

*Full Length Research Paper*

# Effect of essential oil of the leaves of *Eucalyptus globulus* on haematological parameters of wistar rats

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The study was designed to evaluate the effect of essential oil extract of *Eucalyptus globulus* on haematological parameters of wistar rats. Twenty-five adult wistar rats weighing between 80 to 130 g were used. The rats were divided into five groups; with group one as the control group. Increasing doses (12.5, 25.0, 50.0, 72.5 mg/ml) of the extract were administered orally daily to the other four groups for a period of four weeks. The animals were sacrificed and the blood collected for haematological parameters using automated haematological analyzer K-X-21 machine. The results indicate significant increases in level of Haemoglobin (Hb), White Blood Cell (WBC), and Red Blood Cell (RBC) but a decrease in the levels of the Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC). The study confirmed that extract oil of eucalyptus globulus have some significant effects on haematological parameters of the wistar rats and these effects are dose dependent.

**Key words:** *Eucalyptus globulus*, oil extract, haematological parameters, wistar rats.

## INTRODUCTION

Man in solving its numerous medical challenges have for ages depends on his immediate environment taking advantages of nature provisions of it beauty for live and survival. They have learnt to depend on plants and in some cases animals in providing solutions to the myriad of their health problems (Oliver, 1960). However, the increasing use of plants for the therapeutic and medicinal use warrants an adequate scientific investigation to confirm the suitability of plants or otherwise for the purpose for which they are used. Most of these medicinal plants are taking as vegetables, smoked leafs as tobacco, while the stems and roots are sometimes cooked for drinking.

*Eucalyptus globulus* is an ever green tree 40 to 70 m tall (Little, 1983) widely planted in the sub tropics. Its roots, stem, leaves and seed have been widely used in traditional folk medicine in many parts of West Africa countries. The plant fresh leaves are sometimes eating as vegetables, while the dry leaves were often smoked as cigarettes, in this case, for asthma treatment while the oil is used in the form of an aperitif as a digestive (Brooker et al., 1999). The stems and roots are cooked as medicinal agents across different ethnics groups within the country. The medicinal uses of *Eucalyptus* are in the treatment of abscess, arthritis, boil, bronchitis, burns, catarrh, diabetes, and dysentery (Watt et al., 1962; Duke and Wain, 1981; List and Horhammer, 1969). It is also to be useful in the various treatments of lung ailment, malaria, bladder and liver infection (Boukef et al., 1976). However, the mechanisms by which extract of *E. globulus* exert it all these activities are not well

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understood. The chemical composition of *E. globulus* are eucalyptol (cineol), terpineol, sesquiterpene, alcohol, aliphatics aldehyde, isoamyl alcohol, ethanol and terpenes (Morton, 1981).

Erythrocytes (red blood cell) which are anucleate matured cells are loaded with the oxygen carrying proteins known as haemoglobin. The normal concentration of erythrocytes in adult blood is approximately 3.9-5.5 million per micro litre in women and 4.1 to 6 million per micro litre in men (Junqueira et al., 2005). Human erythrocytes life span in circulation ranges from 90 to 120 days, while the worn out red blood cells are removed from the circulation by macrophages of the spleen and bone marrows. The effects of *Eucalyptus* globules on haematological parameters are not documented.

Leukocytes (white blood cells) are involved in the cellular and humoral defense mechanisms of the body are responsible for fight against foreign agents. The estimated total number of leukocytes in the blood varies according to age, sex and physiological conditions of the body. In normal healthy adults, they range from 6,000 to 10,000 leukocytes per micro litre blood (Junqueira et al., 2005). Majorities of these white blood cells migrate to the tissue, where they perform multiple functions as tissue macrophage and mostly died by apoptosis.

Blood platelets (thrombocytes) are nonnucleated disk like cell fragments 2 to 4  $\mu\text{m}$  in diameter. Platelets originate from the fragmentation of giant polypoid megakaryocytes that reside in the bone marrow. Platelets count range from 200,000 to 400,000 per micro litre of blood. Platelets have a life span of about 10 days. Platelets function; the role of platelets in controlling haemorrhage can be summarized as primary aggregation, secondary aggregation, blood coagulation, clot retraction and clot removal (Junqueira et al., 2005). The study was designed to see the effect of *Eucalyptus* globules on the haematological parameters using wistar rats.

