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DOSE DEPENDENT HAEMATOLOGICAL EFFECTS OF MONOCROTOPHOS AND AMELIORATIVE ROLE OF *EMBLICA OFFICINALIS* ON WISTAR RAT

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ABSTRACT:

For many years, agriculture has utilised the organophosphate pesticide monocrotophos (MCP). Since it is exposed to the agricultural community on the job, determining its safe and harmful levels has become a critical problem. The goal of this study was to determine if *Emblica officinalis* might protect albino rats from the harmful effects of monocrotophos. For 30 days, monocrotophos was given at sub-lethal dosages of 0.5 mg/kg and 1 mg/kg body weight. Wistar rats that were mature and in good health were divided into five groups: GI, GII, GIII, GIV, and GV. In comparison to the control, the findings obtained showed substantial changes in the values of Hb, RBC, WBC, PCV, MCV, MCH, and MCHC. The drop in RBC indicated that the rats' circumstances were anaemic. In the current study, there was also a notable increase in WBC. But following *Emblica officinalis* (20 mg/kg/bw) therapy, those parameters significantly changed in the monocrotophos-treated group. Therefore, it can be inferred from the aforementioned study that although the entry of these dangerous pesticides (monocrotophos) into our bodies cannot be stopped, the problem can be resolved to a large extent by using these medicinal plant extracts as potent antidotes, normalising the physiology of the body, and maintaining the cellular integrity and normal functioning of the system.

Keywords: Monocrotophos, toxicity, *Emblica officinalis*, albino rats, cellular integrity.

INTRODUCTION:

Pesticides are the agrochemicals used to control the insect and weed population associated with a variety of crops. The usage of pesticides is aimed to improve crop yields to meet the food requirements of the drastically increasing population of the world (Arora et al., 2019). The situation becomes worse with the indiscriminate use of these toxic chemicals, resulting in environmental pollution and toxicity of various nontarget organisms (Bilal & Iqbal, 2019). Continuous use of pesticides has adverse effects on the health of target as well as non-

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target organisms through their carcinogenic, mutagenic, and teratogenic properties (Amanchi & Hussain, 2010) (Gyawali, 2018). Organophosphate pesticides are used extensively both in developing and developed countries resulting in annual exposure of 2–3 million people. These adversely affect almost all the organs including the liver, kidney, intestine, lungs, gonads, and brain (V. Kumar et al., 2010).

Monocrotophos is one of the widely used organophosphorus pesticides. It is a non-specific and systemic insecticide (Fox, 1995) belonging to the vinyl phosphate group. Monocrotophos is manufactured using monocloromonomethyl aetoacetamide and trimethyl phosphate. It is highly toxic in nature and affects many beneficial, non-target organisms like honey bees, fishes (Thangnipon et al., 1995), birds, and mammals (Skripsky & Loosli, 1994). But, it is still in use in some developing countries like India due to lake of awareness and alternative replacements (Banerjee et al., 2000). Studies were carried out on workers working with pesticides without any prevention measures by WHO and it was revealed that, in most of the cases, residues of monocrotophos were detected near about 20 mg, it works by inhibiting acetylcholine esterase and inhibiting plasma acetylcholine esterase was observed in all these cases. The toxicity of pesticides on mammalian species includes the effect on biochemical, neurological and haematological function (David et al., 2014).

Blood is a very good medium for assessing the health status of animals. Haematological indices are the important biomarker for suggesting altered internal and external environment of animals and variations in their indices within an individual can result in inadequate responses to chemical stressors; however, these variations are nonspecific to a wide range of substances (Prashanth MS et al., 2006). Exposure to chemical pollutants especially pesticides is thought to either increase or decrease haematological levels (Kori-Siakpere o, Ec Oboh et al., 2011) often affecting the survival of the exposed animals. Hence, in the evaluation of health conditions and outcomes from pesticide-induced toxicity within organisms, parameters under haematological aspects are exercised as indicators (Pimpo CT et al., 2007).

Emblica officinalis (Amla) also known as Indian gooseberry is perhaps the most important medicinal plant in the Indian traditional system of medicine (Krishnaveni M et al., 2010). Amla is a source of phenolic compounds, which include phenolic acids and flavonoids. Apart from vitamin C, amla also contains cytokine-like substances such as zeatin. The dried Amla fruit is useful in the treatment of haemorrhage, diarrhoea, and dysentery and is the best antioxidant known in India (Liu X et al., 2008). Hence, the present work aims to study the haematological effects of monocrotophos toxicity of albino Wistar rats and also the co-administration of *Emblica officinalis* to evaluate the ameliorative role and modulate this toxic effect.

