

Effect of ethyl acetate root extract of *Guiera Senegalensis* on some hematological parameters on normal rats

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Abstract:

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Lots of herbs have been used in the management and increasing of the blood volume in the body. Effect of ethyl acetate root extract of *Guiera Senegalensis* on some hematological parameters on normal rats during the period of 28 days were investigated. The parameters evaluated include the red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV) and hemoglobin (HB). A total of sixteen (16) adult rats were weighing between 126- 253g were randomly assigned in to four (4) different groups of 4 rats each, the group 1 served as the control baseline, and the group 2 were administered with a dose of 100mg/kg of the plant extract and a normal diet, group 3 and 4 were administered with the dose 200 mg/kg and 400 mg/kg and a normal diet. The plant extracts were administered to the animals for the period of 28 days and at the 7th, 14th, 21st, and 28th day, the hematological parameters were studied and the animals were sacrificed at the end of the 28th day. The result obtained in the study showed a significant increase in the RBC, WBC, it showed no statistically significant changes in the PCV and HB but it increased as the RBC increased.

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1. Introduction

Plants materials have been used for their therapeutic properties since antiquity [1, 2]. Consequently, human medicine is much linked to plant biodiversity and the fact that most of the current modern medicines were first derived from medicinal plants [3]. Across centuries, the global rate of medicinal plants use has been increasing, more so in developing countries. This is mostly attributed to the fact that medicinal plants are readily available, widely accepted by many cultures, and perceived to be safe and efficacious. At present, the estimated prevalence of global medicinal plants' use ranges between 50 and 95% [4, 5]. Due to this high demand for herbal medicine in both developing and developed countries and other factors such as climate change, about 15,000 medicinal plant species worldwide are at risk of extinction [6].

Medicinal plants (medicinal herbs) have been discovered for decades and used in traditional medicine practices ever since prehistoric times. Plants synthesize hundreds of chemical and biochemical compounds for functions including defense against insects, herbivorous mammals, fungi, and diseases. Numerous phytochemicals with established or potential biological activity have been identified in plants. However, as a single plant has widely diverse phytochemicals, the effect of using a whole plant as

medicine is not certain. Further, the pharmacological actions and phytochemical contents, if any, of several plants with medicinal potential remain untapped unassessed by rigorous scientific research to categorically define safety and efficacy [7].

Lots of herbs have been used in the management and increasing of the blood volume in the body, although the active principles some of them have been isolated and are being used. Folk medicine has existed for as long as human beings existed, folk medicine refers to healing practices and ideas of the body physiology and health preservation widely known to much of the population in the culture, transmitted informally as generally knowledge and practices or applied by anyone in the culture [8].

The use of medicinal herbs in the traditional system of medicine is a common practice in many cultures around the world, especially in African society. This practice has gained widespread acceptance in developing as well as developed nations. Researchers are also beginning to appreciate the role of medicinal plants in health care delivery; this is a result of the effectiveness, low cost and the availability of these herbal medicines [9]. It is noteworthy that some orthodox medicine in use today were developed from the biochemical templates obtained from medicinal plants [10], however the widespread use and popularity of herbal medicines do not guarantee their efficiency and safety [11]. Therefore, there

is a need for determining scientific analysis and adequate information on the toxicity of commonly used herbal drugs [12]. One of the ways to determine the safe or unsafe use of medicinal plants is the assessment of how it affects hematological parameters [13]. Changes from normal physiological levels of these parameters offer administration of a chemical agent indication of adverse effects of such agents on living organisms [14].

Guiera senegalensis is (family of combretaceae, commonly known as sabara in Hausa and kashishi in Kanuri) commonly found as a shrub of the savannah region of west Africa, is very well known in its native area, generally occurs as a shrub that can grow to a height of 3 to 5 m according to habitat. Its stem presents numerous knots that send out branches. The ash-grey stem and branches have fibrous or pubescent bark and bear opposing, short petiolated oval leaves, sometimes mucronate, sometimes even cordate at their base, about 2 to 4 cm long by 1 to 2 cm wide. These grey-green leaves, darker on their upper surface, display black spots on their lower surface and are slightly downy on both sides. These features lend the plant an overall silver green colour that is conspicuous in brush land [15]. The literature reports several recorded uses for *G. senegalensis* in traditional medicine to treat various illnesses [16]. Its leaves extract is used against dysentery, diarrhea, gastro intestinal pain, fever, rheumatism and in the blood volume [15]. The plant produces tannins 3,4,5-Tri-O-gallopliquenic acid and several alkaloids of the human family, it is one of the most important west African medicinal plant often use in the treatment of microbial infections, the most frequently used part of the plant is the leaves [16]. The main aim and objective of the research is to investigate effect of ethyl acetate root extract of *Guiera Senegalensis* on some hematological parameters on normal rats

2. Materials and methods

2.1 Plant and animal Materials

Plants (roots) and Animals (rats). Fresh roots of *Guiera senegalensis* were collected from Jere L.G.A of Borno State. The identification of plants was certified by the Department of Biological Science, University of Maiduguri.

