



Effect of Ethanolic Extracts leaves of *Eucalyptus camaldulensis* on haematological parameters of Males wistar rats

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Abstract : The study was designed to evaluate the effect of Ethanolic Extracts leaves of *Eucalyptus camaldulensis* on haematological parameters of wistar rats. Twenty-five adult males 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 blood analyzer 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 leaves extract of *Eucalyptus camaldulensis* have some significant effects on haematological parameters of the males wistar rats and these effects are dose dependent.

Key world : *Eucalyptus camaldulensis* , haematological wistar rats , WBC , RBC , MCHC , MCV , haematological blood analyzed.

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 (1). 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 camaldulensis* is an ever green tree 40 to 70 m tall(2), 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 (3). 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 (4),(5),(6),(7). It is also to be useful in the various treatments of lung ailment, malaria, bladder and liver infection (8),(9). However, the mechanisms by which extract of *E. camaldulensis* exert it all these activities are not well understood. The chemical composition of *E. camaldulensis* are eucalyptol (cineol), terpineol, sequiterpene, alcohol, aliphatics aldehyde, isoamyl alcohol, ethanol and terpenes (10). 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 3.95.5 million per micro litre in women and 4.1 to 6 million per micro litre in men (11). 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 camaldulensis* 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 (12). 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 non-nucleated disk like cell fragments 2 to 4 μm 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 (13). The study was designed to see the effect of *Eucalyptus camaldulensis* on the haematological parameters using males wistar rats.

Material and methods

Animals:

The present study has been conducted in the animal house of the College of Veterinary Medicine, AL-Qadisiya University during the period extended from October , 2015 to February, 2016, and continued in the laboratory to March , 2016.

Twenty five adult Wistar rats weighing between 80 to 130 g were used for the study. Male rats were allowed to acclimatize to the animal house environment before beginning of the experiment. Animals were housed in polypropylene cages inside a well-ventilated room. Each cage consists of five rats. Male rats were fed on the standard chow , and drinking water throughout the experiment. Room temperature was maintained at (23 \pm 2 $^{\circ}\text{C}$) the light-dark cycle was on a 12 hr light/dark cycle with light on at 06:00 a.m. and off at 06:00 p.m. during the experimental periods.

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 Ethanolic Extracts(*Eucalyptus camaldulensis*):

20gm of *Eucalyptus camaldulensis* powdered leaves were taken and extracted with soxhlet apparatus ethanol (70%) Within 24 hours, and then taking the extract and place it in a ptry dish and put in the oven at a temperature of (40 $^{\circ}\text{C}$) within 48 hours, The result of extract was stored at (4 $^{\circ}\text{C}$) until use (14).

Administration of extract:

Administration of the aqueous extract was done orally by means of calibrated syringe with attached rubber cannula. The control group oral intubation (stomach tube) just water. The experimental groups of 1, 2, 3, and 4 received extract of *E. camaludelsis* 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 blood analyzed 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 ($p < 0.05$).

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 leaves extract. The control group have a mean haemoglobin concentration of 9.63 ± 0.3 mg/dl and 12.1 ± 0.2 mg/dl for the group 4 with high dose of the leaves 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).

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

Groups	Haemoglobin conce.(mg/dl)	Red blood cell counts ($\times 10^6$)
Control	9.60 ± 0.3	4.55 ± 0.04
G1	9.77 ± 0.2	4.66 ± 0.01
G2	9.98 ± 0.6	4.82 ± 0.01
G3	10.44 ± 0.3	4.95 ± 0.02
G4	$12.22 \pm 0.2^*$	$6.44 \pm 0.15^*$

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

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

Groups	(MCV)	(MCH)	(MCHC)
Control	54.5 ± 0.5	22.5 ± 0.5	40.02 ± 1.0
G1	57.6 ± 0.5	$15.5 \pm 0.5^*$	$25.00 \pm 0.01^*$
G2	61.5 ± 0.5	17.5 ± 0.1	29.5 ± 0.01
G3	63.5 ± 0.5	17.5 ± 0.5	35.00 ± 0.02
G4	$65.5 \pm 1.0^*$	18.5 ± 0.5	37.5 ± 0.5

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

The mean haemoglobin concentration for the control group was 21.5 ± 0.5 pg and for the group 1 was 14.5 ± 0.5 pg which statistically significant. These values increases with the increase in the concentration of the leaves 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 $5.55 \pm 0.5 \times 10^9/L$ and that for the group 4 was $8.3 \pm 0.3 \times 10^9/L$. 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 leaves extract (Table 3).

