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Sub-Chronic Safety Assessment on the Use of *Solanum aethiopicum* (L.) Leaf Extract as Blood Booster in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author ACI designed the study, performed the statistical analysis and wrote the protocol. Author ASD managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The effect of *Solanum aethiopicum* (SA) on the haematological indices of Wistar rats was investigated in this study.
Methodology: A total of 20 male Wistar rats with an average 172.45±0.15 g were distributed into four groups (A – D) and allowed to acclimatize for two weeks. Group A served as the control, while groups B, C, and D were given aqueous extracts of SA at doses of 75 mg/kg, 150 mg/kg, and 225 mg/kg per body weight, respectively, every 48 hours for 30 days. After the exposure period, a final evaluation and sacrifice of the rats was performed. Blood sample collection was carried for full blood count and blood film preparation.
Results: The result of this study showed that leaf extract of *Solanum aethiopicum* caused a significant increase in white blood cells (18.18±0.78 - 27.08±2.68 x 10^3/µl), especially lymphocytes

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(13.58±2.48 - 30.95±4.65 x 10^3/µl) in group of rats when compared to control. On the contrary, there was a non-significant reduction in red blood cells (7.78±0.04 - 7.19±0.45 x 10^6/µl), hemoglobin (16.92±0.62 - 14.55±0.95 g/dl), haematocrit (41.49±0.29 - 38.38±1.68 %), mean corpuscular hemoglobin (21.71±0.91 - 20.30±0.10 ρg) when compared to the control. Platelet (451.25±87.25 - 724.75±249.25 x 10^3/µl) and Plateletcrit (0.36±0.07 - 0.50±0.17 %) was significantly higher in treated group, while mean platelet volume (8.21±0.31 - 6.98±0.07 µm^3) and platelet distribution width (18.68±1.38 - 15.93±0.73 %) was low when compared with control.

Conclusion: The current study has demonstrated that the leaves of *Solanum aethiopicum* may be safe to consume in regulated amount, as it has been shown to boost blood indices. These plant extracts may be utilized as a blood promoting potentials as it has been shown to strengthens the body's immune system particularly cell-mediated immunity, have no hemotoxic impact on the red blood cell and its indices and improve the ability for the body to repair itself as seen from the platelet count and its indices.

Keywords: Blood film; haematology; full blood count; wistar rats; Solanum aethiopicum.

1. INTRODUCTION

Solanum aethiopicum is a complicated species with four unique morphological groupings that were formerly considered separate species [1]. It additionally qualifies as a hypervariable species since it has several forms and kinds that differ morphologically, as well as hundreds of local variants [2]. *S. aethiopicum* is divided into four separate groupings based on its application. Gilo, Shum, Kumba, and Aculeatum are the four groupings [2]. The Gilo group produces various ranged shaped edible fruits (varying from a circularly depressed form to an oblong outline); the Kumba group has a strong principal stem with enormous hairless leaves that is able to be harvested as a green vegetable, and subsequently produces huge edible fruits. The Shum family is a brief, much-branched species with little hairless leaves and branches that are widely plucked as an attractive green vegetable, but the small (1.5 cm across) highly unpleasant fruits are not edible [3]. The plant is often grown as an ornamental plant.

It has a greater concentration of anthocyanin, phenols, glycoalkaloids (solasodine), and amide proteins. The presence of glycoalkaloids accounts for its bitterness. Patients with liver problems and/or diabetes benefit from eggplant's therapeutic characteristics [4]. However, due to the plant's association with the Solanaceae (nightshade) family, the fruit was once thought to be exceedingly poisonous. Solanum species (eggplants) are members of the Solanaceae family and the genus Solanum. The plant is an economically significant vegetable that is widely produced throughout the world's tropical regions [4]. Solanaceae is a flowering plant family comprised of about 75 genera and 2000 species. This includes herbaceous plants, but the fruit of Solanum species is a berry, and the seeds have a larger endosperm, and they are mostly produced for food and medicine [5]. *Solanum aethiopicum's* low soluble carbohydrate content has the capacity to limit glucose absorption into the bloodstream, hence managing blood sugar levels, making it an excellent dietary alternative for diabetics [1].

