

EFFECT OF CLOMAZONE ON TLC, DLC, RBC, HAEMOGLOBIN AND HAEMATOCRIT OF FEMALE WISTAR RATS

Satyendra Kumar Kashyap^{1*}, Vandana Garg²

1- **Research Scholar***, Zoology, D.N. (P.G.) College, C.C.S. University Meerut (U.P.) India.

2- **Associate Professor**, Zoology, D.N. (P.G.) College, C.C.S. University Meerut (U.P.) India.

Abstract

This study was conducted for the evaluation the effects of clomazone on Total Leukocyte count (TLC), Differential Leukocyte count (DLC), Red Blood Cells (RBC), Haemoglobin (Hb) and Haematocrit (Hct) on female wistar rats. 20 animals were divided into three treatment groups 250, 500 and 1000 mg/kg b.wt. and one control group, each group contained 5 female wistar rats. animals of three treatment groups were administered doses of Clomazone i.e. 250, 500 and 1000 mg/kg b.wt. orally for 90 consecutive days whereas, only corn oil was administered in control group animals in same manner. All animals were sacrificed on the 91st day of experiment and blood was collected for the analysis of TLC, DLC, RBC, Haemoglobin and Haematocrit from retro-orbital sinus. Statistically significant ($p < 0.05$) decrease of TLC was found in 250 and 500 mg/kg b.wt. female rats and statistically significant ($p < 0.05$) increase E% was found in 500 mg/kg b.wt. when compared with control group.

Keywords: Clomazone, Wistar rat.

I. INTRODUCTION

TLC, DLC, RBC, Haemoglobin and Haematocrit are very important indices of the physiological and pathological status for animals and humans (Adeneye et al., 2006). These indices are diagnostic tools for routine clinical evaluation of the state of health. TLC, DLC, RBC, Haemoglobin and Haematocrit provide useful information on the adverse effects of foreign components on the blood (Mishra M, et al. 2012). Clomazone 2-[(2-chlorobenzyl)]-4,4-imethyl-1,2-oxazolidin-3-one is a well-known broad-spectrum herbicide which is used on rice, peas, pumpkins, soybeans, sweet potatoes, winter squash, cotton and tobacco fields to control annual grasses and weed etc. It was produced first in the early 1980s which selectively blocks both chlorophyll and carotenoid synthesis in weeds (Ferhatoglu and Barrett, 2006) and widely use in agriculture. It belongs to the chemical group isoxazolidinone, acts as an inhibitor of carotenoid synthesis and its herbicidal activity is due to its metabolites, such as 5-ketoclomazone (EPA, 2007), (Ferhatoglu et al., 2005). Because of the broad-spectrum activity and low production costs, it is frequently used against broadleaf weeds and grass in the cultivation of soybeans (Vyas et al., 2000). It is also used for control of broad-leaf and grassy weeds such as barnyard grass (*Echinochloa crus-galli*), crab grass (*Digitaria spp.*), foxtails (*Setaria spp.*), and those infest soybean, tobacco, rice and other row crops (Scott et al. 1995; Lee et al. 2004; Schocken 1997). Farmers are repeatedly using the herbicides, which may not kill the crop plant but may get accumulated in leaves, fruits or grains and consumption of such products may result in to slow accumulation in our body. So This study was performed to observe the effects of Clomazone on TLC, DLC, RBC, Haemoglobin and Haematocrit of the female wistar rats after repeated oral exposure of 90 consecutive days.

II. MATERIAL AND METHODS

Wistar rats used for the study were randomly divided into 4 groups and housed in polycarbonate cages in an air-conditioned room with 12 air changes per hour, 19-25°C temperature, 30-70% relative humidity and 12-hour light/ dark cycle. Animals were provided with standard laboratory animal diet and filtered water *ad-libitum*. Three doses of Clomazone 250, 500 and 1000 mg/kg b.wt. were formulated in corn oil fresh daily and orally administered once daily for 90 consecutive days by using disposable syringes tipped with an oral gavage needle (18 gauge) whereas, corn oil was administered orally into the control group animals. At the last day of study before collection the blood 1-2 drop of Paracaine 0.5% for local anesthesia was given to avoid pain and fasting Blood samples were collected from all the animals. The blood was collected under isoflurane anesthesia through retro-orbital sinus puncture with the help of a fine capillary tube in K3 EDTA (1.8mg/ml of blood) tubes for TLC, DLC, RBC, Haemoglobin and Haematocrit analysis. Blood parameters were evaluated by using BC-5000 vet Haematology System (Mindray).

