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Effect of Methanolic Leaf Extract of Solanum incanum on Full Blood Count Parameters in Wistar Rats

Ibrahim Aliyu Bagudo* and Yakubu Abdulrahman

Department of Haematology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Author for Correspondence*: aliyubagudo1@gmail.com/aliyu.ibrahim@udusok.edu.ng/ +234-706-276-6659. https://dx.doi.org/10.4314/sokjmls.v9i4.21

Abstract

The plant called *Solanum incanum* is known for its medicinal properties, including its ability to boost blood. The various phytochemical components of *Solanum incanum* are thought to have therapeutic impacts on blood-related parameters. These effects have not been rigorously examined through scientific studies to validate and confirm the therapeutic assertions regarding their effect on red bloods, white blood cells and platelets. The aim of this study was to determine the effects of methanolic leaf extract of *Solanum incanum* on full blood count in Wistar rats. The experimental Wistar rats groups were administered with methanolic leaf extracts of *Solanum incanum* at dose of 500mg/kg and 1000 mg/kg orally once per day for a period of fourteen days. Blood of 3 mL was collected from each rat into an EDTA tube, red blood cells, white blood cells and platelets parameters were determined using three parts haematology analyzer (Rayto 7200, China). Phytochemicals constituents were determined using standard methods. Fourteen days after administration of methanolic leaf extracts of *Solanum incanum* at the dose level of 500 mg/kg and 1000mg/kg there was significant increase in white blood cell count (WBC) and Neutrophils. There was no statistically significant difference in red blood cell count (RBC), platelet count and their indices but a slight increase was observed in their mean values compared to the controls. In conclusion, the methanolic leaf extract of *Solanum incanum* had phytochemicals that significantly increase white blood cells counts but red blood cells and platelets were not significantly increase.

Keywords: *Solanum incanum,* red blood cells, white blood cells, platelets and Wistar rats

Introduction

Full blood count parameters play a crucial role in assessing health and diagnosing diseases. Establishing reference ranges and understanding the factors that can influence these parameters are important for accurate interpretation and diagnosis. Further research is needed to explore the relationship between Full blood cell parameters and various diseases and conditions, as well as the impact of different factors on these parameters (Rappai *et al.*, 2019).

Solanum incanum, also known as bitter apple or thorn apple, is a plant species that has been used in traditional medicine for various purposes. It has been reported to have pharmacological potential and is used to treat a range of human diseases (Jima and Megersa, 2018). The plant is native to the Americas but has been widely used in folk medicine worldwide (Salehi *et al*., 2019). It is known for its medicinal properties, including its ability to boost blood and treat gastrointestinal diseases, intestinal parasites, liver pain, and diabetes (Chidambara, 2023). The plant has been found to possess antimicrobial properties, making it potentially useful in the treatment of various infections (Sbhatu and Abraha, 2020). Additionally, *Solanum incanum* has been used to treat stomach ache, cowdriosis, dermatophilosis skin lesions, foot rot, pasteurellosis, black legs, fasciolosis, and snake bites (Mekonnen *et al*., 2022). Overall, *Solanum incanum* is a valuable medicinal plant with antimicrobial, cytotoxic, and genetic diversity

properties. Its phytochemical compounds and traditional uses make it a promising candidate for further research and potential applications in various industries. The plant is rich in phytochemicals such as alkaloids, saponins, flavonoids, glycosides, terpenoids, and steroids, which contribute to its medicinal properties. These phytochemicals have been shown to have antimicrobial, antioxidant, and anti-inflammatory effects (Sbhatu and Abraha, 2020). Additionally, *Solanum incanum* has been found to have hypoglycemic effects, making it potentially beneficial for individuals with diabetes (Owoicho *et al*., 2021). Not much research has been done on its effect on red blood cells, white blood cells and platelets parameters, there is still limited data and information, notably in north-western Nigeria. Thus, this research is aimed at determining the effect of *Solanum incanum* leaf extract on Full blood count as well as adding to the possibility of implementing the use of *Solanum incanum* in the management haematological disorder.