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MATERIAL AND METHODS

Ethical approval

Ethical approval was obtained from the Ministry of Fisheries, Animal Husbandary and Dairying, Committee (CPCSEA), Government of India, New Delhi (SBCP/2021-2022/CPCSEA/IAEC/I(1)/F16/206).

Animals and chemicals

Healthy adult albino rats (*Rattus norvegigus*, Wister Strain) (weight approx. 170-220 g) were obtained from a local supplier and used in this study. The animals were housed individually in the laboratory in plastic cages at 21-24°C ± 3°C temperature and in the uniform light-dark cycle (14:10:L:D). The animals were fed with standard commercial pellet feed and water was given *ad libitum* throughout the course of the study. The animals were quarantined for 10 days before the beginning of the experiments. Monocrotophos of commercial-grade 36% SL (Hilcron) of Hindustan Insecticides LTD was obtained locally. All other chemicals used for experiments were purchased from Merck or Hi-Media and were of analytical grade.

Selection of dose

According to (Finney, 1971) using the probit analysis technique the oral LD 50 of Monocrotophos for a rat is 12mg/kg/body weight, which was similar to that calculated by Janardhan et al., 1986. Two doses were selected for the experimental studies; the low sublethal dose was taken as 1/10=0.5mg/kg body weight and the high sublethal dose was taken as 1/5=1mg/kg/body respectively for seven days a week for 30 days of acute sublethal oral toxicity. 20 mg/kg/d body weight of *Emblica officinalis* was used as a dose for the amelioration of monocrotophos toxicity.

Plant material

For this study we used *Emblica officinalis* were collected from outfield near Rani Anna Government College for women, Tirunelveli, Tamil Nadu. The plant was authenticated by comparison with reference specimens preserved at the St. Xavier's College, Tirunelveli, Tamil Nadu. Voucher Herbarium specimens were kept in the Herbarium for future references. The fruits were washed, dried and crushed to powder. 20mg crushed powder was taken for the experiment, the material was mixed with 1ml of water. It was homogenized and centrifuged at 2000 rpm for 5 minutes. The solvent and other chemicals were of analytical grade and purchased from local commercial sources.

Experimental Design

Five groups each containing six animals were used in this experiment and the dose level used were:

Group1 (GI) : control rats received only the standard diet and distilled water.
Group2 (GII) : rats treated with low dose MCP (0.5 mg/kg b.w)
Group3 (GIII) : rats treated with high dose MCP (1mg/kg b.w)
Group4 (IV) : rats treated with low dose MCP 0.5mg/kg + 20mg/kg *Emblica Officinalis*
Group5 (V) : rats treated with high dose MCP 1mg/kg + 20mg/kg *Emblica Officinalis*

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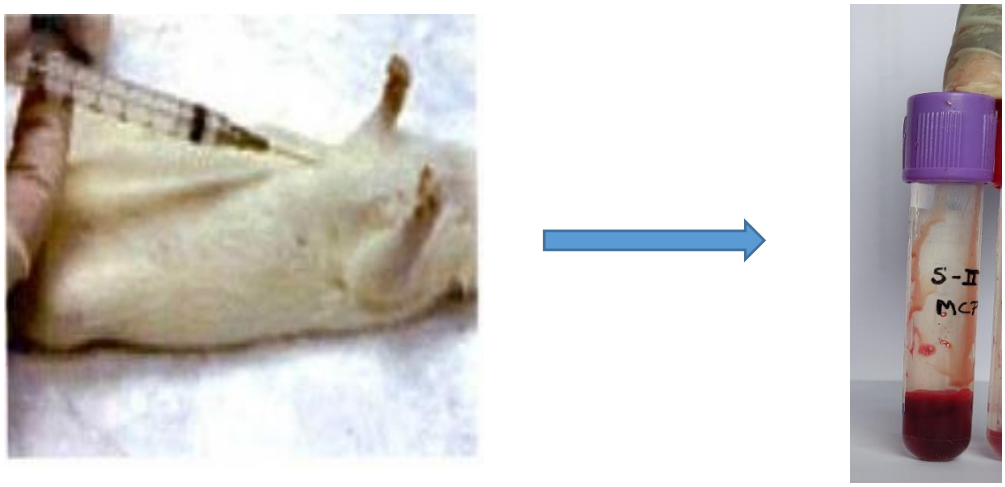
Haematological indices