III. STATISTICAL ANALYSIS

All data was analyzed by one-way analysis of variance (ANOVA) and post Dunnett test between treated and control groups by the using validated software. Statistically significance of data has been reported at the 5% significance level ($p < 0.05$). A level of P value less than 0.05 was considered to be significant.

IV. RESULTS & DISCUSSION

There was increase of Total Leucocyte Count (TLC) found in 500 mg/kg b.wt. female rats whereas, decrease of TLC was found in 250 and 1000 mg/kg b.wt. female rats. The increase of TLC was found statistically non-significant ($p > 0.05$) but decrease of TLC was found statistically significant ($p < 0.05$) when compared with control group. Elevation in TLC of female rats may be indicative of activation of defense and immune system of the body (Yousef et al., 2003) and the increased defense mechanism against probable attack of toxic molecules whereas, decrease of TLC in female rats was found statistically significant ($p < 0.05$) and it may be a sign of an inflammatory response, most commonly as a result of infection. The changes did not consider relevant because these effects appeared to be unrelated to either the anesthetic dose or due to the individual animal biological variation therefore changes might not be considered as treatment related. There was slight decrease of Neutrophils (N%) was found in 250, 500 and 1000 mg/kg b.wt. female rats. These changes in the N% were found statistically non-significant ($p > 0.05$) when compared with control group. There was slight decrease of Lymphocytes (L%) was found in 500 mg/kg b.wt. female rats whereas, increase L% was found in 250 and 1000 mg/kg b.wt. female rats. These changes in L% were marginal and statistically non-significant ($p > 0.05$) when compared with control. There was increase of monocytes (M%) was found in 250, 500 and 1000 mg/kg b.wt. female rats, the increase in the (M%) was found statistically non-significant ($p > 0.05$) when compared with control. There was increase of Eosinophils (E%) was observed in 500 and 1000 mg/kg b.wt. female rats, whereas, decrease of Eosinophils (E%) was observed in 250 mg/kg b.wt. female rats. The increase in the values of E% in 500 mg/kg b.wt. female rats were found statistically significant ($p < 0.05$) when compared with control group and the alterations in 250 and 1000 mg/kg b.wt. female rats showed statistically non-significant ($p > 0.05$) when compared with control group. Significantly increase in Eosinophils counts in the females of 500 mg/kg b.wt. was not considered relevant because these effects appeared to be unrelated to either the anesthetic effect or due to the individual animal biological variation therefore changes might not be considered as treatment related. There was increase of Basophils (B%) was observed in 500 mg/kg b.wt. female rats, whereas, decrease of basophils (B%) was observed in 250 and 1000 mg/kg b.wt. female rats. The alterations in Basophils (B%) of female rats were found statistically non-significant ($p > 0.05$) when compared with control group. There was decrease of RBC was observed in 250, 500 and 1000 mg/kg b.wt. female rats. The decrease of RBC was found marginal and statistically non-

significant ($p>0.05$) when compared with control group. There was decrease of haemoglobin and hematocrit was found in 250, 500 and 1000 mg/kg b.wt. female rats. The changes in the values of hemoglobin and hematocrit were statistically non-significant ($p>0.05$) and in biological limits when compared with control group. The non-significant effect of Clomazone on the RBC, Hemoglobin and Hematocrit was observed in the all treatment group of female rats when compared to their control counterparts. This is an indication that there was no destruction of matured RBC's and no change in the rate of production of RBCs (erythropoiesis) in female rats due to the exposure of clomazone.

V. CONCLUSION

Based on results studied it is concluded that upon repeated oral administration of clomazone for consecutive 90 days, did not cause any adverse effect on Total Leukocyte count (TLC), Differential Leukocyte count (DLC), Red Blood Cells (RBC), Haemoglobin (Hb) and Haematocrit (Hct) of female wistar rats. The potential for toxic effects is no serious concern for this herbicide so farmers/users but they should make aware about hazardous effects and toxicity as well as it should be avoided in public places, schools and strict regulation of the use of Clomazone in our environment.

