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Effects of Ethanol leaf extracts of *Mangifera indica* and *Gongronama latifolium* on some Haematological Parameters of Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effects of ethanol leaf extracts of *Mangifera indica* and *Gongronema latifolium* on some haematological parameters (Haematocrit, HCT; Mean corpuscular Haemoglobin concentration, MCHC; Neutrophils, NEUT; Red cell Distribution width, RDW; and mixed blood cell count, MXD) in albino wistar rats were evaluated. Sixteen animals (185-223g) were assigned four groups of four rats each. Groups 1 and 3 were treated with 200mg/kg *Mangifera indica* and *Gongronama latifolium* respectively.

Group 2 was treated with 200mg/kg of both plant leaf extracts at 50:50 dosage ratio. Group 4 was not treated and served as control. Treatment was done daily via oral route for one month. The animals were allowed free access to commercial rat mash and water throughout one month

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treatment. At the end of the treatment, no significant difference (P>0.05) was found in the HCT, MCHC and MXD levels in all the treatment groups when compared to the control. No significant difference (P>0.05) was also found among the treatment groups. NEUT levels in groups 1,2 and 3 animals recorded significant increase (P<0.05) when compared to the control but, no significant difference (P>0.05) was observed among the treatment groups. RDW recorded significant decrease (P<0.05) in groups 1,2 and 3 when compared to the control, but not significant different (P>0.05) was found among the treatment groups. These results implied that ethanol leaf extracts of *Mangifera indica* and *Gongonema latifolium* have no adverse effects on the haematological indices studied.

Keywords: Haematological parameters; Mangifera indica; Gongronema latifolium; wistar rats; ethanol leaf extracts.

1. INTRODUCTION

"Plants have been used for food, medicine, building materials and fuel for many centuries until now, the World Health Organization (WHO) noted that of the 119 plant-derived pharmaceutical drugs, 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as Herbal plants medicine by native cultures" [1]. Many of such plants known to be used primitively to elucidate symptoms of illness have been screened to have both good and adverse effects on human, some of which include *Mangifera indica* and *Gongronema latifolium.* In many tropical and subtropical climates, M. indica is the sole specie that is farmed, and its fruits are widely available. The tree is widely cultivated throughout Africa, and in Nigeria, it may be found in abundance in the states of Benue and other regions of the nation.

Mangifera indica is used as medication to treat a variety of conditions, including diabetes, anemia, diarrhea, dysentery, jaundice, and asthma [2,3], (Botool et al. 2018). "Several studies have been proven the pharmacological potential of different parts of mango (*M. indica*) trees such as leaves, bark, fruit peel and flesh, roots and flowers as anticancer, anti-inflammatory, anti-oxidant, antibacterial, antifungal, anti-plasmodial and antihyperlipidemic in nature" [4-6]. "*M. indica* plant has been reported to contain phytochemicals such as phenolic acids,benzophenones, and antioxidants such as flavonoids, ascorbic acid, carotenoids and tocopherols" [7,8], (Akpakpan et al. 2020); [8].

"*Gongronema latifolium* is an important plant as well as a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [9]. It contains essential oils, Saponins and pregnanes among others, aqueous and ethanol extracts of *G. latifolium* exhibit hypoglycermic, hypolipidemic and antioxidant properties" [10]. "The fresh leaves and stem can be chewed or the sap extracted with water or palm wine and taken as herbal medicine. The leaves are rich in fats, proteins, vitamins, minerals and amino acids, including leucine, valine, phenyl alanine, aspartic acid, glycine and glutamic acid" [11-13]. "A decoction of leaves or leafy stems of *G. latifolium* is commonly taken to treat diabetes and high blood pressure, asthma and patients chew fresh leaves to relieve wheezing" [12].

However, the two plant leaves (that is *M.indica* and *G. latifolium* can be toxic to human thereby affecting some physiological parameters like haematological indices [14]. Haematological parameters include the blood related parameters such as red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb), haematocrit (pack cell volume) etc

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Samples

The fresh leaves of *mangiferia indica* and *Gongronema latifolium* were collected at different locations within Ikot Ekpene and Essien Udim Local Government Area, both in Akwa Ibom State, Nigeria. The two plants were authenticated by a Taxonomist in the Department of Botany and Ecological studies, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

"The two plants leaves were plucked from their stems, washed with tap water to remove dirt, sliced separately with knife into tiny pieces and dried separately at room temperature for 3 days.