## MATERIALS AND METHODS

### Animals

Twenty five adult Wistar rats weighing between 80 to 130 g were used for the study. The rats were purchased from the animal house of Faculty of Pharmacy, Obafemi Awolowo University Ile-Ife, Nigeria. They were bred for weeks in the animal house of Department of Anatomy, University of Ilorin. The rats were fed with pellets grower mash obtained from Bendel feed mill, Yoruba road, Ilorin and water *ad libitum* during the breeding period designed to acclimatize the rats. They were exposed to 12 h of light and darkness per day. The animals were care for in accordance with the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences (NIH, 1985).

The rats were randomly grouped into five groups. A: control group, and groups 1 to 4 were the experimental groups. Each of the rats were marked at the tail with different colours of pen marker and put into different segments of the cage, according to their group. The rats were sacrificed after four weeks of extract administration

using cervical dislocation. Blood from each rat was collected into labelled heparinised bottle to prevent coagulation of the blood and analyzed for the haematological parameters.

### Preparation of extract

Fresh leaves of the plant *E. globulus* were collected around the Department of Anatomy, University of Ilorin, Nigeria. Botanical identification of the plants was done at the herbarium of the Department of Botany, Faculty of Sciences, University of Ilorin. The fresh leaves were dried under laboratory condition as this helps prevent destruction of active constituents of the plant, which may occur on exposure to radiation, and drying lasted for a week.

The plant extract was prepared by the process of hydro distillation using the Clevenger apparatus in which the grinded plant material was heated. The evaporated oil were condensed and decanted into sample bottles and refrigerated.

### Administration of extract

Administration of the aqueous extract was done orally by means of calibrated syringe with attached rubber cannula. The control group received normal saline and DMSO<sub>4</sub>. The experimental groups of 1, 2, 3, and 4 received extract of *E. globulus* at doses of 12.5, 25.0, 50.0, and 72.5 mg/ml respectively. The assigned doses per groups were administered daily and lasted for duration of four weeks.

### Haematological parameters analysis

Evaluation of the haematological parameters was carried out using automated haematological analyzed K-X-21 made by Symex, Kobe, Japan. Sample of blood from the Wistar rats in heparinized bottle were analyzed using this machine for accuracy. Each sample was run twice and the average value calculated and recorded. The coefficient of error of the analyzer machine is less than 5%. Data obtained were presented as mean  $\pm$  standard deviation and in some cases; the use t-test was employed for comparison and the level of significance was predetermined as  $p \leq 0.05$ .

## RESULTS

There was steady increase in the haemoglobin concentration and the estimated total red blood cell counts across the concentration gradient with increasing concentrations of the oil extract. The control group have a mean haemoglobin concentration of  $9.63 \pm 0.3$  mg/dl and  $12.1 \pm 0.2$  mg/dl for the group 4 with high dose of the oil extract. The estimated total red blood cell counts for the control group was  $4.45 \pm 0.04$  ( $\times 10^6$ ) and  $6.16 \pm 0.15$  ( $\times 10^6$ ) for the group 4. There was statistical significant difference between them (Table 1).

The mean haemoglobin concentration for the control group was  $21.5 \pm 0.5$  pg and for the group 1 was  $14.5 \pm 0.5$  pg which statistically significant. These values increases with the increase in the concentration of the oil extract from group 2 to group 4. Similar pattern were observed for the mean corpuscular haemoglobin concentration (Table 2).

The estimated total white blood count for the control group was  $5.55 \pm 0.5 \times 10^9/\text{L}$  and that for the group 4 was

**Table 1.** Haemoglobin concentration (mg/dl) and total estimated Red Blood Cells counts ( $\times 10^6$ ) in adult wistar rats.

Experimental group	Haemoglobin concentration (mg/dl)	Red blood cell counts ( $\times 10^6$ )
Control	9.63 $\pm$ 0.3	4.45 $\pm$ 0.04
Group 1	9.70 $\pm$ 0.2	4.52 $\pm$ 0.01
Group 2	9.90 $\pm$ 0.6	4.79 $\pm$ 0.01
Group 3	10.30 $\pm$ 0.25	4.87 $\pm$ 0.02
Group 4	12.10 $\pm$ 0.2*	6.16 $\pm$ 0.15*

(\*) indicates statistical significant at  $p \leq 0.05$ . Data were expressed as mean  $\pm$  standard deviation.

**Table 2.** Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, and Mean Corpuscular Haemoglobin Concentration in adult wistar rats.

