

Comparative toxicity study of aqueous, ethanol and methanol leaf extracts of *Simarouba glauca* on hematological indices in adult normotensive male Wistar rats

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Abstract

Background

The study focused on the toxicological evaluation of leaf extracts of *S. glauca* on some hematological indices of male *Wistar* rats.

Methods

Thirty (30) male *Wistar* rats were divided into ten groups of three rats each. Test rats were given AESG, EESG or MESG at doses of 500, 1000 and 2000 mg/kg body weight respectively; the control group was provided with food and water *ad libitum* daily for thirty (30) days. At the end of the study, the fasted rats were sacrificed and haematological assessment was conducted.

Results

The data obtained indicates elevated ($P < 0.05$) RBC levels of experimental rat administered EESG and MESG at 500, 1000 and 2000 mg respectively. Reduced ($P < 0.05$) haemoglobin concentration at AESG and MESG 500 mg, AESG, EESG and MESG 1000 mg; EESG 2000 mg. The haematocrit/PCV levels were only slightly reduced ($P < 0.05$) and elevated ($P < 0.05$) at EESG 1000 and 2000 mg respectively. Platelets count was elevated ($P < 0.05$) at MESG 500 mg, lowered at AESG 1000 mg and EESG 2000 mg. The WBC count was elevated ($P < 0.05$) at AESG and EESG 1000 mg; elevated ($P < 0.05$) at EESG and MESG 2000 mg. The lymphocytes count was elevated ($P < 0.05$) at EESG 500, 1000 & 2000 mg; at AESG 1000 mg. The monocytes count was elevated ($P < 0.05$) at AESG 500, 1000 & 2000 mg, elevated ($P < 0.05$) AESG and EESG 1000 & 2000 mg respectively, and elevated ($P < 0.05$) at MESG 2000 mg.

Conclusion

Oral administration of leaf extracts of *S. glauca*, especially at higher doses elicit disturbances in haematological indices.

Introduction

Haematopoietic system is one of the most susceptible targets of toxic compounds, especially in the bone marrow where the production of red blood cell occurs [1]. Adverse effects of plant compounds of medicinal importance often elicit inflammatory responses associated with toxicity of these biological compounds. However, herbal preparations which are assumed to be safe may contain contaminants such as heavy metals [2], aflatoxins and pathogenic microbes due to the manner in which they are prepared or as a result of acquisition of metals (e.g. cadmium) from the soil [3, 4]. There is also the

notion amongst the large population of herbal remedy users that these herbal medicines are not toxic because they are derived from natural sources [5].

Pluripotent stem cell of the bone marrow is the source of red blood cell, white blood cells and the platelets; as such, toxicological events associated with drugs like chloramphenicol and its depressant effect on the bone marrow has been reported [6]. Similarly, phytochemicals from plants may also affect the bone marrow; thus, interfering with the production of blood cells [7]. Furthermore, the activities of numerous enzymes involved in the synthesis of blood components in the bone marrow are also possibly affected by phytochemical constituents of medicinal plants [8]. This suggests that any substance which affects the enzyme activities of bone marrow may adversely compromise the synthesis of blood cells.

The hematopoietic physiology is very sensitive to toxins, hence data obtained after exposure of an animal to certain compounds may be used to evaluate the pathological or physiological status of the test animal [9]. Also, Tohti *et al.* [10] reported decrease in the white blood cell count (WBC) due to the presence of some phytochemicals such as saponins and cardiac glycosides.

Like erythrocytes, leukocytes are constitutively produced throughout adult life from haematopoietic stem cells in the red bone marrow; they are released into the circulation where they perform defense tasks; then removed from the blood by the liver and spleen [11]. Unlike erythrocytes, these large, nucleated, and translucent cells undertake significant protective functions. They are often capable of phagocytosis and are highly specialized to defend the body against various microorganisms and others like, tumor cells or foreign substances [11]. In general, leukocytes are motile and very flexible; most of these cells are found in body tissues, as opposed to the bloodstream [12]. Some specific molecules that are released by damaged, abnormal, and dead cells, or by foreign invaders, attract leukocytes by chemotaxis to the sites of injury, infection, and inflammation [12]. Although all five types of leukocytes contribute to the same general function; each type of these cells undertake a specific function in the defense system. For example, neutrophils and monocytes are capable of the process of phagocytosis of various pathogens. While neutrophils are the more abundant phagocytic cells which are short-lived, monocytes are significantly more efficient as they differentiate into macrophages which can perform phagocytosis of damaged, abnormal or dead cells and tissues at the sites of injury or inflammation [12]. During infectious responses, neutrophils are produced more rapidly, and the immature forms of these cells, called band cells (or stab cells), may appear in significantly greater numbers in the peripheral blood [11–12].