The dried leaves were later ground separately using clean, dry mortar and pestle and 350g each of the samples were soaked in 150 ml of 70% ethanol for 72 hours at room temperature" [15]. The macerated leaf extracts were differently filtered using Whatman No. 1 filter paper by means of funnel. The filtrates were separately concentrated at 40 -50^{o}C in a water bath for 3 consecutive days after which slurry form of the extracts obtained and preserved in a refrigerator at 4^{o}C for further use.

2.2 Experimental Design, Grouping and Treatment of Animals

A total of sixteen healthy adult male albino wistar rats weighing 185-223g were obtained from the disease-free stock of the animal house of the Department of Biochemistry, University of Calabar, Cross River State, Nigeria. "The animals were housed in a cage with four sizeable compartments of wooden bottom and were mesh top randomly assigned four animals per four groups. The rats were maintained under standard conditions of temperature and natural light-dark cycle for 7 days acclimatization in the animal house, AkwaIbom State Polytechnic, Ikot Osurua. Groups 1 and 3 were treated with 200mg/kg *M.indica* and *G.latifolium* respectively, while group 2 was treated with 200mg/kg combined extracts of the two plants at 50:50 dosage ratios. Group 4 was not treated with the extracts and served as normal control. Treatments were done daily via oral route for a period of one month. The animals were allowed free access to commercial rats mash and water" [16].

2.3 Collection of Blood Sample

At the end of one month treatment, the animals were fasted for 12hours and were anaesthetized under chloroform vapour and were sacrificed by dissecting medioventrically and blood was collected via cardiac puncture by means of syringe and needle into a sterile EDTA sample bottle and were taken to the laboratory for the determination of haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), neutrophils counts (NEU), Red cell Distribution width (RDW) and mixed blood cell count (MXD).

Determination of Haematological indices. Full blood count (FBC) was determined using SYSMEX automated haematology analyzer (model KX-21n)-SYSMEX, corporation Kope, Japan.

Each blood sample was mixed well and then approximately 20µl was aspirated into the instrument and the start button was pressed on. The results of the analysis was displayed after some seconds which the analyzer generated a paper copy of the results on thermal printing paper.

2.4 Statistical Analysis

Data were analyzed using SPSS (version 23). The descriptive data were expressed as mean ± standard error of mean (SEM) and one way analysis of variance (ANOVA) was used for analytical assessment. Statistical significance were recorded when the P-values obtained was <0.05.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1. Mean Haematological indices of normal Albino wistar Rats treated with Ethanol leaf Extracts of *M.indica* and *G. latifolium*

	Haematological parameters				
Group	**HCT(C/C)**	**MCHC (%)**	**NEUT (%)**	**RDW (%)**	**MXD (%)**
1	48.13±2.57	29.82±0.47	35.96±0.02	25.78±5.03	3.83±0.17
2	47.10±1.27	25.28±4.97	39.38±5.65	28.75±5.11	4.53±0.46
3	47.98±2.14	29.90±0.76	32.33±3.88	30.08±0.59	3.85±0.32
4	48.73±1.32	24.65±2.52	13.28±1.16	48.75±1.32	4.35±0.18

Data present as mean ±SEM, N=4

3.2 Discussion

Many of the pharmaceuticals available to physicians have a long history of use as herbals including. Opium, aspirin, digitalis and quinine [17]. The World Health Organization (WHO) estimates that 80 percent of the the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care [2].

Pharmaceuticals are prohibitively expensive for most of the world's population, half of which lives on less than & 2US pay day [18]. Therefore, the use of, and search for drugs and dietary supplements derived from plants have accelerated in recent years (Tietz, 1996). That made this study to focused on the evaluation of the effects of ethanol extracts of *Mangiferia indica* and *Gougronema latifoluim* leaves on some haematological indices in normal male albino Wistar rats.