S. aethiopicum juvenile fruits are eaten fresh or cooked as vegetables in stews. The leaves and shoots are prepared in the same way that vegetables are. They are collected from the same plants as the fruit or from distinct leafy cultivars. The fruits of bitter cultivars are used as medicine in several African countries. The roots and fruits can be used as a carminative and sedative, as well as to treat colic and high blood pressure; a sedative to treat uterine issues; an alcoholic extract of the leaves can be used as a sedative, anti-emetic, and to treat tetanus after abortion; as well as crushed and macerated fruits can be used as an enema. In south-eastern Nigeria, the Igbo tribe traditionally greet visitors by offering fruits. *S. aethiopicum* is occasionally grown as an ornamental plant. Some cultivars are used as rootstock for tomatoes and eggplant on occasion.

S. aethiopicum is one of the plants sought for due to its availability and cost. As a result, *S. aethiopicum* has a wide range of applications in ayurvedic medicine. Concurrently, another pertinent scientific concern is the adverse effects of *S. aethiopicum*. Interestingly, no study documented the detrimental potentials of SA after a thorough literature search, although other studies reported promising outcomes in the application of *S. aethiopicum* in therapeutic

medicine. A few research have indicated that *S. aethiopicum* fruit extracts exhibit anti-inflammatory, lipid-lowering, hypotensive, antibacterial, and anticancer effects [6]. However, there is a dearth of information on the effects of this vegetable's aqueous extract on hematological profile of rats.The aim of this study is to investigate the effect of the aqueous extract of *S. aethiopicum* on the hematological profile of male Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of *Solanum aethiopicum* Leaf Extract

Fresh leaves of *Solanum aethiopicum* (African garden eggplant) were purchased from Effurun Market in Effurun, Delta state in January, 2021; the taxonomic identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria. The plant was analyzed to determine its Phytochemical composition [7-10].

The purchased leaves were air-dried to crispiness in the laboratory (prevailing room temperature of 30 ± 2°C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles using Viking Exclusive Joncod pulverizing machine (Model: YLH2M2 - 4). The crude aqueous extract was prepared by decoction; where 50 g of the leave powder extracted with 200 mls of distilled water for 8 hrs. The mixture was decanted and filtered using sterile whatman paper No 1. The filtrate was evaporated to dryness using a freeze dryer and reconstituted in distilled water to appropriate concentrations.

2.2 Experimental Setup

Male Wistar rats (6-7 weeks old) with an average weight of 172.45±0.15 g were obtained from the Anatomy Department, University of Benin, Nigeria. The rats were distributed randomly into four groups of six animals each for group A to D and allowed to acclimatize for 2 weeks. During acclimatization, the animals were housed in wooden cages with wire mess covers and fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo state, Nigeria) and given distilled water *ad labitum.* After acclimatization, the rats were given different treatment protocol: Group A which was the Control (CTR) was given distilled water; Group B, C and D rats were gavaged 75 mg/kg b/w (EGG 1),150 mg/kg b/w (EGG 2), 225 mg/kg b/w (EGG 3) of aqueous extract of SM for 30 consecutive days (once every 48 hour) respectively. The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo state, Nigeria®) *ad libitum*. At the end of exposure period, survivors were fasted overnight and sacrificed under slight Anesthesia; then blood was collected from the inferior vena cava of the rats with plain 10 ml sterilized syringe into a vial containing 0.5 ml EDTA for haematological analysis under a light anaesthesia. The blood sample was gently homogenised to ensure proper mixing of the blood with the anticoagulant, before taking it to the laboratory for analysis.

2.3 Laboratory Analysis

Hematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx corporation kobe, Japan) following the manufacturer's instructions. Briefly the sample was mixed and placed in contact with the sample probe for aspiration.

2.4 Data Analysis

All statistical analyses were analyzed with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean±SE (n=5). One-way ANOVA was used to determine the differences among various groups. When the corresponding F test for differences among the treated group means was significant pair wise, comparisons between treated groups and corresponding negative control were determined using multiple comparison procedure of the Dunnett post-hoc test and differences were considered significant at $p<0.05$ levels of significance.

3. RESULTS AND DISCUSSION

The result of the phytochemical composition of *Solanum aethiopicum* is shown in Table 1. It reveals the presence of phytochemicals such as phenols, tannins, anthocyanin, flavonoids, alkaloids, and saponin in the aqueous leaf extract of *Solanum aethiopicum*.