The increase in application of medicinal plants cannot be overemphasized; toxicity and safety of herbal products remains a mainstay in evaluation and development of herbal medicine for treatment and (or) management of various diseases in humans [13]. *Simarouba glauca*, paradise tree or “Laxmitaru” belongs to the family *Simaroubaceae* [14]. *S. glauca* has a long history of herbal medicine application considering documented evidence of pharmacological potentials in literatures [14]. The stem-bark and leaf extracts of *S. glauca* contain triterpenes, useful in curing amoebiasis, diarrhea and malaria [15]. Chemicals present in leaf, fruit, pulp and seed of *S. glauca* have been reported to possess analgesic, anticancer, antimicrobial, antiviral, astringent, cardio-protective, emmenagogue, stomachic, tonic,

vermifuge properties [15–18]. The present study focused on the toxicological evaluation of leaf extracts of *S. glauca* on some selected hematological indices of male *Wistar* rat

Materials And Methods

Collection of *S. glauca* leaves and preparation of Extracts

Leaves of *S. glauca* were collected from *Cercobela* Farms®, Ubiaja, Esan South East Local Government Area of Edo State, Nigeria. The plant was authenticated and at the Department of Plant Biology and Biotechnology, University of Benin with a herbarium voucher specimen N0. UBH₅382. The leaves were rinsed with tap water and air-dried at room temperature at the Department of Biochemistry, University of Benin, for twenty-eight (28) days. Leaves were pulverized and sieved of a tiny pore mesh at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, to obtain a fine powder. A 500 g of the leaf powder was soaked in 2.5 L of distilled water, ethanol and methanol solvent of 99% purity w/v respectively; stirred at intervals for 24 h, and filtered. The leaf powdered material was re-extracted in another 2.5 L of distilled water, ethanol and methanol solvent of 99% purity w/v respectively; stirred at intervals for another 24 h. Both filtrate portions were decanted; freeze-dried in line with the method previously reported by Osagie-Eweka *et al.* [19]. The percentage yield of extraction was 6% w/w. Plate 1 below shows a section of the paradise tree (*S. glauca*) with fruits in its natural habitat.

Experimental animals

A total of 24 male *Wistar* rats weighing between 184 and 200 g were used for the study. The animals were housed in metabolic cages, fed with normal commercial pellets (Livestock Feeds®) and drank water *ad libitum*. They were maintained under laboratory conditions of 12 h light/ 12 h dark cycle and were acclimatized for two weeks prior to commencement of studies. All experiments were conducted in accordance with the internationally accepted guidelines for laboratory animal use. The protocols were approved by the Faculty of Pharmacy, University of Benin Ethics Committee with reference number EC/FP/021/11.

Oral administration of Leaf Extracts of *S. glauca*

The study was conducted as prescribed in the OECD No. 425 test guidelines [20], as described by [21, 22]. The rats were randomly allotted into four (4) groups (n = 6). Test animals received oral doses of 500, 1000, and 2000 mg/kg body weight respectively of leaf extracts daily for thirty (30) days while the control group received only water *ad libitum*.

Collection of samples and specimens

On day 30, the rats were fasted overnight; the following day, each group of rats were anesthetized in a chloroform-saturated chamber and sacrificed. Blood sample was withdrawn from the thoracic aorta into an ethylenediaminetetraacetic (EDTA) acid specimen bottles.

Haematological analyses

The Erma haematology analyzer and Automated/Electronic cell counters (Located at the Hematology unit of University of Benin Teaching Hospital (UBTH)) was utilized to analyze the whole blood collected from *Wistar* rat as previously reported by Clark *et al.* [23]. The hematological parameters include measurement of red blood cell count (RBC), hemoglobin (Hgb), Hematocrit (HCT), white blood cell count (WBC) and WBC differentials (lymphocytes and monocytes count).

Statistical analyses

Data are expressed as mean $\pm$ SD (standard deviation). Differences between means of test groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered significant at $P < 0.05$. All statistical analyses were conducted using GraphPad prism®, version 9.0.

