The Pharmaceutical and Chemical Journal, 2020, 7(3):38-43

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Haematological Assessment of Ethanolic Leaf Extract of *Cymbopogon citratus* in Wistar Rats

Nosiri, C. I.[1]*, Nwaogwugwu, C. J.[1], Idume, J.N.[2], Iheanacho, U.E.[3], Nwankwo, V. C.[1] Onwuasoanya W.E.[1]

[1]Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria
[2]Department of Medical Laboratory Science, School of General and Applied Sciences, Shehu Idris College of Health Sciences and Technology, Makarfi, Kaduna State, Nigeria
[3]Department of Medical Biochemistry, Abia State University, Uturu

Abstract This study was designed to examine the effect of ethanolic leaf extract of *Cymbopogon citratus* on hematological indices of wistar rats. Treatment was carried out using 20 rats divided into four groups of 5 rats each. Group I served as control and received only feed and water, while groups 2, 3 and 4 were given 600,800 and 1000mg/kg body weight of extract respectively. At the end of the 14th day of treatment, the rats were sacrificed and their blood samples collected in an EDTA container for hematological indices using an automated cell counter. The results showed a significant increase ($p<0.05$) in Red blood cells, Packed cell volume, and Hemoglobin in the rats administered with 600mg/kg b.wt and 800mg/kg b.wt of the extract. There was a significant increase ($p<0.05$) of White Blood Cells in the 600mg/kg b.wt rats but a decrease in the rats given 1000mg/kg b.wt of the extract when compared to the control. A significant decrease in granulocyte was observed of the rats administered with the extract when compared to the control while the number of lymphocytes increased. The values of Mean corpuscular volume, Mean corpuscular hemoglobin, and Mean corpuscular hemoglobin concentration and platelet show significant increases ($p<0.05$) in the rats administered with the extract when compared to the control. From these observations, it may be inferred that the ethanolic leaf extract of *Cymbopogon citratus* is not toxic hence it stimulated erythropoeisis, immune state of the body and can aid in blood clotting on prolonged administration. With all these stimulatory activities, it can therefore be concluded that the plant may be of benefit in the management of hematological disorders.

Keywords *Cymbopogon citratus*, hematological, hemopoeisis, immune, clotting

Introduction

Hematology helps to comprehend the relationship between the characteristics of blood with the environment as they are of ecological and physiological interests [1]. It encompasses the study of the morphology and number of cellular components of the blood especially the Erythrocytes (red blood cells), Leucocytes (white blood cells), and the Thrombocytes (platelets) and how they are being used in the monitoring and diagnosis of diseases [2]. The distribution of oxygen from the lungs to the periphery through the pulmonary arteries, removal of carbon dioxide back to the lungs from the tissues through the systemic capillaries and hemostasis of the acidic and basic values of the body are all functions of the Red blood cells [3]. The Leucocytes made up of lymphocytes and myeloid