Experimental group	Mean corpuscular volume (MCV)	Mean corpuscular haemoglobin (MCH)	Mean corpuscular haemoglobin concentration (MCHC)
Control	53.5 $\pm$ 0.5	21.5 $\pm$ 0.5	39.01 $\pm$ 1.00
Group 1	57.5 $\pm$ 0.5	14.5 $\pm$ 0.5*	24.00 $\pm$ 0.01*
Group 2	60.5 $\pm$ 0.5	16.0 $\pm$ 0.1	28.50 $\pm$ 0.01
Group 3	62.5 $\pm$ 0.5	16.5 $\pm$ 0.5	34.00 $\pm$ 0.02
Group 4	63.0 $\pm$ 1.0*	17.5 $\pm$ 0.5	36.50 $\pm$ 0.50

(\*) indicates statistical significant at  $p \leq 0.05$ . Data were expressed as mean  $\pm$  standard deviation.

**Table 3.** White blood cells count ( $\times 10^9/l$ ), estimated Neutrophil counts (%), and estimated total Lymphocyte counts (%) in adult wistar rats.

Experimental group	White blood cells count ( $\times 10^9/l$ )	Neutrophil counts (%)	Lymphocyte counts (%)
Control	5.55 $\pm$ 0.50	10.0 $\pm$ 0.10	81.5 $\pm$ 0.5
Group 1	5.65 $\pm$ 0.05	10.0 $\pm$ 0.10	84.5 $\pm$ 0.1
Group 2	6.70 $\pm$ 0.10	10.0 $\pm$ 0.10	88.0 $\pm$ 1.0
Group 3	7.20 $\pm$ 0.10*	12.0 $\pm$ 0.10	90.0 $\pm$ 0.1
Group 4	8.30 $\pm$ 0.10*	15.0 $\pm$ 0.10*	91.5 $\pm$ 0.05*

(\*) indicates statistical significant at  $p \leq 0.05$ . Data were expressed as mean  $\pm$  standard deviation.

8.3  $\pm$  0.3  $\times 10^9/L$ . The neutrophil counts was stable in the control, group 1 and 2 but steadily increases from group 3 to group 4 and this was significant. Similar observations were not recorded in the lymphocytes counts which show a linear increase along the concentration gradients of the administered oil extract (Table 3).

## DISCUSSION

The study demonstrated the effect of varied concentration of oil extract of *E. globulus* on the haematological parameters in adult Wistar rats. The apparent increase in the haemoglobin concentrations across the experimental groups was dose dependent and this may be due to increased iron concentration present in the extract. This finding was corroborated by Osawa and Namiki (2005) that observed increased iron concentrations of various

extract of *E. globulus*. Though, the haemoglobin value was 9.6 mg/dl on the average for the control group, the steady rise in the values with increase in concentrations of the extract across the experimental groups may also be due to the presence of some of the phytochemical contents of eucalyptus globulus that may have increased the size of the red blood cells. This observation was corroborated Yakubu et al., 2008; Oyesomi and Ajao, 2011 where the roles of phytochemical agents in reproductive hormones and testis were demonstrated respectively.

The reduction of the mean corpuscular volume (MCV) and that of the mean corpuscular haemoglobin concentration (MCHC) by the oil extract of *Eucalyptus globulus* across the increased concentration gradient administered to wistar rats may be due to the decrease in size of the red blood cells produced which may partly explained the findings of the increase in the estimated

total red blood cell counts that was recorded from the study. Though, some of the constituents of the extract may stimulate production of blood cells; these may be immature and may be of irregular shapes and sizes. The mechanisms by which this carried out is not fully understood and beyond the scope of the present study. However, this observation was corroborated by Osawa and Namiki (2005) and Medubi et al. (2010) in their various related studies.

The present study demonstrated a gradual increase in the estimated total white blood cell count (WBC) from that of those of the control groups and these appears to dose dependent. This may be result from the immune busting activities of some this medicinal plants as demonstrated by Adefolaju et al. (2009) where aqueous extract of plant have Hepatoprotective activity in rats. However, the selective busting of the lymphocytes component of the differential counts was not clearly understood and that post a future challenge for research. In conclusion, the study shows that the essential oil extract of *E. globulus* administered at increasing dosage used as outlined in the present study for the duration of one month enhanced the haemopoietic activities in wistar rats. The mechanisms for the observed increased may be due to the presence of some constituents of iron which are of great importance in the production of blood.

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