Study Location

The study was carried out in the Department of Haematology, School of Medical Laboratory Science Usmanu Danfidiyo University Sokoto, Animal House, Faculty of Pharmacology and Toxicology, Usmanu Danfodiyo University Sokoto, and Usmanu Danfidiyo University Teaching Hospital Sokoto, Nigeria.

Ethical Clearance

Ethical clearance was obtained from the Ethics and Research Committee of Usmanu Danfodiyo University, Sokoto. Animals were handled according to the guidelines for handling laboratory animals and ethical guidelines of the Animal Ethics Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto.

Plant Collection and Authentication

Solanum incanum leaves were collected from Kara market Sokoto, Sokoto State. The plant was recognized and authenticated at the herbarium unit of the Department of Pharmaceutical Sciences at Usmanu Danfodiyo University, Sokoto State. The identification was conducted by established standards and issued a voucher number of PCG/UDUS/Sola/0008.

Preparation of Extract

Solanum incanum leaves was purchased from Kara market Sokoto, Nigeria. It was air-dried at room temperature until completely dried. The leaves was crushed into semi powdered form using mortar and pestle and then soaked in 90% methanol and kept at room temperature free of dust for 48 hours with occasional shaking. It was first sieved using soft cotton cloths and then Whatman filter paper. The extract was poured into a wide mouth container and kept at room temperature for methanol evaporation and complete dryness of the extract.

Phytochemicals Screening

The methanolic leave extract of *Solanum incanum* was analyzed qualitatively for bioactive compounds such as carbohydrate using Molisch test and Fehlings solution, Tannins using Ferric chloride test, Flavonoid using Alkaline test and Schinodis test, Phenol using Ferric chloride test, Protein using Xanthoprotic test, Saponin using Frothing test, Cardiac glycosides using Keller Kiliani test, Alkaloid using Meyers test, Interphenoid using Salkankis test (Sbhatu and Abraha, 2020).

Experimental Animals

Twenty-seven Wistar rats weighing 150±20g, were purchased from the animal House in UDUS, Sokoto State. The animals were housed under standard conditions, having free access to feed and water and were allowed to acclimatize for 14 days prior to the experiment.

Study Design

Acute Oral Toxicity

A total of twelve Wistar rats were used for acute oral toxicity study and was perform in two phases, phase I and phase II in accordance with Lorkes method (Lorke, 1983).

Phase I of Acute Toxicity Study

Nine Wistar rats were divided into three groups: group I, II and III respectively, containing three rats each. Single dose of 10mg, 100mg and 1000mg of the extract were administered to the animals in groups I, II and III per kg of their body

weight by intragastric gavage using Oral cannula. Observations for signs of toxicity were made and recorded within the first 4 hours and subsequently for 24 hours after administration of the extract. The rats were monitored closely for behavioral changes and mortality for up to 14 days. In the absence of mortality phase II of acute toxicity study was carried out as follows;
Three rats were divided into three groups: I, II and III respectively, of one rat each. Single doses of 1600mg, 2900mg and 5000mg of the extract was administered respectively to group I, II and III rats per kg body weight by intragastric gavage using oral cannula. Observations for the signs of toxicity were made and recorded within the first four hours and subsequently for 24 hours after. The rats were closely monitored for behavioural changes and mortality for up to 14 days.

Animal Grouping and Treatment
Fifteen (15) Wistar rats were employed for the study. The experimental animals were placed into three groups at random, with each group comprising five animals (n = 5). Group 1: Wistar rats were given a normal meal and water for 14 days; Group 2: Wistar rats that were treated with the methanolic leaf extract of *Solanum incanum* at 500mg/kg per body weight daily for 14 days and Group 3: Wistar rats were treated with the methanolic leaf extract of *Solanum incanum* at 1000mg/kg per body weight daily for 14 days.

Blood Sample Collection and Processing
The animals were anaesthetized in a covered transparent plastic container containing cotton wool soaked with 10 ml chloroform on day 15 that is 24 hours after the last treatment with the extract. Three milliliters (3 ml) blood samples was collected from the animals through cardiac puncture using 5 ml syringes, and the blood samples from each animal were emptied into an EDTA anti-coagulated tube. These samples were analyzed in Haematology Laboratory, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Laboratory Analysis
Assay of Full Blood Count
A Full blood count (FBC), also known as a complete blood count (CBC), is a common blood test that provides detailed information about the types and numbers of cells in the blood, including red blood cells, white blood cells, and platelets. This analysis was done with aid of three parts haematology analyzer (Rayto 7200, China).