Results

Effects of *S. glauca* Leaf Extracts on Red Blood Cell Count, Platelet Count, Haemoglobin Concentration and Haematocrit level of male Wistar rat

The data presented in Fig. 1 indicate marked increases ($P < 0.05$) in Red Blood Cell (RBC) count of experimental rats administered EESG and MESG at 500, 1000 & 2000 mg/kg body weight respectively when compared with the control rats whereas there were no observed differences in the RBC of rats administered AESG at respective doses when compared with the control rats administered feed and water *ad libitum* only. The data presented in Fig. 2 indicate observed reduction ($P < 0.05$) in haemoglobin concentrations (Hgb) across all groups of rats administered all respective extracts at 500 mg/kg; however, AESG & MESG particular show marked ($P < 0.05$) decreases in Hgb concentration at 500 mg; whereas, in rat administered EESG 500 mg/kg, the reduction in Hgb concentration was not significant ($P > 0.05$) when compared with the control. Furthermore, Fig. 2 reveals reduction ($P < 0.05$) in Hgb concentrations of experimental rats administered 1000 mg/kg across all extracts; whereas only rat administered EESG 2000 mg/kg indicated observed reduction ($P < 0.05$) in haemoglobin concentration; compared to the control group. Figure 3 reveals that doses administered at 500 or 1000 mg/kg across respective extracts did not significantly ($P > 0.05$) indicate differences in the ratio of the volume of red blood cells to the total volume of blood (haematocrit/PCV) when compared with the haematocrit level of the control group. Albeit, there was marked increase in the haematocrit level of the experimental animal administered AESG 2000 mg/kg, compared with the control group. The data presented in Fig. 4 indicate significant ($P < 0.05$) increase in platelet count at MESG 500 mg/kg, significant decrease ($P < 0.05$) at AESG 1000 mg/kg and EESG 2000 mg/kg, compared to the platelets count of the control group; whereas, there was no significant ($P > 0.05$) differences in platelet count at other doses across different extracts when compared with the control group.

Effects of *S. glauca* Leaf Extracts on White Blood Cell Count and Differentials of Male Wistar Rat.

The data presented in Fig. 5 indicate significant ($P \leq 0.05$) rise in the White Blood Cell (WBC) count of experimental rats administered AESG and EESG 1000 mg/kg respectively, when compared with the control group. The data further reveal that rats administered EESG and MESG 2000 mg/kg respectively likewise indicate significant ($P \leq 0.05$) rise in WBC count; whereas, there were no significant ($P \leq 0.05$) differences in WBC count of rats administered the extracts at 500 mg/kg compare to the control. Figure 6 show increased ($P \leq 0.05$) lymphocyte count in experimental rats administered EESG 500, 1000 & 2000 mg/kg respectively; AESG 2000 mg/kg. Whereas, there were no significant differences ($P \leq 0.05$) in the lymphocyte count of other extracts; at varying doses, when compared to the control. The data presented in Fig. 7 indicate significant ($P \leq 0.05$) rise in the monocytes count of experimental rat administered AESG or EESG 1000 mg/kg respectively; whereas at MESG 1000 mg/kg, there was no significant ($P \leq 0.05$) difference in monocytes count; compared to the control. The data further indicated significant ($P \leq 0.05$) rise in monocytes count of rats administered AESG, EESG or MESG at 500 & 2000 mg/kg respectively; when compared to the monocytes counts of the control.

Discussion

Safety of medicinal plants remains a focus in alternative medicine application; in the management of various diseases in human life. Regardless of the general perception that medicinal plants are without adverse effects, it is indeed of scientific importance to evaluate the safety and (or) potential toxic effect(s) of these medicinal plant(s) compounds on relevant organs and tissues [24, 25]. Studies have reported experimental designs directed at evaluating the bioactive compounds inherent in medicinal plants for safety purposes [26, 27].

The haematopoietic system is susceptible to inflammatory conditions often linked to foreign compounds, be it medicinal, anthropogenic and (or) allopathic drugs. Alterations in red blood cell (RBC) counts, haemoglobin concentrations (Hgb), and haematocrit (HCT) are prominent indicators of anaemic conditions; whereas alterations in white blood cell (WBC) count and its agranulocyte differentials (lymphocyte and monocyte counts) are indicators of infections, inflammatory conditions; as well as tissue and organ damage. There are several reports in the literatures detailing the effects of the application of medicinal plants. Olaniyan *et al.* [28] reported that aqueous and methanol extracts of *N. campestris* did not result to significant changes ($P > 0.05$) in the haematological profile of rats that received the entire test doses; whereas, Musila *et al.* [29] reported that administration of *C. volkensii* to test animals indicated observed changes in the RBC counts.