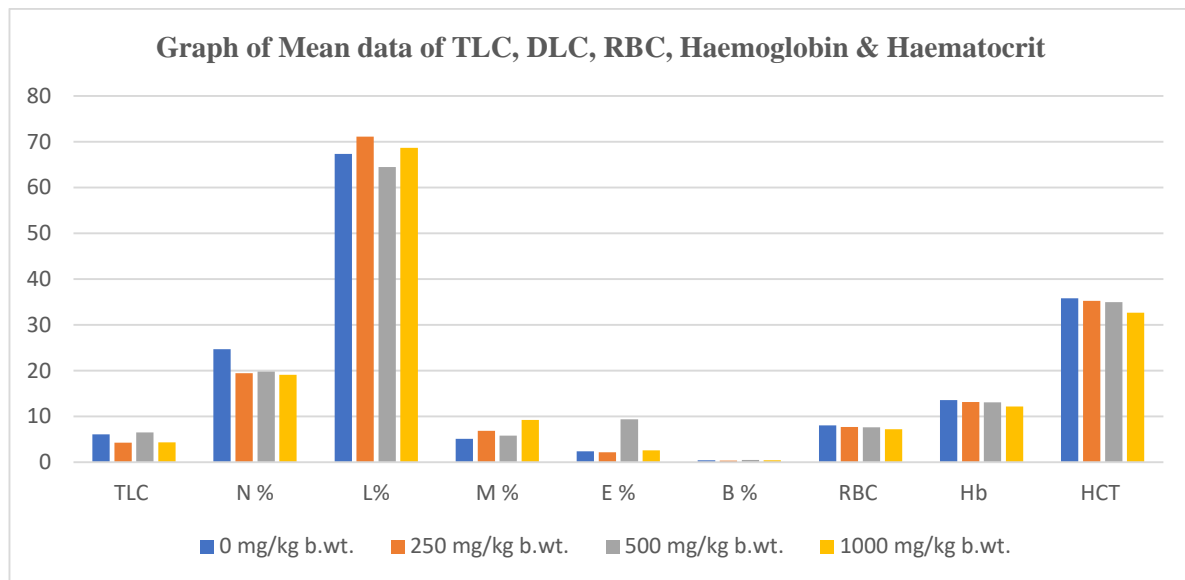
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Table-1: Mean data of TLC, DLC, RBC, Haemoglobin & Haematocrit

Parameters	0 mg/kg b.wt.	250 mg/kg b.wt.	500 mg/kg b.wt.	1000 mg/kg b.wt.
TLC (k/μl)	6.07 \pm 1.09	4.25 \pm 0.40	6.54 \pm 1.58	4.33 \pm 0.96
DLC	N%	24.70 \pm 4.50	19.44 \pm 2.73	19.82 \pm 3.71
	L%	67.34 \pm 4.47	71.16 \pm 3.72	64.48 \pm 2.03
	M%	5.10 \pm 0.69	6.84 \pm 1.98	5.84 \pm 1.65
	E%	2.42 \pm 0.35	2.20 \pm 0.51	9.36 \pm 3.96
	B%	0.44 \pm 0.05	0.36 \pm 0.11	0.50 \pm 0.10
RBC (m/ μl)	8.03 \pm 0.41	7.70 \pm 0.41	7.64 \pm 0.80	7.24 \pm 1.20
Hb (g/dl)	13.58 \pm 1.13	13.18 \pm 0.57	13.08 \pm 1.23	12.18 \pm 1.93
HCT%	35.84 \pm 2.67	35.28 \pm 0.90	34.96 \pm 3.19	32.64 \pm 5.25

Keys: TLC= Total Leukocyte count, DLC= Differential Leukocyte count, N%= Neutrophils %, L%= Lymphocyte %, M%=Monocyte %, E= Eosinophils %, B%= Basophils %, RBC= Red Blood Cells, Hb= Haemoglobin, Hct= Haematocrit.



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