Data Analysis
The data obtained was analyzed using statistical package for social sciences (SPSS) version 20.0 and all data were expressed as mean ± standard deviation (SD). Comparisons were determined using one way analysis of variance (ANOVA) and Post hoc analysis of multiple comparison using Bonferonni, difference between groups were considered significant at p- value less than or equal to 0.05 ($p \leq 0.05$).

Results
The results for the effect of methanolic leaf extract of *Solanum incanum* on full blood count parameter are presented in the tables 1-5.

Table 1: Shows the phytochemical constituents of methanolic leaf extract of *solanum incanum* and indicates Carbohydrates, Tannins, Flavonoid, Phenol, protein, saponin, cardiac glycosides, alkaloid and triterpenoids were observed to be present.

Table 2: Shows the result of acute oral toxicity in Wistar rats administered with methanolic *Solanum incanum* extract. The result showed neither death nor abnormal behavior were recorded following the administration of 5000 mg/kg body weight of the *Solanum incanum* leaf extract. The animals were apparently healthy and showed no signs of toxicity at this dose extract. Thus, the LD_{50} was greater than 5000 mg/kg per body weight.

Table 3: Shows the effect of methanolic leaf extract of *Solanum incanum* on Red blood cells count, Haemoglobin concentration, Haematocrit and Red blood cells indices in Wister rats, where there is no statistical differences($p \leq 0.05$) among control group1 and treated group 2 and group 3. However, an increase in RBC count, Haemoglobin concentration and Haematocrit values were observed in treated group 2 and group 3 compared to control group 1. Whereas, in red blood cells indices there was no clear distinction among the control group1 and treated groups 2 and group 3.

Table 4: Effect of methanolic leaf extract of *Solanum incanum* on White blood cells count in Wistar rats. The values for WBC Count in group3 show significant increase ($p \leq 0.05$) compared to group 2 and control group 1. Similarly, the values for Neutrophils differential count shows significant increase ($p < 0.05$) in group 2 and group 3 compared to control group 1. However, the values for the Lymphocytes differential count and the average for Monocytes, Basophils and Eosinophils differential count indicates no significant differences ($p \leq 0.05$) among the control group1 and the treated group 2 and group 3.

Table 5: Effect of methanolic leaf extract of *Solanum incanum* on Platelets count and Platelet indices in Wistar rats. The result shows that there are no statistical differences ($p \geq 0.05$) between control group 1 and treated groups. However, an increase in Platelet count was observed in treated group 2 and group 3 compared to control group 1. Whereas, in platelet indices there was no clear distinction changes between the control group1 and treated group 2 and group 3.

Table 1: Phytochemicals constituents of methanolic leaf extract *Solanum incanum*

Phytochemicals	Result
Carbohydrates	+
Tannins	+
Flavonoid	+
Phenol	+
Protein	+
Saponin	+
Cardiac glycosides	+
Alkaloid	+
Triterpenoids	+

Key: (+) indicates presence, (-) indicates absence

Table 2: The Acute Oral Toxicity Study of methanolic Leaf extract of *Solanum incanum* in Wistar Rats

Dose (mg/kg)	**Mortality**	**% mortality**
	Phase I	
10	0/3	0
100	0/3	0
1000	0/3	0
	Phase II	
1600	0/1	0
2900	0/1	0
5000	0/1	0

Table 3: Effect of methanolic leaf extract of *Solanum incanum* on Red blood cells in Wistar rats