Blood was drawn by cardiac puncture in anesthetized rats. Whole blood for haematogram was collected in vials containing the anticoagulant, ethylene diamine tetra-acetic acid (Figure 1). Total erythrocytes (RBC), Total leukocyte count (WBC), Packed cell volume (PCV), Mean corpuscular volume (MCV), MCH and MCHC were estimated by using the standard procedure of Rodak (Rodak LC et al., 1995), Hemoglobin (Hb) by the method of Van Kampen and Zijlstra(Van Kampen E, Zijlstra WG et al., 1961).

Figure 1: Blood sample collection by cardiac puncture in anesthetized rat in the labortary



Statistical analysis

Continuous observation, data collection and statistical analysis of the above parameters like pesticide-treated rats, herbal-treated rats and control was done as the mean ± standard error of the mean (SEM) obtained from triplicates. The data were subjected to one-way ANOVA followed by Dunnett's test, and all groups were compared with normal control *P<0.05, **P<0.01, ***P<0.001.

RESULT AND DISCUSSION:

The present investigation indicated significant variations in haematological indices of rats exposed to different sublethal doses of monocrotophos for 30 days which have been presented in Table 1 and Graph 1. No changes were observed in control rats which advertised normal count.

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Table 1: Effect of *Emblica officinalis* on monocrotophos treated rats showing haematological changes

Group	RBC	WBC	HB	PCV	MCV (µm^3)	MCH (pg/cell)	MCHC (g/dl)
Control (GI)	8.76±0.58	6.21±0.31	22.1±0.22	67.11±4.04	70.1±1.02	24.12±0.31	36.12±0.41
MCP 0.5mg/kg (GII)	5.32±0.97*	11.03±0.65*	17.11±0.49**	61.17±0.46*	83.12±0.74	34.43±0.21*	45.37±0.57
MCP1 mg/kg (GIII)	4.41±1.22*	14.02±0.22*	15.5±0.13	42.13±1.03	86.3±0.97	38.10±0.42*	58.62±0.78
MCP 0.5mg/kg + Amla 20mg/kg (GIV)	6.76±0.51**	8.10±0.05**	19.1±0.41*	64.25±3.48	82.10±0.81	29.12±0.17*	41.02±0.03*
MCP1 mg/kg + Amla 20mg/kg (GV)	6.12±0.84**	10.13±0.21*	17.5±0.27**	66.14±2.47*	72.17±0.56**	35.02±0.22*	47.12±0.77*

n=6, Data were expressed as Mean± SEM, one-way ANOVA followed by Dunnett's test, all groups were compared with Normal control, *p< 0.05, **p<0.01, ***p< 0.001