leucocytes are immune system cells produced or derived from the multipotent cell of the bone marrow called the hematopoietic stem cell. Platelets help in stopping bleeding by clogging and clumping injuries of blood vessel [4].
There is no plant in the universe that does not have a function, either it functions as food or it functions as medicine. Traditionally, *Cymbopogon citratus* has been used as a remedy in a large number of medical conditions. This is as a result of excessive number of bioactive constituents and secondary metabolites it contains. *Cymbopogon citratus*, commonly known as lemon grass is a tropical plant from South Asia and Southeast Asia. Cymbopogon is a genius of about 55 species, which are indigenous in tropical and semi-tropical areas of Asia. They are cultivated in South and Central America, Africa and other tropical countries. Cymbopogon are tufted perennial grasses with numerous stiff stems arising from a short, rhizomatous rootstock, as with citrus flavor, and can be dried and powdered or used [5]. The name Cymbopogon is coined from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement. *Cymbopogon citratus* is commonly used in teas, soups and curries. It is also suitable for poultry, fish and seafood. This tropical grass grows in dense clumps and can grow to 6 fit (1.8m) in height and about 4 fit (1.2m) in width, with a short rhizome [6]. *Cymbopogon citratus* contains an essential oil that its chemical constituent varies according to the geographical origin. The compounds identified in this grass are mainly terpenes, alcohol, ketones, aldehyde and esters. Some of the reported phytoconstituents are essential oils that contain mainly of citral, which is a mixture of two stereoisomeric monoterpenes aldehyde; the trans isomer geranial (40-62%) dominates over the cis isomer neral (25-38%), citronellal, terpionolens, geranyl acetate, myrecene and terpinol methylheptenone [7]. The grass also contains reported phytoconstituents such as flavonoids and phenolic compounds. The flavonoids contain quercetin, kaempferol and apoginin at the aerial parts while phenolic compound composed of elimicin, catecol, chlorogenic acid, caffeic acid hydroquinone, luteolin and isoroentin 2-D- rhamnoside [8]. Studies have also shown that *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties. Various other effects like antimalaria, antimutagenecity, antimycobacterial, antioxidants, hypoglycemic and neurobehavioral have also been studied. In the traditional medicine of Brazil, *Cymbopogon citratus* is believed to have anxiolytic, hypnotic and anticonvulsant properties [9]. Also in the folk medicine of India, the leaves of the plant are used as stimulant, antiperiodic, and anticatarrhal, while the essential oil is used as carminative, depressant, analgesic, antipyretic and antifungal agent. Blood is a good indicator for diagnosis and monitoring of diseases. Phytochemical constituents and pharmacological properties especially the chlorogenic acid and anti-inflammatory properties respectively have been reported in Lemon grass, therefore based on this background there is need to assess the hematological indices using animal model in this study.

Materials and Methods

Collection and Identification of Sample

Cymbopogon citratus (lemongrass) was collected from Okigwe in Imo State in Nigeria. This was transported to the Biochemistry Departmental Animal House of Abia State University Uturu in a polyethylene container where the analysis was carried out and authenticated by the taxanomist in the Department of Plant Science and Biotechnology, Abia State University, Uturu where the voucher specimen is deposited.

Sample Preparation

The lemongrass was collected in bulk for preparation of extracts. The grass was washed and air dried. It was ground to powder with the use of an electric blender. Oil was extracted from the powdered lemongrass of weight (105g) with ethanol as a solvent for two hours using soxhlet extractor. The crude extract was heated in the water bath to ensure that the ethanol in the oil evaporated. The volumes of the extract to be administered to the animals were calculated based on their body weight.

Experimental Design

The wistar rats used for this study were purchased from the Abia State University Uturu. A total of twenty (20) male rats weighed 133-180g were used. The rats were housed using a plastic cage and placed on commercial growers

feed purchased from Eke Okigwe market, Imo State as produced by Nigeria Flour Mills. The rats were allowed to acclimatize for 14 days before the commencement of the experiment. Ethical principles were strictly adhered to while handling the animals and at the end of acclimatization, the animals were grouped into four groups each containing five (5) rats and were administered orally with different doses of the extracts according to their body weight. The animals were grouped as follows: Group 1: Served as normal control receiving only feed and water, Group 2: Received 600mg/kg of extract, Group 3: Received 800mg/kg of extract., Group 4: Received 1000mg/kg of extract respectively for 14 days.

Blood Collection

Fourteen (14) days after administering the rats with the ethanolic extract of *Cymbopogon citratus*, they were fasted overnight, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture using syringe and needle and blood samples from each animal collected into EDTA (Ethylene diamine tetra acetic acid) container for haematological analyses.