Parameters	Group1	Group2	Group3	F-value	p-value
RBC($\times10^{12}$/L)	6.22 ± 0.58 [a]	6.81 ± 0.34 [a]	6.58 ± 0.37 [a]	2.019	0.179
HGB (g/dL)	12.74± 1.42 [a]	13.58 ± 0.72 [a]	13.70 ± 1.15 [a]	0.986	0.404
HCT (%)	35.44 ± 2.37 [a]	38.01 ± 0.56 [a]	37.32 ± 3.13 [a]	1.455	0.275
MCV (fL)	57.16 ± 2.74 [a]	55.93 ± 2.89 [a]	56.62 ± 2.22 [a]	0.249	0.784
MCH (pg)	20.46 ± 0.74 [a]	19.95 ± 1.11 [a]	20.78 ± 0.86 [a]	0.956	0.414
MCHC(g/dL)	35.88 ± 2.17 [a]	35.73 ± 1.88 [a]	36.72 ± 0.94 [a]	0.451	0.648
RDW_SD(fL)	34.38 ± 3.39 [a]	35.36 ± 3.33 [a]	32.74 ± 2.86 [a]	0.791	0.478
RDWCV (%)	15.80 ± 1.89 [a]	17.80 ± 3.52 [a]	15.18 ± 2.19 [a]	1.263	0.321

Key: Group 1= control, Group 2 = treated with 500mg/kg of methanolic leaf extract of *Solanum incanum*, Group 3 = treated with 1000mg/kg of methanolic leaf extract of *Solanum incanum*, SD = Standard deviation. Values are expressed in mean ± SD. Values carrying superscripts different from the negative control across each group are statistically significant at ($p \leq 0.05$).

Table 4: Effect of methanolic leaf extract of *Solanum incanum* on White blood cells in Wister rats

Parameters	Group1	Group2	Group3	f-value	p-value
WBC($\times10^{9}$/L)	13.12 ±7.83[a]	21.75 ±2.19[ab]	23.86 ± 6.77[b]	3.957	0.050*
NEUT($\times10^{9}$/L)	0.90 ± 0.35 [a]	1.65 ± 0.17 [b]	1.38 ± 0.31 [b]	7.418	0.009*
LYM ($\times10^{9}$/L)	11.6 ± 7.56 [a]	19.00 ± 2.59[a]	21.38 ±7.00 [a]	3.167	0.082
MXD ($\times10^{9}$/L)	1.04 ± 1.07 [a]	1.10 ± 0.32 [a]	1.10 ± 0.31 [a]	0.012	0.988

Key: (*) means p-value is significant ($p \leq 0.05$). Key: Group 1= control, Group 2 = treated with 500mg/kg of methanolic leaf extract of *Solanum incanum*, Group 3 = treated with 1000mg/kg of methanolic leaf extract of *Solanum incanum*, SD = Standard deviation. Values are expressed in mean ± SD. Values carrying superscripts different from the negative control across each group are statistically significant at ($p \leq 0.05$).

Table 5: Effect of methanolic leaf extract of *Solanum incanum* on Platelets in Wistar rats

Parameters	Group1	Group2	Group3	f-value	p-value
PLT($\times10^{9}$/L)	554.0± 252.70 [a]	791.3± 261.30 [a]	613.0± 110.90 [a]	1.425	0.282
PDW (fL)	8.82 ± 0.35 [a]	8.88 ± 1.06 [a]	8.88 ± 0.56 [a]	0.012	0.988
MPV (fL)	7.22 ± 0.35 [a]	7.25 ± 0.49 [a]	7.30 ± 0.44 [a]	0.045	0.956
P - LCR (%)	8.94 ± 1.53 [a]	9.15 ± 3.25 [a]	9.26 ± 2.61 [a]	0.021	0.979

Key: Group 1= control, Group 2 = treated with 500mg/kg of methanolic leaf extract of *Solanum incanum*, Group 3 = treated with 1000mg/kg of methanolic leaf extract of *Solanum incanum*. Values are expressed in mean ± SD. Values carrying superscripts different from the negative control across each group are statistically significant at ($p \leq 0.05$).