Table 1. Phytochemical composition of *Solanum aethiopicum* leaf extract

S/N	Content	Composition
1	Phenol	+
2	Tannins	+
3	Anthocyanin	+
4	Flavonoids	+
5	Alkaloids	+
6	Saponin	+

NB: + indicates present and – indicates absent

Table 2. Hematological profile of male wistar rats administered crude aqueous leaf extract of SA

	CTR	EGG 1	EGG 2	EGG 3	P-Value
White Blood Cell (x 10^3/µl)	18.18±0.78	24.45±0.95	25.25±1.15	27.08±2.68	P<0.05
Lymphocytes (x 10^3/µl)	13.58±2.48	19.60±0.40	21.10±0.10	30.95±4.65	P<0.05
Mid-Sized cell (x 10^3/µl)	3.36±0.44	2.65±0.25	2.50±0.50	2.73±0.28	P>0.05
Granulocyte (x 10^3/µl)	2.08±0.42	1.20±0.20	1.65±0.65	1.40±0.70	P>0.05
Lymphocyte (%)	77.73±13.83	66.40±1.40	83.70±4.10	59.95±16.45	P>0.05
Mid-Sized cell (%)	17.50±4.40	14.30±0.80	9.85±1.65	7.28±3.63	P<0.05
Granulocyte (%)	9.43±4.77	15.30±0.60	6.45±2.45	22.78±12.83	P<0.05
Red Blood Cell (x 10^6/µl)	7.78±0.04	7.73±0.41	7.33±0.20	7.19±0.45	P>0.05
Hemoglobin (g/dl)	16.92±0.62	15.75±0.05	15.35±0.05	14.55±0.95	P>0.05
Hematocrit (%)	41.49±0.29	40.55±0.25	39.05±0.75	38.38±1.68	P>0.05
Mean Corposcular Volume (µm^3)	53.41±0.71	52.65±3.05	53.35±2.45	52.85±0.15	P>0.05
Mean Corposcular Haemoglobin (ρg)	21.71±0.91	20.45±1.15	21.00±0.50	20.30±0.10	P>0.05
Mean Corposcular Haemoglobin conc.(g/dl)	40.85±1.25	38.85±0.15	39.30±0.90	38.40±0.30	P>0.05
Coefficient of variation RDW (%)	16.00±0.50	17.20±0.10	18.95±1.55	17.43±0.07	P>0.05
Standard deviation of RDW (µm^3)	30.93±0.73	30.00±1.90	33.00±0.90	31.15±1.05	P>0.05
Platelet (x 10^3/µl)	451.25±87.25	456.50±1.50	443.00±53.00	724.75±249.25	P<0.05
Mean Platelet Volume (µm^3)	8.21±0.31	6.75±0.15	7.80±0.30	6.98±0.07	P>0.05
Plateletocrit (%)	0.36±0.07	0.31±0.01	0.34±0.03	0.50±0.17	P<0.05
Platelet Distribution Width (%)	18.68±1.38	16.20±1.30	17.70±1.90	15.93±0.73	P>0.05
P-LCR (%)	11.14±0.86	5.85±0.65	11.80±1.60	7.00±0.70	P>0.05

NB: RDW = Red cell distribution width; All values are expressed as Mean ± S. E., N = 5; P<0.05 and P>0.05 indicates significant and non-significant difference respectively

In Table 2, the leaf extract of *solanum aethiopicum* caused a significant increase in white blood cells (18.18±0.78 - 27.08±2.68 x 10^3/µl), especially lymphocytes (13.58±2.48 - 30.95±4.65 x 10^3/µl) in group of rats when compared to control. On the contrary, there was a non significant reduction in red blood cells (7.78±0.04 - 7.19±0.45 x 10^6/µl), hemoglobin (16.92±0.62 - 14.55±0.95 g/dl), haematocrit (41.49±0.29 - 38.38±1.68 %), mean corpuscular hemoglobin (21.71±0.91 - 20.30±0.10 ρg) when compared to the control. Platelet (451.25±87.25 - 724.75±249.25 x 10^3/µl) and Plateletcrit (0.36±0.07 - 0.50±0.17 %) was significantly higher in treated group, while mean platelet volume (8.21±0.31 - 6.98±0.07 µm^3) and platelet distribution width (18.68±1.38 - 15.93±0.73 %) was low when compared with control.