Measurement of blood parameters

Blood samples were analyzed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) with standard calibration, according to the manufacturer's instructions for analysis of human blood (Instruction manual for the Coulter Model S-plus. 2nd ed. Bedfordshire, UK: 1979) and accurately programmed for the analysis of red blood cell (RBC) count, total white blood cell (WBC) count, hemoglobins (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobins concentration (MCHC), RBC distribution width (RDW), MPV, PDW, and P–LCR.

Statistical Analysis

Results were expressed mean±SEM (Standard Error of Mean). Statistical analysis was performed by one –way analysis of variance (ANOVA) with the RTM statistic software package, vision 3.03. The normal distribution of the data and the homogeneity of variance were tested by Barlett homogeneity test. One way ANOVA with a Turkey test post-hoe was used to identify statistical differences among groups. A p-value of <0.05 was considered statistically significant.

Table 1: The Effect of Ethanolic Extract of *Cymbopogon citratus* Oil on the Hematological Parameters of Wistar Rats after 14 Days Administration

Parameters	**Group 1 Control**	**Group 2 (600mg/Kg)**	**Group 3 (800mg/Kg)**	**Group 4 (1000mg/Kg)**
RBC $\times 10^{12}$/L	6.95 ± 0.21^{a}	8.71 ± 0.13^{c}	7.38 ± 2.20^{b}	6.80 ± 0.42^{a}
PCV %	49.70 ± 0.57^{a}	52.70 ± 0.57^{b}	51.10 ± 3.54^{b}	49.65 ± 0.64^{a}
Hb g/dl	12.95 ± 0.21^{a}	15.05 ± 0.21^{c}	14.35 ± 0.78^{c}	12.65 ± 0.35^{a}
WBC $\times 10^{9}$/L	87.85 ± 2.47^{a}	137.55 ± 2.05^{c}	87.40 ± 54.59^{a}	80.45 ± 9.55^{b}
PLATELET $\times 10^{9}$/L	471.50 ± 20.51^{a}	557.50 ± 71.42^{b}	521.00 ± 257.39^{b}	772.50 ± 27.58^{c}
MCV fl	72.35 ± 0.35^{a}	75.70 ± 0.71^{c}	76.05 ± 1.48^{c}	74.90 ± 0.85^{b}
MCH pg	17.50 ± 0.42^{a}	25.90 ± 0.42^{c}	21.65 ± 5.30^{c}	18.60 ± 0.57^{b}
MCHC g/L	249.00 ± 1.41^{a}	282.00 ± 15.56^{c}	285.00 ± 1.41^{c}	262.00 ± 14.14^{b}

Values represent the mean ±SEM for five animals per group N=5. Values marked with the same alphabet (a) are not significantly different from normal control (P>.05).

Table 1 result showed the effect of ethanolic extract of *Cymbopogon citratus* oil on the changes in hematological parameters of wistar rats with is a slight increase in RBC and Hb counts in the rats administered with 600mg/kg body weight and 800mg/kg body weight of the extract, but a slight decrease in the rats administered 1000mg/kg when compared to the control. Also it caused an insignificant increase in PCV% of 600mg/kg body weight and 800mg/kg body weight whereas there was no difference in 1000mg/kg body weight when compared to the control.

From the Leucocyte result, the oral administration of ethanolic extract of *Cymbopogon citratus* oil did not cause a significant difference in the WBC count of test rats administered with 800mg/kg body weight and 1000mg/kg body weight, but there was a significant increase in the test rats administered with 600mg/kg body weight (from 87.85±2.47 to 137.55±2.05) when compared to the control. The thrombocyte result showed a significant increase in the platelet count of the test rats when compared to the control. However, the highest increase was seen in the test rats administered with 1000mg/Kg body weight. The result also showed a significant increase in the levels of MCV, MCH and MCHC of the test rats when compared to the control.