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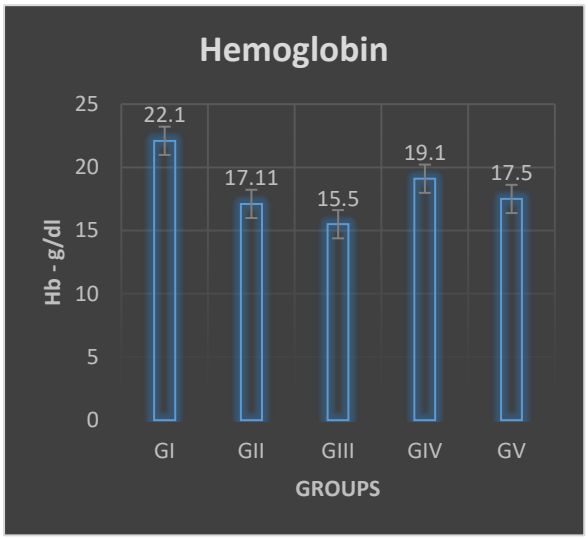
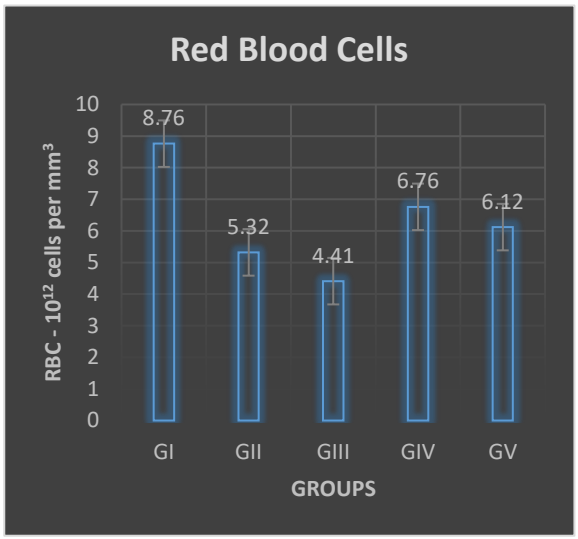
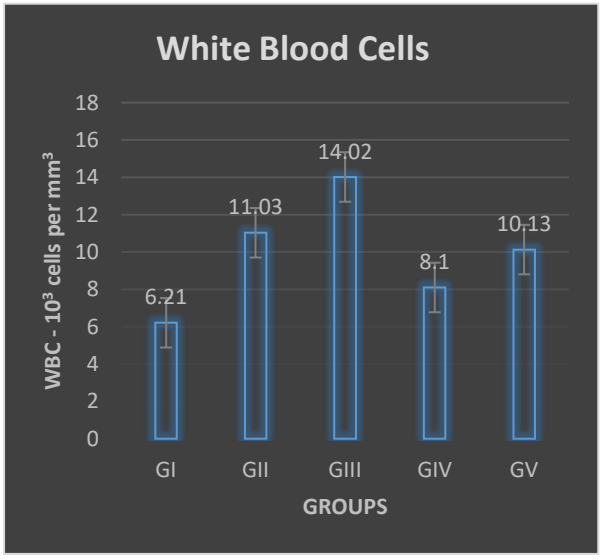
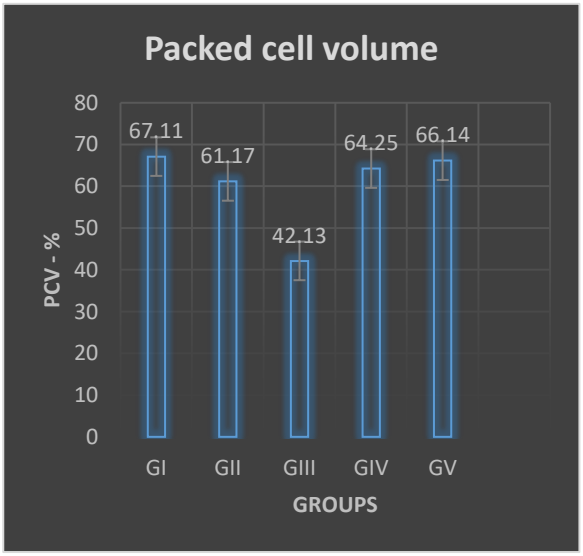
Graph 1: Effect of *Emblica officianlis* on monocrotophos treated rats showing haematological changes









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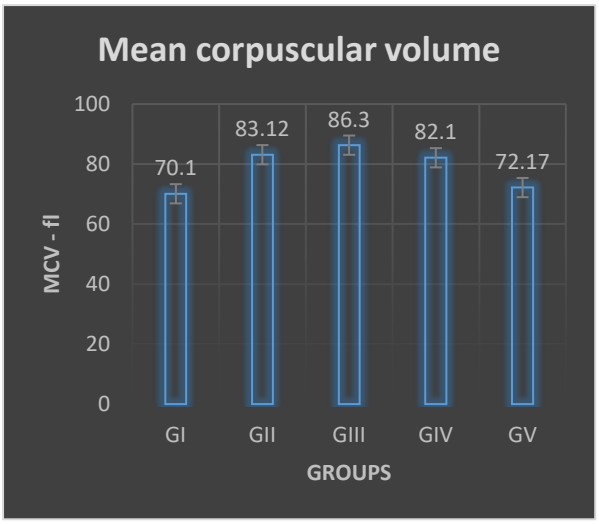