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

Groups	WBCs ($\times 10^9/l$)	Neutrophil counts (%)	Lymphocyte counts (%)
Control	5.56 \pm 0.5	11.0 \pm 0.1	82.5 \pm 0.5
G1	5.77 \pm 0.5	11.0 \pm 0.1	85.5 \pm 0.1
G2	6.78 \pm 0.1	11.0 \pm 0.1	89.0 \pm 1.0
G3	8.20 \pm 0.1*	13.0 \pm 0.1	91.0 \pm 0.1
G4	9.20 \pm 0.1*	16.0 \pm 0.1*	93.5 \pm 0.5*

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

Discussion

The study demonstrated the effect of varied concentration of Leaves extract of *E. camaludeinesis* on the haematological parameters in adult males 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 (15), that observed increased iron concentrations of various extract of *E. camaludelsis*. 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 camaludelsis* that may have increased the size of the red blood cells. This observation was corroborated (16),(17) 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 leaves extract of *Eucalyptus camaludelsis* across the increased concentration gradient administered to males 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 (18) and (19) 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 (20) 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 leaves extract of *E. camaludelsis* 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.

Reference

1. Oliver B (1960). Medicinal plants in Nigeria. Ibadan College of Arts and Sciences and Technology, Ibadan. p 358.
2. Wallis, I.R.,Foley,W.J.(2005).The rapid determination of sider oxylonalsin *Eucalyptus* foliage by extraction with sonication followed by HPLC. *Phytochem. Anal.*16, 49–54.
3. Brooker SG, Cammbie RC, Cooper RC (1999). *New Zealand medicinal plants*. Heinemann.
4. Watt JM, Breyer-Brandwork MJ (1962). “Medicinal and poisonous plants of southern and eastern Africa”, E&S Livingstone Edinburgh.
5. List PH, Horhammer L (1969). *Harger’s Handbuck der pharmaceutischen praxis*. 2(6). Springer-Verlag, Berlin.
6. Duke JA, Wain KK (1981). *Medicinal plants of the world*. Computer index with more than 85,000 entries vols.
7. Ying Wang, Yanmei Liu, Study .(2009).the chemical constituents of the essential oil of the Leaves of *Eucalyptus globulus* Labill China Aihua Song a, *Asian Journal of Traditional Medicines*, (4).

8. Boukef K, Balanshad G, Lallemand M, Brenard P (1976). Study of flavonic heterosides and aglycones isolated from the leaves of *Eucalyptus globulus* (Hot Abstract 47:1899).
9. Luzar J. (2007). The Political Ecology of a Forest Transition: *Eucalyptus* forestry in the Southern Peruvian. *Ethnobotany Research & Applications*. World Watch Institute.
10. Cunningham, G.M., W.E.Mulham, P.E.Milthorpe and J.H.Leigh.(1981). *Plants of Western New South Wales*, Soil Conservation Service of New South Wales.
11. Junqueira LC, Carnerio L, Robert OK (2005). *Textbook of basic histology*, Lange publication, 9th Edition, Pg. 218-230.
12. Junqueira LC, Carnerio L, Robert OK (2005). *Textbook of basic histology*, Lange publication, 9th Edition, Pg. 218-230.
13. Junqueira LC, Carnerio L, Robert OK (2005). *Textbook of basic histology*, Lange publication, 9th Edition, Pg. 218-230.
14. Harborne.(1999). *Phytochemical dictionary: Handbook of bioactive compounds from plants* 2nd (Edn.).
15. Osawa T, Namiki (2005). A novel type of anti-oxidant isolated from leafwax of *Eucalyptus* leaves; and its suitabilities as an anticoagulant for biochemical and haematological analysis. *Afr. J. Bio.*, 4(7): 679-689.
16. Yakubu MT, Akanji MA, Oladiji AT, Olatinwo AWO, Adesokan AA, Yakubu MO, Owoyele BVO, Sunmonu TO, Ajao MS (2008). Effect of *Cnidioscolous aconitifolius* (Miller) I. M. Johnston Leaf extract on Reproductive Hormones of Female Rats. *Iranian J. Reprod. Med.*, 6(3): 149-155.
17. Oyesomi TO, Ajao MS (2011). Histological effect of aqueous extract of *Anacardium occidentale* (cashew) stems bark on adult wistar rat testis. *Med. Prac. Rev.*, 2(7): 73-77.
18. Osawa T, Namiki (2005). A novel type of anti-oxidant isolated from leafwax of *Eucalyptus* leaves; and its suitabilities as an anticoagulant for biochemical and haematological analysis. *Afr. J. Bio.*, 4(7): 679-689.
19. Medubi LJ, Ukwenya VO, Aderinto OT, Makanjuola VO, Ojo OA, Bamidele O, Ajao MS (2010). Effects of administration of ethanolic root extract of *Jatropha Gossypifolia* and prednisolone on the kidneys of wistar rats. *Electron. J. Biomed.*, 2: 41- 48.
20. Adefolaju GA, Ajao MS, Olatunji L A, Enaibe BU, Musa MG (2009). Hepatoprotective effect of aqueous extract of water leaf (*Talinum Triangulare*) on carbon tetrachloride (CCl₄) induced liver damage in wistar rats. *Int. J. Pathol.*, 8(1): 1-9.