Table 2: The Effect of Ethanolic Extract of *Cymbopogon citratus* Oil on the Differential WBC of Wistar Rats after 14 Days Administration

Parameters	Group 1 Control	Group 2 (600mg/Kg)	Group 3 (800mg/Kg)	Group 4 (1000mg/Kg)
Neutrophils %	52.50±0.71^{c}	44.50±3.54^{c}	48.00±11.31^{b}	48.50±0.71^{b}
Lymphocytes %	39.50±0.71^{a}	48.00±2.83^{c}	46.50±9.19^{b}	45.00±0.00^{b}
Monocytes %	3.50±0.71^{a}	3.50±0.71^{a}	3.50±2.12^{a}	3.50±0.71^{a}
Eosinophil %	3.50±0.71^{c}	3.00±0.00^{b}	2.00±0.00^{a}	2.50±0.71^{a}
Basophil %	1.00±0.00^{c}	1.00±0.00^{c}	0.00±0.00^{a}	0.50±0.71^{b}

Values represent the mean SEM for N=5. Values marked with the same alphabet (a) are not significantly different from normal control (P>.05).

The result on Table 2 above showed that the ethanolic extract of *Cymbopogon citratus* oil caused a significant decrease in the values of neutrophil of the test rats when compared to that of control. Also a significant increase in the levels of lymphocytes of the test rats was seen when compared to the control. The result also showed no difference in the monocyte% between the rats administered with the ethanolic extract of lemongrass oil and the control. In addition, the extract caused an insignificant decrease in the eosinophil of the test rats administered with 600mg/kg body weight of the extract when compared to that of control while those of 800mg/kg body weight and 1000mg/kg body weight of the extract shows a significant decrease. The levels of basophil of the test rats administered with 600mg/kg body weight and that of control is the same, while that of 800mg/kg body weight and 1000mg/kg body weight showed little or no value.

Discussion

In the universe, the use of medicinal plants by humans without the scientific knowledge of the phytochemical constituent and standard dose coupled with a scarcity of adequate scientific information on their safety have raised concern regarding their toxicity [10]. In order to find out the safety of drugs and medicinal plants for human consumption, toxicological evaluations are carried out on various experimental animals to predict toxicity and provide guideline for selecting a safe dose in humans. Hematological parameters have been associated with health and are of diagnostic significance in routine clinical evaluation of the state of health [11]. It can also be used to assess the hematological functions of a plant extract in an organism. This present studywas aimed at examining the effect of ethanolic leaf extract of *Cymbopogon citratus* on hematological indices of wistar rats.

In this hematological analysis, the results showed an increase in RBC, PCV, Hb levels, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), and Mean corpuscular hemoglobin concentration (MCHC) values of the rats administered with the extract when compared to that of control. The increase in RBC, PVC and Hb is in line with the study on murine model where the ethanolic leaf extract of *Cymbopogon citratus* caused an increase in RBC counts without causing any form of toxicity [12]. It has also been reported to possess an erythropoietic boosting effect on a human volunteer study [13]. Increase in the production of RBCs may be an indication of increased bone marrow function and sufficient erythropoeisis induced by the extract [14]. Also MCV increase in levels may lead tomore reticulocytes (immature RBCs). Looking at the elevation in RBC, it is expected to result in an increase of MVC on administration of *C. citratus* while increase in Hb leads to an increase in MCHC and MCH indices. Individual RBC counts are related to the MCV, MCH and MCHC while total population of RBC cells are related to hematocrit and hemoglobin profiles, it could then be implied that although the extract of *C. citratus* may have

stimulated RBC and hematocrit production, it could also inhibit the incorporation of Hb into RBC hence reducing oxygen exchange [15]. The rats administered with 600mg/kg body weight and 800mg/kg body weight showed an increase in PCV% when compared to the control, whereas there was no significant difference in the rats given 1000mg/kg body weight of the extract when compared to control. Also, there was an increase in Hb concentration of the rats administered with 600mg/Kg body weight and 800mg/kg body weight when compared to the control. However, there was no difference in the Hb concentration of the rats given 1000mg/kg body weight and that of control. This could mean at a safe dose, the ethanolic leaf extract of *Cymbopogon citratus* could increase blood formation and pigmentation and also the oxygen carrying capacity of the blood.