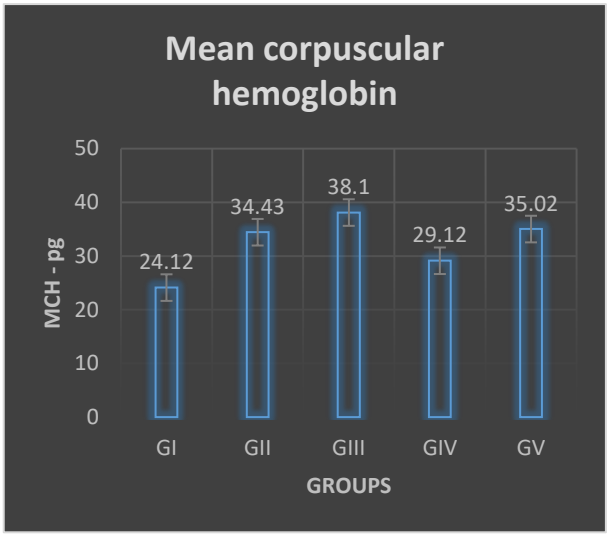

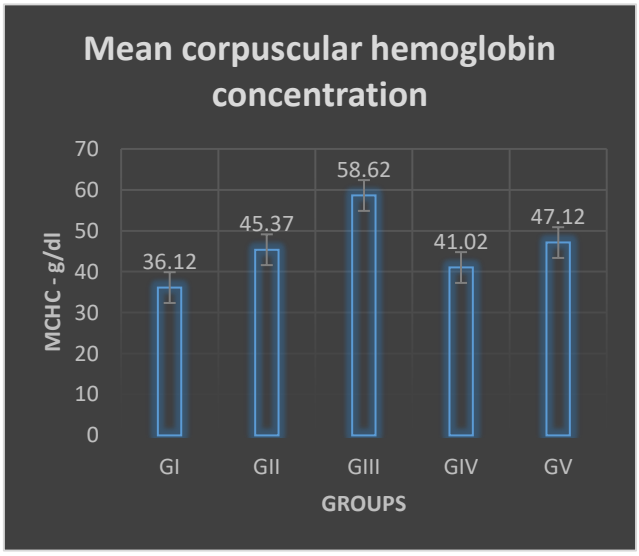

Monocrotophos exposure leads to marked alteration in haematological parameters and was found to be a potent toxic agent. Changes in haematological indices are considered to be one of the crucial biomarkers of physiological stress (Akinrotimi et al., 2009). The present investigation indicated significant variations in haematological indices of rats exposed to oral administration of different sublethal doses of MCP at the rate of 0.5mg/kg and 1mg/kg/bw for 30 days. The rats produced a significant decrease in Hb, RBC and PCV whereas co-administration of amla 20mg/kg/bw brought mild to moderate improvement in all these parameters. The Hb value of GI was recorded at 22.1±0.22. In the low dose MCP GII the value of Hb was decreased high significantly ($p<0.01$) (17.11±0.49**) and high dose MCP GIII also declined (15.5±0.13). but when amla was fed with GIV & GV the Hb value was observed rapidly increased significantly ($p<0.05$) in GIV (19.1±0.41*), high significantly ($p<0.01$) in

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GV (17.5±0.27**). It is observed from the table that RBC in the GI was (8.76±0.58) and MCP treated group GII & GIII there was fall in the value of RBC was noted significantly ($p<0.05$) (5.32±0.97* & 4.41±1.22*). When the co-administration of MCP and amla-treated group GIV & GV the RBC counts were observed to increase highly significantly ($p<0.01$) (6.76±0.51** & 6.12±0.84**). According to PCV in GI it was 67.11±4.04. it was declined in MCP treated groups GII &GIII, in GII significantly ($p<0.05$) (61.17±0.46*) & GIII (42.13±1.03). MCP with amla group GIV & GV the PCV value rose to GIV (64.25±3.48) & GV it was significantly ($p<0.05$) increased (66.14±2.47*).

Reduced levels of RBC count, Hb concentration, and PCV values in the blood of rats revealed probable damage to red cells owing to ribosomal abnormalities. These changes in Hb, RBC, and PCV values resulted in hypochromic anaemia, which was linked to an iron deficit. The lower RBC, Hb, and PCV counts seen in mice treated with monocrotophos were explained by previous research (Ali and Abdul, 1993). The acute haemorrhage that causes the blood to dilate owing to the input of cells and fluids from bodily reserves may be the reason for the considerable drop in RBC, Hb, and PCV (Celik and Suzek, 2009; Kalender et al., 2006). However, the Monocrotophos-induced deficiencies were lessened in rats treated with amla, leading to the restoration of RBC, Hb, and PCV seen in this work.