2.2 Extract preparation

The roots of *Guiera senegalensis* were washed to remove particles and dust, the washed roots were shade dried and heated at 80°C for 10 minutes and 60°C for 30 minutes [17]. The dried roots were ground to fine powder using pestle and mortar and stored in cellophane bags at room temperature before use. About 200g of the weighed powdered dried root (sample) was portioned using solvent of different polarities, methanol, ethyl acetate, n-butanol and water. The crude extracts obtained will be concentrated to dryness at 40°C - 45°C using a water bath, the concentrated (extract) were weighed, labeled and kept for use. [17, 18]. The ethyl acetate extract was used for this study.

2.3 Experimental animals

Sixteen male and female rats were weighed between 126-253g and were divided into four groups of rats each in a cage. The groups are as in equations 1 to 4:

$$A = \text{control baseline} + \text{distilled water} \quad (1)$$

$$B = \text{Normal diet} + \frac{100\text{mg}}{\text{kg}} \text{ ethyl acetate extract} \quad (2)$$

$$C = \text{Normal diet} + \frac{200\text{mg}}{\text{kg}} \text{ ethyl acetate extract} \quad (3)$$

$$D = \text{Normal diet} + \frac{400\text{mg}}{\text{kg}} \text{ ethyl acetate extract} \quad (4)$$

There were caged and maintained under standard condition throughout the experiment and are feed for 28 days, saw dust was used regularly for the bedding of the cages. The sawdust was used as sufficient bedding for the animals and the bedding was changed after every two days.

2.4 Extract administration

Animals in group (1) served as the control baseline, hence were served with a normal diet of food and distilled water, they were maintained on the commercial preparation of the growers marsh and distilled water. Animals in group (2) were given the normal diet, distilled water and were intubated orally with a dose of 100mg/kg of the extract in a solution form. Animals in group (3) were given the normal diet, distilled water and are also intubated orally with a dose of 200mg/kg of the extract solution. Animals in group (4) are also given normal diet, distilled water and are intubated orally with 400mg/kg extract solution. The animals were intubated every day in the morning for a period of 28 days (4 weeks). The extract was given based on their body weight.

2.5 Sample collection (blood).

At the end of every 7th day (week), blood sample was collected and was used for hematological parameters test, the parameters that are been tested for are as follows:-

- Red blood cell (RBC)
- White blood cell (WBC)
- Packed cell volume (PVC)
- Hemoglobin (HB)

The blood sample was collected through the tail of the rats for testing and at the end of the 28th day the rats were sacrificed and the blood was collected through the hearts of the animals.

2.5.1 PCV(hematocrit method)

The blood was collected in a hematocrit pipette and one end was sealed. Then it is put in a centrifuge machine for spinning, the blood is spun for 5 minutes until the RBC. The heaviest part of the blood goes to the bottom of the tube and then reading of PCVis done with a measuring hematocrit meter and the result was obtained [19].

2.5.2 RBC (heamocytometric method)

Principle- Is based on counting the number of RBC in a known small volume of accurately diluted blood, blood is diluted exactly 1:200 with hayem's solution (an isotonic RBC diluting fluid) in a pipette and the RBC in a small volume of

the diluted blood are counted in a counting chamber known as haemocytometer [20].

The RBC pipette was cleaned and allowed to dry with the bead moving freely inside the bulb. The tail of the animal was cut off and the first drop of the blood was discarded, the point was then held to the base of the drop, tilted slightly downwards, tip uppermost and allowed filling up to 0.5mark. The pipette was withdrawn and kept horizontally to prevent blood shifting wipe the outside of the pipette with filter paper to remove any blood, if the blood is above 0.5mark bring it down to the mark by dabbing on filter paper. Immediately draw up hayem's solution (RBC), diluting fluid from the watch glass up to 101 mark being careful not to overshoot, rolling the rubber tube without squeezing for 1 minute, mixing blood and diluting fluid thoroughly in the bulb.