In the total white blood cell (WBC) counts, the rats administered with 600mg/kg body weight showed an increase while there was no difference in the rats administered with 800mg/kg body weight, whereas the rats administered with 1000mg/Kg body weight decreased significantly when compared to the control. This could mean that the ethanolic leaf extract of *Cymbopogon citratus* may have increased the immune cells that are producing antibodies against pathogens [16]. From the results of the differential WBC, the values of the granulocytes which are neutrophil, basophil and eosinophil decreased significantly for the rats administered with the extract. The decrease seen in basophil and eosinophil count was in line with the decrease seen in human volunteers administered with 2, 4 or 8g of lemongrass tea for 30 days, however there was a contrast in the decrease in neutrophil count in this present study when compared to the increase in neutrophil of human volunteers administered with lemongrass tea for 30 days [13]. This difference could be as a result of the experimental subjects used. The decrease in neutrophil observed in all treatment rats is similar to the study reported using the leaf extract of *P. cispum* [17]. Neutrophil plays an important role in defense mechanism of the body. It functions by attaching to the walls of the blood vessels, blocking the passageway of germs that try to gain access to the blood through a cut or infectious area. In other words, the neutrophil provides the first line of defense against the invading microorganisms [18]. The apparent reduction in the levels of granulocytes in this study may be to the advantage of the animals, because this is an indication that the animals were not adversely affected in any way that could elicit their responses. There was no difference in the value of monocyte of the rats fed with the extract when compared to the control. This is in line with the work of [17] who reported a non-significant value in the monocyte level in the Wistar albino rats administered with the ethanolic leaf extract of *Petroselinum crispum*.

The level of lymphocytes increased in the rats administered with the extract especially in the test group given 600mg/Kg body weight. This finding was in line with the increase seen in lymphocytes count of human volunteers fed with 2, 4 or 8g lemongrass tea for 30 days [13]. This could suggest that lemongrass could influence defense mechanism, so the continuous exposure of the body system to this plant may cause lymphocytosis, which may account for the use of this plant as a medicinal plant [19]. The result also showed that there was a significant increase in platelet counts of rats administered with the extract when compared to the control. Under normal healthy body condition, platelets are produced from the megakaryocytes within 4 to 6 days [20]. This platelet boosting effect might be due to the vitamin C content of *C. citatus* leaf, which acts as an anti-oxidant [13] that prevents free-radical mediated damage of platelets [21]. This could suggest that the extract have the ability to enhance the process of thrombocytosis that encourages hemostasis.

Conclusion

In this present study, it was observed that the oral administration of ethanolic leaf extract of *Cymbopogon citratus* could be used to treat anemia and therefore may improve bone marrow failure as well as serve as a therapeutic agent to increase lymphocyte count. This result correlates the traditional use of lemongrass as an anti-anemic tonic. Therefore, *Cymbopogon citratus* may be beneficial in boosting erythropoiesis likely due to some nutritional and phytochemical constituents, antioxidant and pharmacologic properties.

References

[1]. Ovuru, S.S. and Ekweozor, I. (2004) Haematological changes associated with crude oil ingestion in experimental rabbits. *African Journal of Biotechnology*, 3: 346-348.