Precisely, the study considered the impacts of ethanol leaves extracts of the two plants on haematoirit (HCT), mean corpuscular haemoglobin concentration (MCHC), nuetrophils (NEU), red cell distribution width (RDW) and mixed blood cell count (MXD) in male albino wistar rats.

No statistical significant difference was found in the HCT, MCHC and MXD level in all the extracts treated groups when compared to the control group. Haematocrit (HCT) evaluates the percentage of the blood that is made up of red blood cells which helps in transporting oxygen throughout the body (Parves et al. 2004). Low levels of haematocrit leads to pale complexion, weakness, fatigue, low energy, trouble breathing, irregular heartbeats cold hands and feet [19]. Both high and low haematocrit levels can be detrimental to people's health and can be as a result of a variety of conditions and lifestyle factors. The mean corpuscular haemoglobin concentration (MCHC) was also lower in the treated groups of animals, but were higher than the value in the control group. MCHC reflect the average haemoglobin contents of red blood cells in slightly different ways. Although MCH expresses the average content (mass, weight) of haemoglobin per red cell, MCHC expresses the average weight of haemoglobin per unit volume of the red cells. MCHC are parts of red cell indices (parameters reflecting size and haemoglobin content of red cell) that have traditionally been used to aid in the differential diagnosis of anemia [9,20].

However, there was a significant increase in the concentration of neutrophils (NEU) in all the treated groups when compared to the control group. A normal neutrophil count is between 2500C/µl and 7000 C/µl. The process of measuring the absolute neutrophil count is automated by the analyzer and shows in some complete blood counts as the neutrophil automated count. Neutrophilia is diagnosed when the complete count shows an absolute neutrophil count over 7000C/µl. Neutrophils help prevent infections by blocking, disabling, digesting or warding off invading particles and microorganisms. They also communicate with other cells to help the repair cells and mount a proper immune response. Increase in neutrophil levels usually occur naturally due to infections or injuries. It may rise in blood in response to some medications such as corticosteroids, beta -2-agonists, and epinephrine, surgery or accidents, physical or emotional stress surgical removal of the spleen [20]. However, the increased level of neutrophils in the treated groups of animals under study signified *M.indica* and *G.latifolium* leaves administration.

Moreover, the significant decrease in the mean red cell distribution width (WRCDW) in all the treated groups of animals when compared to the control signified the activity of the bioactive compounds in the plant leaves extracts. Similarly, the significant decrease in the RDW in all the treatment groups could be attributed to lysis of blood cells during the time the animals were sacrificed. RSW reduced below the normal range of 40.0-55.0 ml, indicating that the red blood cells varies little in size and can trigger macrocytic and microcytic anemia [9]. This decrease in the level of red cell distribution width (RDW) in the treated groups of animals signified the *G. latifolium* and *M.indica* the administered dosage had no adverse effect on the hematropoiesis process.

Mixed blood cell count (MXD) is the collective measurement of the levels monocytes, eosinophils, basophils in the blood which are very significant than the neutrophiles and lymphocytes. The results are expressed in percentage, and the MXD normal range for the blood test value is considered to be between 5-10%. MXD fights against bacteria, parasitic and fungal infections in the body. "A significant rise in the percentage count of MXD is indication of a corresponding rise in eosinophils, monocytes and basophils. This may lead to a general immunological response as a number of systems

employing antibodies were evolved to disturb the schistosomes" [21]. A low MXD blood test score indicates that the body's defenses against specific illnesses, including cancer, HIV, bone marrow disease, lymphoma, lupus, and serious infections, have been compromised [19,21]. The decrease may be caused by saponin found in plant extracts, which has been shown to lower various hematological parameters in albino rats, likely as a result of blood cell lysis [22-28].

4. CONCLUSION

There was no adverse effects of ethanol leaves extracts of *Mangiteria indica* and *Gongronema latifolium* on some haematological indices studied. However, the plant extracts regulated the haematogical parameters within their normal ranges, therefore, they could be used to formulate blood boosting medication to be used by human to enhance normal haemotogical indices.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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