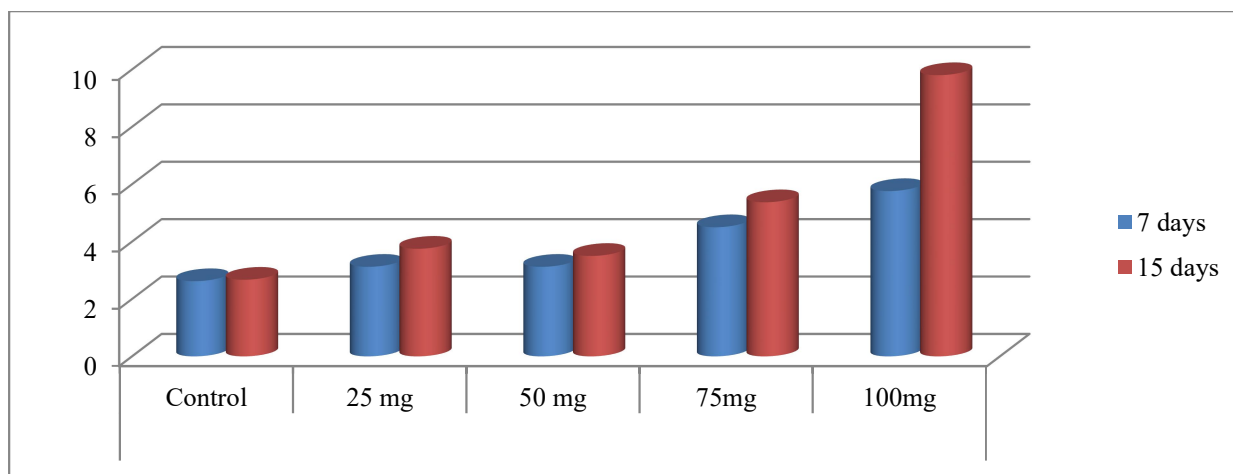
**Fig 7. Effect of different dose of Malathion on MCH value (pg)**



**Fig 8. Effect of different dose of Malathion on MCHC (g/dl)**



**Fig 9. Effect of different dose of Malathion on P.T./sec**



**Fig 10. Effect of different dose of Malathion on C.T (min)**

Bharti (1994) also reported toxicity of insecticide and behaviour of the blood clam of *Anadara granosa*.

#### **ESR and MCV**

Erythrocyte sedimentation rate and Mean corpuscular volume observations are given in table 1. The concentration of the erythrocytes in the blood was found increased during study period. The ESR represents (Fig. 5) a non-specific response to tissue damage and denotes the presence of disease, nephritis, acute hepatitis etc. When a dose of 25mg per kg body weight per day of Malathion following variations in the ESR observed insignificant an increase after 7 days of treatment was found as 9.09% while after 15 days an increment of 6.8% was observed. But with a dose of 50mg per kg body weight per day ESR was found increased after 7 days by 11.36% and after 15 days of treatment the ESR increased by 26.09% .With the dose of 75mg per kg body weight per day and 100 mg per kg body weight per day dose of malathion at 7 days the ESR increased by 37.45% while after 15 days it increases by 53.36%.

#### **MCV (Mean Corpuscular Volume)**

With dose of 25mg per kg body weight per day of Malathion an increase after 7 days by 1% and 15 days of treatment by 5.57% was observed with a dose of 50mg per kg body weight per day MCV increases after 7 days by 0.07% and after 15 days it was observed as 3% while with a dose of 75 mg/kg body weight/day and 100mg per kg body weight per day of Malathion it was observed as 2.9% and after 15 days of treatment the MCV increases to 6.8% as shown in fig 6.

#### **MCH (Mean corpuscular Hemoglobin)**

MCH observed is indicated in fig 7 the amount of haemoglobin in the red blood cells was found correlated with the MCV and MCHC. The value present in table 1 was calculated by the formula and found that the dose of Malathion causes variations in the MCH. MCH decreased after 7 days of treatment by 6.9% and after 15 days of treatment the MCH decreased by 10.5%. Findings were in agreement with those of the observations of Kumar *et al.* (2001) who reported that dimecron cause the changes of haematological parameter in fish *Heteropneustes fossilis*.