In the present study, the marked increase in RBC counts of experimental animals administered ethanol and methanol leaf extracts of *S. glauca* at the respective doses indicates erythropoietic potential; perhaps attributable to inherent phytochemicals and abundant co-factors in the leaf extract of *S. glauca* such as Fe^{2+} and vitamin B complex required for synthesis of erythrocytes. The findings are consistent with the earlier reports stressing the roles of phytochemicals and vitamins in synthesis of erythrocytes [19, 30–32]. Polycythemia may be avoided at relatively low doses as may be required.

The data also agrees with the findings of Muriithi *et al.* [33] who reported significant increase in RBC counts speculatively relying on the possible stimulating effect of erythropoietin by inherent phytochemicals in leaf extract of *S. Incanum*. Although, the increased Hgb concentration reported by Muriithi *et al.* [33] is at variance with the outcome of the present study; perhaps due to difference in isoforms of similar phytochemicals of interests. The significant reduction in Hgb concentrations, particularly at 500 and 1000 mg doses indicate that extracts interfered with the oxygen binding capacity of the RBC, although, by a yet to be identified phytochemical.

Administration of leaf extracts of *S. glauca* at respective doses did not indicate significant alteration to the percentage by volume of the red cells in the total blood test rats, although, there was observed significant reduction in haematocrit level at AESG 1000 mg; which further suggest that extract may not have resulted to hemorrhagic bleeding or heavy loss of blood. The significantly low platelet counts recorded in test animals administered AESG 1000 mg and EESG 2000 mg may be attributed to the adverse effect of a yet to be identified phytochemical inherent in the leaf extract, as similarly observed in reduced haemoglobin. The thrombocytopenic state observed at AESG 1000 mg may raise concerns for suspected bleeding of no deleterious effect; which may be reversed at lower doses or withdrawal of extract. It is important to note that the haematocrit level was similarly significantly affected at AESG 1000 mg. Contrariwise, it may be considered that the extract demonstrated some noticeable degree of anti-platelets activity, being a considerable mechanism in the management of agglutination associated with complicated cardiovascular conditions.

White Blood Cells (WBC) function to fight infections; are mobilized when the body system encounters foreign elements. Studies have reported the immune-stimulatory effects of certain herbal extracts [25, 34]. In the present study, it is strongly suggested that the markedly elevated WBC counts, lymphocytes and monocytes differentials observed at varied doses may not be unconnected with cellular inflammatory responses elicited by inherent phytochemicals of the leaf extracts of *S. glauca* [17, 25, 34], which may have signaled, stimulated and activated the release of T-helper cells, macrophage colony stimulating factor, interleukins IL-2 IL-4 and IL-5, proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells and differentials [35, 36].

Conclusion

The present study has revealed that oral administration of the considered extracts of *S. glauca* did not elicit anaemia; rather stimulated marked increases in red blood cell counts, an indication of the seemingly erythropoietic potentials of the extracts, with observed interference with the oxygen-binding capacity of the blood. The extract also exhibited some sort of anti-platelets activity. The alterations recorded in the white blood cell (WBC) counts and differentials suggests obvious immune-stimulatory responses elicited by yet to be identified phytochemicals of the leaf extract(s) of *S. glauca*. Future studies shall focus on isolating and identifying the phytochemical inherent in the leaf extracts of *S. glauca* responsible for the elicited responses characterized by marked alterations in WBC counts and agranulocytes differentials, the

interference with the oxygen-binding capacity of the blood, as well as the disturbances observed in platelets counts.

Declarations

Availability of data and materials

All data and materials are available upon request.

Competing Interest

Authors state no conflict of interests.

Funding

None declared.

Author Contributions

SDEO, NEJO and EKIO contributed to the conceptualization and data curation; SDEO, NEJO and EGM carried out the formal analysis and investigation; SDEO and EGM participated in data analyses. SDEO, NEJO, EGM and EKIO contributed to writing of the original manuscript. All authors read and approved the final version of the manuscript.