Bring the tip of the pipette in contact with the exposed part of the counting chamber and raise the pipette toward vertical position until diluted blood flow under the cover slide by capillary action. It was examined using a low power (x10) objective lens and then using a high (x40) objective lens. Count all the number of red blood corpuscles in each 80 small square i.e. 5 sets of small squares each having 16 smaller squares (5x16 = 80) [20].

2.5.2.1 Calculations

Area of 1 small square (marked R):

$$\frac{1}{5} \times \frac{1}{5} = \frac{1}{25} sqmm$$

In RBC count, 5 small square (marked R) are counted Thus the area of Small Square:

$$- 5 \times \frac{1}{25sqmm} = \frac{1}{5} sqmm$$

Depth of the chamber = $\frac{1}{10} mm$

Volume of the 5 small squares is:

$$\frac{1}{5} \times \frac{1}{10} cumm = \frac{1}{50} cumm$$

Let the number of the cell counted in 5 small squares be X, therefore a cubic mm of diluted blood contain:

$$X \times 50cells$$

Dilution factor for RBC count is 1:200

Therefore 1 cubic millimeter of undiluted blood contain

$$X \times 50 \times 200cells = X \times 10,000. [20].$$

2.5.3 HB estimation (Sahli's method)

Principle: - HB estimation has visual matching colors for accurate estimation; the transmitted light energy may be

transformed into auto electric energy by means of photoelectric cell and the result read on a calorimeter.

Take 2mls of the 0.04% ammonia solution in a fulse, add 2ml of blood to the solution with the pipette provided, sucking the solution up to 2 or 3 times to wash the pipette oxy-HB is found. Add more of the 0.04% ammonia solution to the graduated mark on the tube to mark a total volume of 8mls in the tube. Proceed to the calorimeter and read from the sheet provided before using the instrument [21].

2.5.4 WBC (heamocytometric method)

These are counted in similar manner to RBC (RBC), the procedures are basically the same except that in WBC count the pipette upper mark, diluting fluid (Tur's) and the diluting factor is different.

Normal white blood count: In both men and women 2300 – 8600cells/cubic.mm of blood

2.5.4.1 Calculation

Area of the WBC square 1sq.mm

Depth $\frac{1}{10} mm$

Volume of one square $\frac{1}{10} cu. mm$

Volume of 4 WBC square $= \frac{1}{10} \times 4 cubic. mm$
 $= \frac{4}{10} cu. mm$

Dilution factor 1:20

If X = number of cells present in 4/10 cu.mm of diluted blood therefore 1 cubic.mm of diluted blood contain

$$X \times \frac{10}{4} cells$$

So, 1 cubic mm of undiluted blood contains

$$X \times 10 \times \frac{20}{4} or X \times 50 cells. [20].$$

2.6 Statistical analysis

The results are presented as mean \pm SD (standard deviation). The difference between the mean values was analysed using ANOVA, and the differences were considered significant at (P < 0.05). The instat computer software was used.

3. Results

Effects of ethyl acetate root extract of *Guiera senegalensis* on some hematological parameters in normal rats are shown in Tables 1 to 4.

Table 1: Effects of ethyl acetate root extract of *Guiera senegalensis* on Red Blood Cell (RBC) (10⁶mm³)

	0day	7 th day	14 th day	21 st day	28 th day
Control	8.2 \pm 0.24	8.8 \pm 0.35	9.5 \pm 0.11	6.7 \pm 0.10	7.1 \pm 0.11
100mg/kg	9.3 \pm 0.47	10 \pm 0.40**	10.3 \pm 0.11***	9.0 \pm 0.10***	7.7 \pm 0.10 ^{ns}
200mg/kg	8.8 \pm 0.25	9.3 \pm 0.25 ^{ns}	12.4 \pm 0.19***	8.6 \pm 0.28***	6.7 \pm 0.10 ^{ns}
400mg/kg	7.1 \pm 0.25	7.6 \pm 0.25**	13.2 \pm 0.00***	6.6 \pm 0.00 ^{ns}	8.15 \pm 0.90*

Values are mean \pm SD, NS- not significant, P (>0.05) – ns, P (<0.05) - *, P (<0.01) - **, P (<0.001) - ***. When compared with the control group. N – 4 (no of replicates).