[2]. Merck, M. (2012) Haematologic reference ranges. Mareck Veterinary Manual. Retrieved from http://www.merckmanuals.com/.
[3]. Jagger JE, Bateman RM, Ellsworth ML, Ellis CG (2001) Role of erythrocyte in regulating local O2 delivery mediated by hemoglobin oxygenation. *Am J Physiol Heart Circ Physiol,* 280: H2833-2839.
[4]. Laki, K. (1972). Our ancient heritage in blood clotting and some of its consequences. *Ann N Y Acad Sci*, 202: 297-307.
[5]. Kumar, S., Dwivedi, S., Kukreja, A., Sharma, J. and Bagchi, G. (2000). Cymbopogon: The Aromatic Grass Monograph. Lucknow India Centr. Inst. Med. Aromat. Plants, 456-460.
[6]. Reitz, R. (1982). Flora ilustrada catarinense. Itajaí. *Amer. Fern. J. Publ.*, 1309–14.
[7]. Ming, L. Figueiredo, R., Machado, S. and Andrade, R. (1996). In Proceedings of the International Symposium on Medicinal and Aromatic Plants. Mexico: Acta Horticulturae Leiden. Yield of essential oil of and citral content in different parts of lemongrass leaves (Cymbopogon citratus (DC.). Stapf.) *Poac.*, 555–9.
[8]. Miean, K. and Mohamed, S. (2001). Flavonoid (Myricitin, Quercetin, Kaempferol, Luteolin, and Apigenin) Content of Edible Tropical Plants. *J. Agric Food Chem.,* 49:3106–12.
[9]. Filipoy A (1994). Medicinal plants of the Pilaga of Central Chaco. *J Ethnopharmacol.,* 44:181–93.
[10]. Saad, B., Azaizeh, H., Abuhijleh, E. and Said, O. (2006). Safety of traditional Arab herbal medicine. J. Pharmacol., 3: 350-354.
[11]. Muyibi, S., Olorode, B., Onyeyili, P., Osunkwo, U., Muhammed, B. and Ajagbonna, O. (2000). Hematological and histopayhological changes of Cassia Occidentalis leaf extract in rats. Nig. *J. Nat. Prod. Med*., 4: 48-52.
[12]. Vasudevan, D. M., Sreekumari, S. and Kannan, V. (2013). Textbook of biochemistry for medical students 7th edition, Japee Brothers medical Publishers, 176-180.
[13]. Ekpenyong, C.E., Daniel N.E and Antai A.B. (2015).Tea anf Erythropoeisis Boosting Effects: Potential Use in Prevention and Treatment of Anemia. *Journal of Medicinal Food*, 18(1) 118-127
[14]. Koffuor, G., Amoateng, P. and Andey, T. (2011). Immunomodulatory and erythropoietic effect of aqueous extract of the fruits of Solanum torvum swartz (Solaneceae). *Pharmacogno.*3:130-4.
[15]. Yakubu, M.T, Akanji, M.A. and Oladji, A.T. (2007) Haematological evaluation in male albino rats following chronic administration of aqueous extracts of Fedogia agrestis stem. The Pharmacognosy Magazine 3: 34.
[16]. Kytridis, V. and Manetas, Y. (2006). Mesophyll versus epidermal enthocyamins aas potential in vivo antioxidants evidence linking the putative antioxidant role of the proximity of oxy-radical source. *J. Exp. Bot.* 57: 2203-2210.
[17]. Awe, O., Banjoko, O. (2013). Biochemical and haematological assessment of toxic effects of the leaf ethanol extract of Petroselinum crispum (Mill) Nyman ex A.W. Hill (Parsley) in rats. *BMC Complem. & Altern. Med*., 13:75-80.
[18]. Sembulingam, K., and Sembulingam, P. (2016). Essential of Medical Physiology, 7th edition. The Health Sciences Publishers. 64-104.
[19]. Keenwe, M. and Bekalo, I. (1996). Ethno vertinary Medicine in Kenya, a field manual of practical animal health care practices. *Nai., 60:789-792.*
[20]. Choi, S., Nichol, L., Hokom, M., Hornkohl, C., and Hunt, P. (1995). Platelets generated in vitro from proplatelet-displaying human megakaryocytes are functional. *Blood,* 85(2):402-413.
[21]. Olas, B. and Wachowicz, B. (2012). Resveratrol and vitamin C as antioxidants in blood platelets. *Thromb. Res*., 106(2):143-148