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Ethics approval and consent of participate

Not applicable

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Plates

Plate 1 is available in the Supplementary Files section

Figures

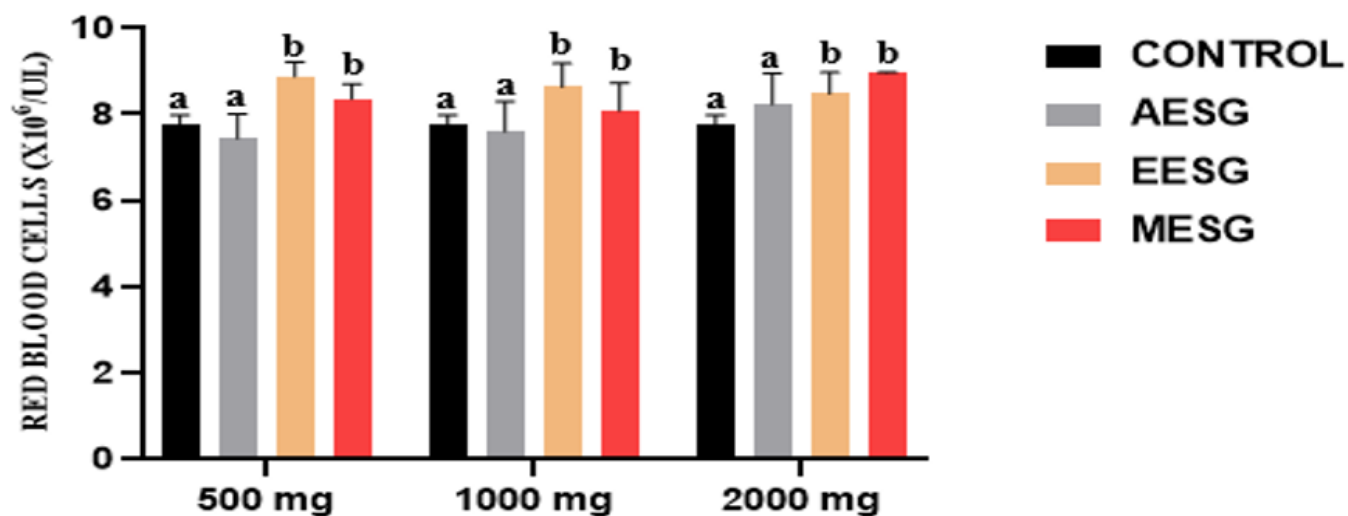


Figure 1

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on Red Blood Cells Counts (RBC) of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p \leq 0.05$). Data are presented as Mean $\pm$ SD.