The structure, function, metabolic transformation, and concentration of biomolecules, enzymes, and even metabolic pathways can all be significantly altered by the injection of a chemical agent [1]. These changes, which could occur quickly or gradually, could trigger various biochemical processes that result in comparable pathological, clinical, and laboratory findings [11]. The magnitude of a foreign compound's harmful effect on the blood, including plant extract, can be assessed using haematological measures. It can also be used to illustrate how plant extracts with chemical compounds work in relation to the blood [12]. Hematology, which includes the diagnosis, treatment, and prevention of illnesses of the blood, bone marrow, immune, hemostatic, and vascular systems, is the study of blood and blood-forming organs. Animal diseases are frequently diagnosed and treated using hematologic analysis. Hematology has grown in importance for disease detection and treatment in smaller laboratory animals as analytical methods have gotten more sensitive, necessitating smaller sample volumes.

White blood cells, or leucocytes, are a crucial part of the immune system (host defense), which works to defend the body from infectious disorders brought on by bacteria, fungi, viruses, and parasite invasion [13]. The large rise in white blood cell count seen in this study is consistent with the findings of Alhassan et al. [14], who examined the impact of *S. melongena* fruit extract on the circulation of Wistar rats and discovered an increase in white blood cell count. Both Lowenthal et al. [15] and Saba et al. [16] reported that eggplant may play a role in cellular immunity. The response to numerous stimuli, such as changes in hormone levels, stress, shock, infection, drug use, allergic reactions, and inflammations, can cause changes in white blood cell count. Alkaloids, phytosterol, and certain pigments that may occasionally trigger allergies have all been linked to eggplants [17]. White blood cells may become mobilized as allergic reactions progress, raising their concentration. The differential cell analysis and white blood cell count both indicated that the mean lymphocyte values had increased. An improvement in the body's immunological state, particularly the cell-mediated immune response, is correlated with an increase in the number of white blood cells and lymphocytes in circulation. The results here are consistent with those of Lowenthal et al., [15] and Saba et al. [16] who proposed that administering an aqueous extract of eggplant leaf may benefit the cells engaged in immunity.

The considerable drop in red blood cell count found is consistent with findings from Alhassan et al. [14] who reported that rats given *S. melongena fruit* extract saw a significant drop in red blood cell count. A possible reduction in the blood iron level may be responsible for the reduction in in RBC, heamoglobin and heamatocrit in treated rats. This is as eggplants have been observed to have high concentration of dietary fiber that can bind cations like iron [18]. As a result, iron's bioavailability may be compromised, which could reduce red blood cell production [19]. Ossamulu et al. [17] reported the abundance of saponins, an abundant bioactive ingredient in eggplant which may trigger hemolysis in red blood cells [20]. The non-significant changes observed indicates that *Solanum aethiopicum* may not have any direct hemopoeitic properties. SA leaf extract may also have blood detoxifying potentials, as has been seen in various vegetables [21,22]. Ths may have played a physiological role in maintaining the levels of RBC, HCT, and HCB of treated group of wistar rats.

The major role of platelets is to stop and stop bleeding, repair wounds, and help the body mend itself. As seen in this study, when compared to the control group, the platelet count was higher in the group that received an aqueous extract of *Solanum aethiopicum* PDW and MPV are two platelet indices that could be helpful markers for the early detection of thromboembolic illnesses [23]. MPV levels were reduced but not substantially (p 0.05) different. According to Amin et al. [24], PDW increases in sickle cell anemia, which may be a factor in erythrocyte abnormalities and platelet dysfunction. High MPV concentrations have been linked to vascular diseases such atherosclerosis, coronary artery disease, and ischemic stroke [25,26]. This indicates an impoved blood parameters as the ability of the rats to repair wonds was improved while other platelet indices where no negetively altered. This improved trend might be due to the phytochemicals present in *S, aethiopicum* [27].

4. CONCLUSION

The current study has demonstrated that the leaves of *Solanum aethiopicum* may be safe to consume in regulated amount, as it has been shown to boost blood indices. These plant extracts may be utilized as a blood promoting potentials as it has been shown to strengthens the body's immune system particularly cell-mediated immunity, have no hemotoxic impact on the red blood cell and its indices and improve the ability for the body to repair itself as seen from the platelet count and its indices.

ETHICAL APPROVAL

This research design was reviewed and approved by the College of Science Ethical board, Federal University of Petroleum Resources, Effurun (CS/EMT/2021/006).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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