Table 2: Effects of ethyl acetate root extract of *Guiera senegalensis* on White Blood Cell Count (WBC) (10^4mm^3)

	0day	7 th day	14 th day	21 st day	28 th day
Control	1.41 ± 0.02	1.52 ± 0.07	2.40 ± 0.00	1.22 ± 0.02	1.05 ± 0.01
100mg/kg	1.42 ± 0.03 ^{ns}	1.51 ± 0.02 ^{ns}	2.63 ± 0.01 ^{***}	1.43 ± 0.01 ^{***}	1.27 ± 0.01 ^{***}
200mg/kg	1.39 ± 0.01 ^{ns}	1.46 ± 0.01 ^{ns}	1.61 ± 0.01 ^{**}	1.75 ± 0.01 ^{**}	1.44 ± 0.01 ^{**}
400mg/kg	1.47 ± 0.03 [*]	1.92 ± 0.10 ^{***}	2.43 ± 0.02 [*]	1.27 ± 0.01 ^{**}	1.75 ± 0.01 ^{***}

Values are mean ± SD, ns- not significant, P (>0.05) – ns, P (<0.05) – *, P (<0.01) – **, P (<0.001) – ***. When compared with the control group. N – 4 (no of replicates)

Table 3: Effects of ethyl acetate root extract of *Guiera senegalensis* on PCV (%)

	0day	7 th day	14 th day	21 st day	28 th day
Control	42.25± 1.89	44.25±1.89	45.6±1.00	45.5±1.73	45.5±1.91
100mg/kg	43.75± 0.95	45.75±0.95	49±0.00	46.5±1.00	44.25±2.21
200mg/kg	45.0± 4.96	47.0±4.96	47.75±3.20	46.0±4.50	45.5±3.69
400mg/kg	44.0± 2.94	46.0±2.94	47.0±0.00	43.5±4.12	47.75±0.50

Values are mean ± SD. N – 4 (no of replicates). Statistical analysis shows no significant difference when compared with the control group.

Table 4: Effects of ethyl acetate root extract of *Guiera senegalensis* on HB (g/dl)

	0 day	7 th day	14 th day	21 st day	28 th day
Control	13.4± 0.85	13.62±0.85	15.15±0.70	14.6±1.38	16.21±0.43
100mg/kg	14.27± 0.61	14.47±0.61	16.95±0.97	15.6±0.67	15.7±0.50
200mg/kg	14.12± 1.33	14.32±1.33	16.45±1.47	15.0±2.33	16.45±1.47
400mg/kg	14.8± 1.33	15.0±1.55	16.8±0.00	13.95±2.16	17.0±1.57

Values are mean ± SD, ns- not significant, N – 4 (no of replicates). It shows no statistical significance when compared with the control group.

4. Discussion

Plants generally have varied chemical composition depending upon species, a good number of plants are known to be of economic and medicinal values, those that are of medicinal value are often used as herbal remedy for the restriction and maintenance of good health. Some herbs have been considered as drugs and therefore generally safe and effective, most herbs have been associated with broad actions on a number of physiological systems in concert unlike the pharmaceutical drugs which are usually designed to elicit a specific effect, some researchers on medicinal plants are of the opinion that some herbal plants are usually oriented in the same general therapeutic direction and are complementary or synergistic often nonspecific but very rarely adverse. Despite the widespread use, few scientific studies have been undertaken to ascertain the safety or toxicity risks of many herbal remedies [22].

In this study, the effect of ethyl acetate root extract of *Guiera senegalensis* on hematological parameters shows that it significantly increased RBC at all doses tested when compared with the control group and shows no any statistical significant on Hb, and PCV but it there increase as the RBC increases, however since Hb, RBC and PCV relate to the population of the RBC in the blood, it imply that the extract may neither affects the incorporation of HB into RBC nor the morphology and osmotic fragility of RBC cells produced. However the increase in Hb, RBC and PCV implies that the extract may increase the population of RBC produced from the bone marrow.

The extract significantly increases WBC (WBC) at 100mg/kg and 400mg/kg administered when compared with the control group, this suggests that the extract may contain some bioactive agents that could lead to the stimulation of

WBC production. It has been reported that granulocyte-macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC.

5. Conclusion

The ethyl acetate root extract of *Guiera senegalensis* showed that the RBC, WBC, increased significantly and it showed no significant effect on the PCV and HB but as the RBC increases, it has the ability to stimulate the production of WBC. This may suggest a mild effect on the hematological indices at the doses tested. Therefore the effect of the extract is mild, parameters and dose selective and thus it is safe for oral administration, safe as an oral remedy as used in folk medicine and also it might be used in the treatment of blood disease e.g. anemia, and the result obtained may explain the uses of this plant in traditional medicine to increase and stimulate the production of blood in the body.

Recommendation

Having observed the significant different activity of *Guiera senegalensis*, hereby recommend that research work should continue in this regard to identify the active component of the root so as to be used, also it might possess the increase in the level of blood production from the bone marrow.

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