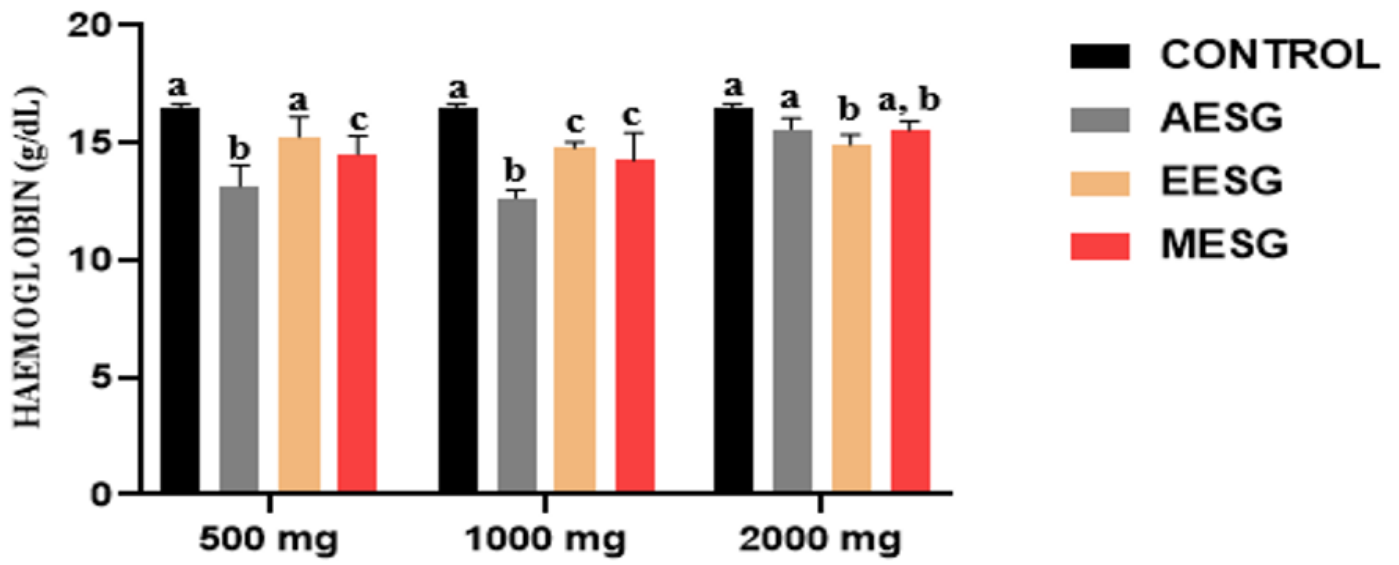


Figure 2

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on Haemoglobin (Hgb) Concentration of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p < 0.05$). Data are presented as Mean $\pm$ SD.

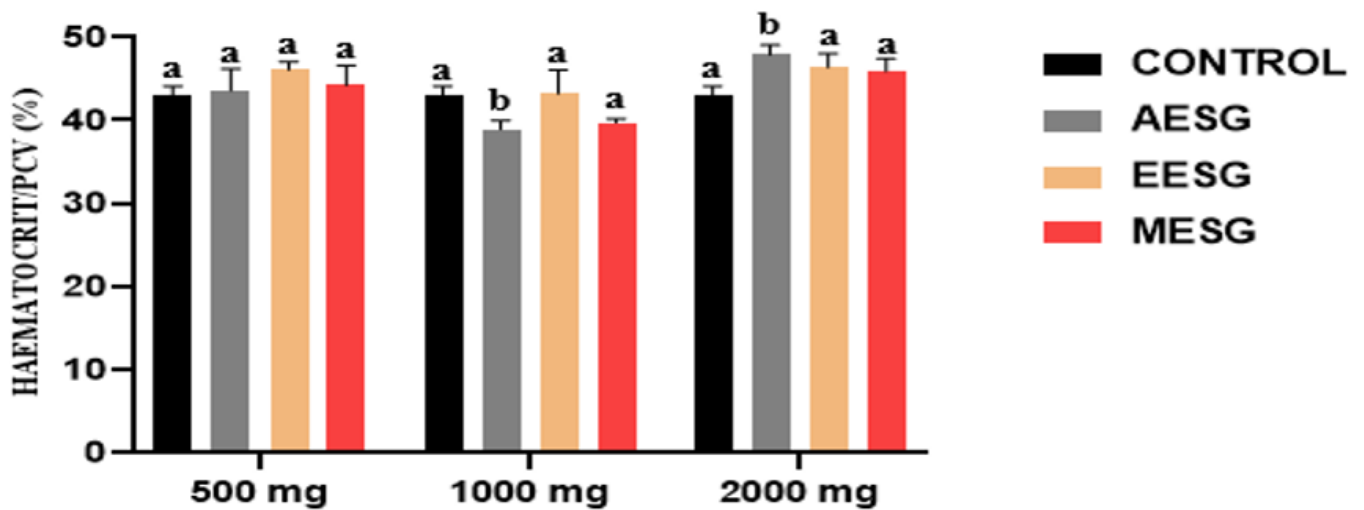


Figure 3

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on Haematocrit (HCT) Concentration of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p \leq 0.05$). Data are presented as Mean $\pm$ SD.