According to reports by Isaac et al., 2013, PCV is mostly linked to the delivery of oxygen and ingested nutrients. Since the current analysis found lower PCV levels, it is impossible to rule out the likelihood that oxygen and nutrition delivery is inadequate. This section of our study supports results by EI-Bakary et al., 1995 by showing that Hb concentration and PCV values are closely correlated with RBC count. However, Amla-treated rats showed improved Monocrotophos-related deficiencies, which led to the repair of RBC, Hb, and PCV seen in this study. According to WBC the value of WBC in GI was 6.21±0.31 and MCP treated Group GII & GIII the value was increased significantly ($p<0.05$) (11.03±0.65* & 14.02±0.22*). Changes of WBC in MCP with amla treated group GIV and GV the values were decreased high significantly ($p<0.01$) in GIV(8.10±0.05**) significantly ($p<0.05$) in GV (10.13±0.21*). At all times when they were exposed to monocrotophos, there was a rise in the overall number of WBCs. The animal increases its protective capacity through hyper-blastic mechanisms under diseased circumstances. Therefore, a rise in WBC counts in the current research likely points to the existence of such a defence mechanism against a potential harmful molecule attack when inducing monocrotophos toxicosis.

In the present study, there is observed significant increased in red cell indicators like MCV, MCH and MCHC. In MCV GI was observed 70.1±1.02. In MCP treated group GII & GIII it was rapidly increased like (83.12±0.74 & 86.3±0.97) and MCP with amla treated group GIV & GV it was slightly decreased in GV it was significantly ($p<0.05$) decreased (82.10±0.81 & 72.17±0.56**). According to MCH in GI it was 24.12±0.31 and MCP treated group GII & GIII the values were rise significantly ($p<0.05$) like (34.43±0.21* & 38.10±0.42*), coadministration of MCP wit amla treated group GIV & GV it was rapidly declined

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significantly ($p<0.05$) (29.12±0.17* & 35.02±0.22*). About MCHC, in GI the value recorded at 36.12±0.41 and MCP treated group GII & GIII its increased (45.37±0.57 & 58.62±0.78). when amla fed with MCP treated group GIV & GV it was rapidly decresed significantly ($p<0.05$) (41.02±0.03* & 47.12±0.77*).

The RBC count, Hb content, and PCV values affect the red cell markers such MCV, MCH, and MCHC. Changes in erythrocyte size, which may be caused by osmotic imbalance in fluid circumstances, may have an impact on MCV across all categories. Anandhkumar et alreports from 2001 showed that endosmosis is the reason of a rise in MCV. It has been discovered that the haemodilution, which results from endosmosis and moves solvent from a less concentrated solution to a more concentrated one, raises the MCV value. The findings of this study suggest that Monocrotophos may be able to physically alter the shape of rat erythrocytes, changing them from their typical discoid shape to alternative forms including echinocytes, dacrocytes, and schistocytes. The transition of echinocytes has an anti-haemolytic effect because it raises the cell-volume ratio and membrane area, allowing the cells to enlarge before lysing. The results of the current analysis showed that the MCH and MCHC levels in the pesticide-treated group were higher. Since it is generated from HB and RBC, any change in the quantities of those two substances will typically affect the MCHC and MCH. Similarly to this, Atamanalp et al., 2002 observed that exposure to cypermethrin significantly increased the levels of MCH, MCHC, and MCV in O. mykiss. Therefore, overall haematological parameters during monocrotophos exposure revealed a considerable change. In the current study, these alterations are more pronounced in high-dose treated rats than in low-dose monocrotophos treated rats. There was a noticeable improvement in those parameters when amla was given MCP as opposed to only being treated with it for 30 days. It was discovered that the groups that had pesticide treatment benefited the most from amla exposure.

CONCLUSION

Total RBC and WBC levels were observed to change when rats were exposed to monocrotophos, suggesting anaemia and the initiation of an immunological response, respectively. The decrease in Hb that was seen during the current experiment suggests that exposed animals may have experienced hypoxic conditions. Significant variations in PCV, MCV, MCH, and MCHC suggest a direct effect on the animal's overall haematological state. However, all the metrics significantly improved after using *Emblica officinalis* as a therapy. *Emblica officinalis* had the greatest ameliorative impact on pesticide-treated groups as a result.

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