#### **MCHC (Mean Corpuscular Hemoglobin Concentration)**

Result of different doses of MCHC are indicated in fig 8. MCHC was found decreased after 7 days of treatment by 5.61%. while after 15 days it decreases by 15.45%. With a dose of 50 mg/kg body weight/day decrease was observed as 19.10% after 7 day and after 15 days of treatment decreased was found as 19.78%. With the dose of 75mg per kg body weight per day and 100mg per kg body weight per day MCHC decreased by 17.90% after 7 days and 10.90% after 15 days these results were found similar to those of Mehreen and Yousafzai (2017) who reported the negative effects of insecticides on rabbit. The Malathion pesticide increases the number of WBCs and RBCs, MCHC but some parameter were found decrease as Hb, MCV, and MCH etc. Atamanalp *et al.* (2002) concludes that the value of MCHC and other parameter of haematology of rainbow trout exposed to cypermethrin. Parma *et al.* (2007) reported the



findings of the effect of cypermethrin similar to our findings in *Prochilodus lineatus*.

#### PT (Prothrombin time)

A Prothrombin time (Fig 9) is determined time in which fibrinogen, Prothrombin was made. When a dose of 25mg per kg body weight per day of Malathion following variations in the PT was observed an increase in 7 days of treatment was found as 11.1%. While after 15 days of treatment PT increases by 8.3%. With a dose of 50mg per kg body weight per day PT was found increased after seven days of treatment by 11% after 15 days of treatment increased was found as 14.97%. With 75mg per kg body weight per day dose of Malathion for 7 and 15 days PT increase by 21.1% after 7 days of treatment and 23.3% respectively. When a dose of 100mg per kg body weight per day animals was administered the PT increase by 43.3% after 7 days of treatment and increase was highly significant as shown in (fig 5) after the treatment of 15 days the increase was observed 60.5%. Hymavathi and Rao (2000) reported that lead also cause haematological changes in *Channapunctatus*.

#### CT (Clotting Time)

The clotting time is the time clinched between haemorrhage to clot the formation. When 25mg per kg body weight per day of Malathion following variations in the CT(Fig-X) were observed an increase in CT was found as 19% after 7 days of treatment and after 15 days of treatment the increase was observed as 43.13%. With a dose of 50mg per kg body weight per day following variation CT increased by 1.9% after Seven days of treatment and after 15 days of treatment the increase was found as 33.5% with a dose of 75mg per kg body weight per day for 7 and 15 days CT was found increased by 7.1% and 10.4% respectively. When animals were orally administered with a dose of 100mg per kg body weight per day the CT (clotting time) increased after 7 days of treatment by 11.9% while an increase after 15 days of treatment was found as 31.9%. The above findings are in agreement to those of Kori-Siakpere and Oboh (2011). Mehra *et al.* (2014) also reported similar findings. Reena *et al.* (1989) find the similar changes in Rat. Zaratzizian *et al.* (1961) injected Malathion i/p in rats @ 500 mg/kg. After 2 hours there was 70.53 and 60% inhibition of brain RBC and plasma ChE respectively. After 125 hours of treatment the

inhibition was found 7.14 and 11%. Distribution and excretion study in cattle were carried out by oral administration of p32 labelled Malathion by O'Brain (1967) who observed that 90% of the drug was excreted in the urine and rest through the faeces. They further observed that 69% of the dose was excreted in 4 days after the first dose and 23% in the next 2 to 3 days. Bigoniyaa *et al.* (2015) find that the exposure of artesunate in rat changes the biochemical and hematological parameter. Garg *et al.* (1989) reported induced haematological and biochemical abnormalities in *Heteropneustes fossilis* by Manganese. Pickering *et al.* (1962) observed deleterious effects at the cellular level in the fish caused by intoxication of Malathion. Similarly Pasarela *et al.* (1962) observed that with 800 ppm of Malathion for 41 to 44 days of experiment in cows no Malathion was found in blood, kidney, heart, muscles and fat. Isaac *et al.* (2013) reported the blood parameter of different breed of rabbit. Magar and Dube (2012) reported that the blood parameter of fish *Channa punctatus* effected by Malathion pesticide. Nagarjuna, (2007) find the similar findings in albino rats. Pimpao *et al.* (2007) previously find the effects of deltamethrin on hematological parameters and biochemical parameter.

#### Conclusion

The animals administered with different doses of Malathion, gradually lost their activity and became lethargic. In all the cases Hb% and RBCs were lowered, also macrocytic anemia was visible as there was an increase in MCV. Erythrocytes in rodent blood were very much enlarged macrocytic anemia, the liver and RBCs are the main storage sites of foliate. It plays an important role in cellular metabolism by acting as co-enzyme for important biochemical reactions. Our findings on WBCs number, PT and CT time increase which indicate the toxic effects of Malathion pesticide. Because this pesticide is generally used in farming practice hence we suggest that the use of pesticide needs very carefull handling with proper precaution. Higher intake of protein diet may further reduce the hazards of pesticides.

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