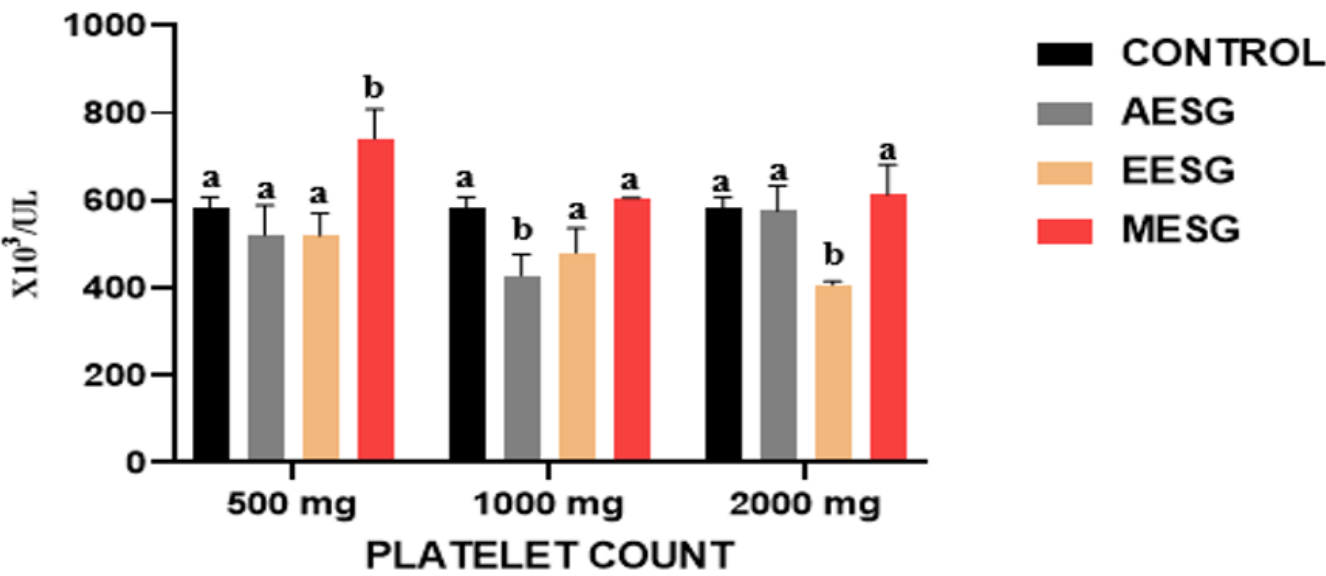


Figure 4

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on Platelets Count of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p \leq 0.05$). Data are presented as Mean $\pm$ SD.

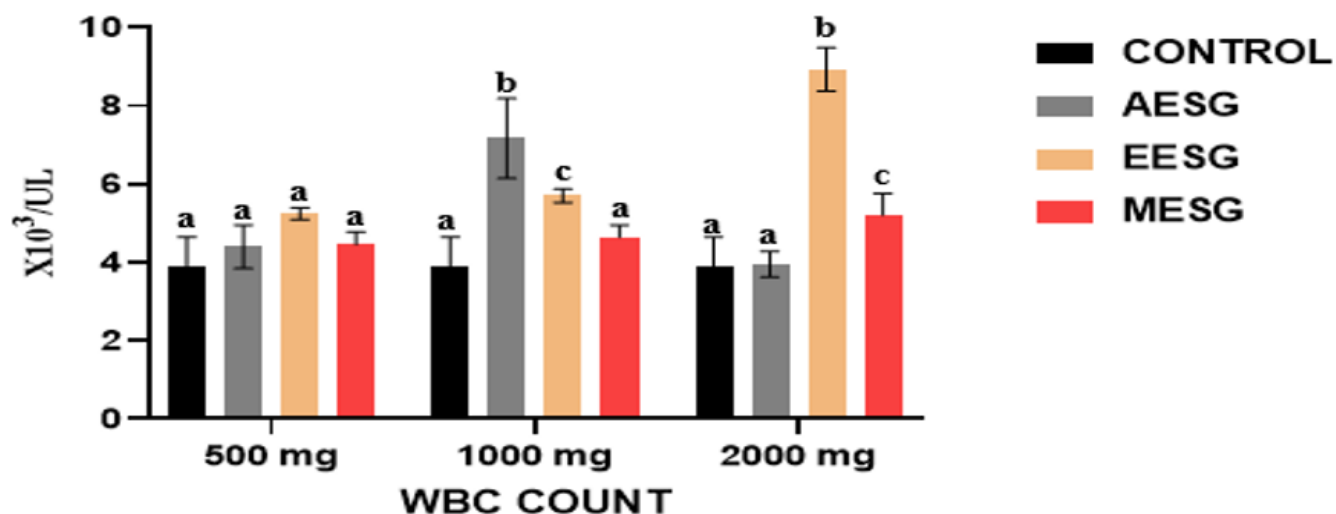


Figure 5

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on White Blood Cell (WBC) Count of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p < 0.05$). Data are presented as Mean $\pm$ SD.

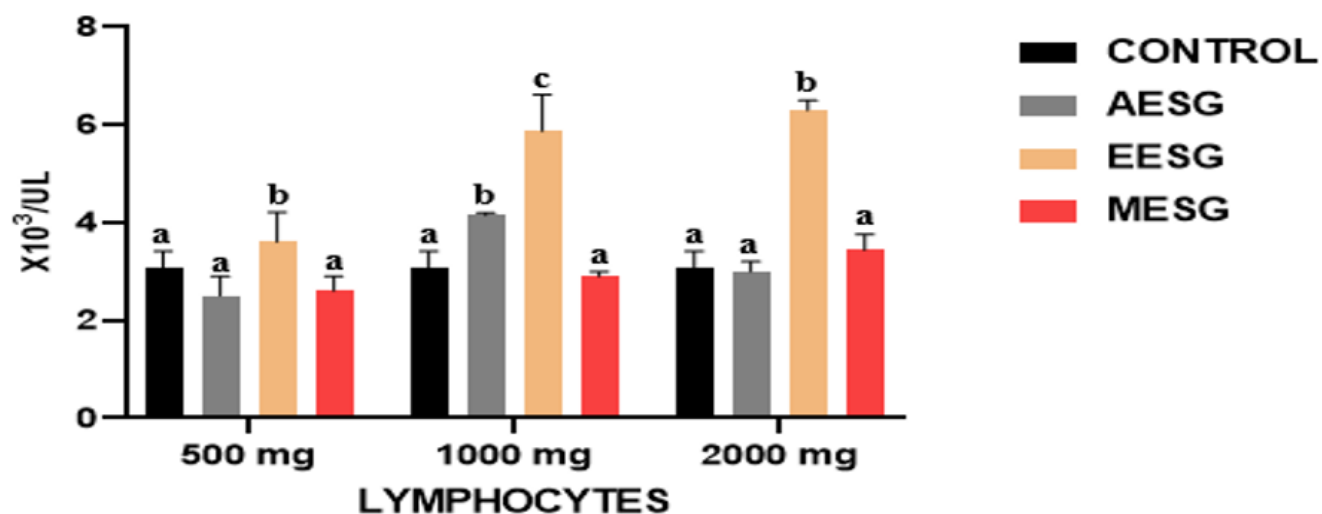


Figure 6

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on Lymphocyte Count of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets

are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p \leq 0.05$). Data are presented as Mean $\pm$ SD.

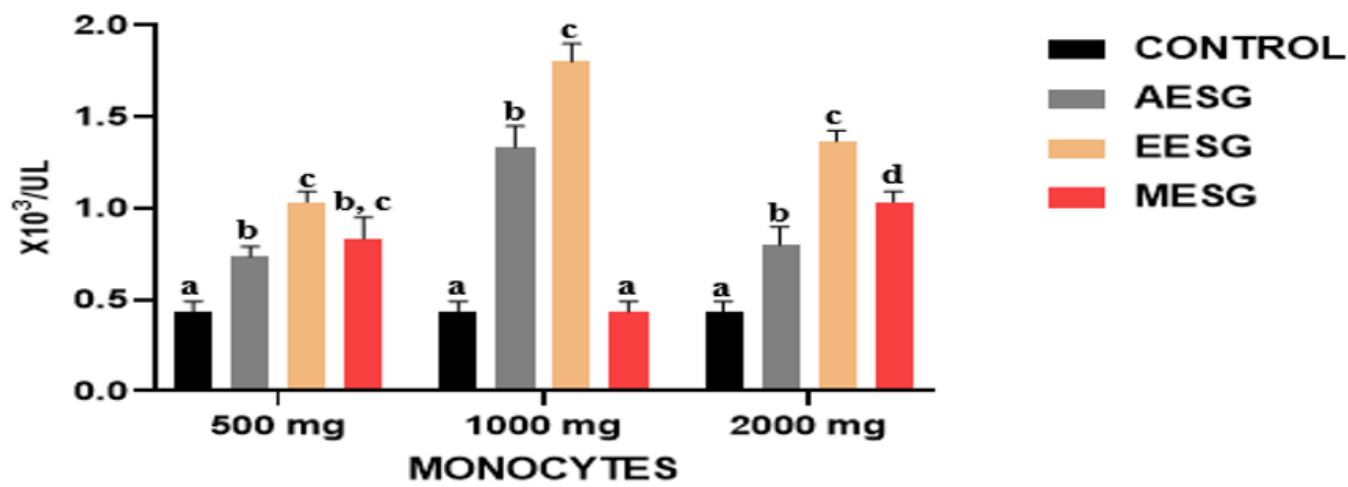


Figure 7

Effect of varying doses of Aqueous (AESG), Ethanol (EESG) or Methanol (MESG) Leaf Extracts of *S. glauca* on Monocyte Count of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p \geq 0.05$); data with different lower-case alphabets are significantly different ($p \leq 0.05$). Data are presented as Mean $\pm$ SD.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Plate1.jpeg](#)