Discussion

The Full Blood Count (FBC) is a haematological test used to assess a patient's overall health. It aids in identifying various conditions like anemia, low platelet count, infections, and leukemia by measuring blood components such as red blood cells (related to tissue oxygenation), white blood cells (related to immunity), and platelets (related to blood clotting). This test provides valuable insights into blood cell production, oxygen-carrying capacity, and immune system function. When interpreted alongside clinical context, the FBC can offer crucial information for diagnosing conditions like anaemia, cancer, clotting disorders, infections, and immune deficiencies, as well as monitoring drug side effects (Erhabor *et al*., 2021). The leaf of *Solanum incanum* is rich in minerals such as potassium and calcium. It is a good source of many phytochemicals used against pathogens and predators as well as for treating many human and animal ailments. In Africa, it is used for treating angina, colic or indigestion, dandruff, fever, general infection, headache, liver pain, painful menstruation, snake bites, sore throat, stomach ache, abdominal pain, skin diseases and wounds. *Solanum incanum* has antioxidant and anti-inflammatory activities, which may be involved in its potential therapeutic effects (Belayneh *et al*., 2021).

The phytochemical result of methanolic leaf extract of *Solanum incanum* in this present study is consistent with the report in a previous report (Sbhatu and Abraha, 2020) that showed that it contains constituents such as carbohydrate, tannins, flavonoid, phenol, protein, saponin, cardiac glycosides, and diterpenoid. Saponin has antifungal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant, and immunomodulatory properties. Tannins have been found to have various therapeutic effects, including anti-inflammatory, antioxidant, antidiabetic, anticancer, and cardioprotective properties. Carbohydrates have been shown to have anxiolytic effects, and increased levels of carbohydrates have been attributed to the therapeutic effects of essential oils on anxiety and depression (Wu *et al*., 2011). Phenolic compounds have been shown to possess a wide range of therapeutic effects, including antioxidant, anticancer, anti-inflammatory, and antimicrobial properties. Alkaloids have been found to possess potential therapeutic effects against neurodegenerative disorders such as Alzheimer's disease, Huntington disease, Parkinson's disease, epilepsy, schizophrenia, and stroke. Also, it was been explored in the context of pain management, with studies demonstrating the potential analgesic effects of crude alkaloids from medicinal plants. Proteins have proven effective in treating diseases such as diabetes, heart disorders, and cancer. Triterpenoids a wide range of pharmacological activities including antiviral and antitumor effects. Cardiac glycosides have been extensively researched, particularly in the context of their potential as anticancer agents and their impact on various physiological processes. Cardiac glycosides have been found to exhibit antineoplastic properties by inhibiting HIF-1α synthesis and blocking tumor growth. Flavonoids have been found to possess multiple therapeutic benefits against cancer, neurodegenerative disorders, and cardiovascular diseases (Zhuang *et al.,* 2017).

This current study revealed the acute toxicity (LD50) was up to 5000mg/kg per body weight without any mortality. This result is similar to a study reported by Belayneh and his Colleagues (2021) where there was no mortality and toxicity of *Solanum incanum* leaf extract in animals at a limit dose of 2000mg/kg. This may be the reason some individuals consumed it without any health-related issue.

The finding in this study revealed the values of the red blood cell count in the negative control group compared to the groups administered with *Solanum incanum* has no statistical significance but there was little increase in the values compared to the control. This finding is in similar with a study reported by Hassan *et al.* (2022) on their study on the therapeutic effect of methanolic leaf extract of *Solanum incanum* in copper- induced cardio toxicity. However, this is in contrast to a report by Muriithi *et al.* (2015) who found an improvement in erythrocytic pararmeters after oral administration of methanolic leaf extract of *Solanum incanum*. The difference may be due to phytochemical constituents of the extract.

The finding in this study showed the values of the total white blood cell count in the negative control group compared to the groups administered with *Solanum incanum* has a statistical significance difference indicating methanolic leaf extract of *Solanum incanum* increased the total white blood cell and neutrophils. This finding is in line with research reported by Muriithi *et al.* (2015) who found an increase in total white blood cell count after oral administration of methanolic leaf extract of *Solanum incanum*. *Solanum incanum* leaf is rich in flavonoids, saponin which have immunostimulatory effects on immune system thereby increasing white blood cells.

The methanolic leaf extract of *Solanum incanum* had no notable increase effect on platelet count in Wistar rats. Despite being no statistically significant differences among the control group and treated groups in terms of platelet indices, a non-statistically significant increase in platelet count was observed in treated groups compared to the control group. This suggests that the methanolic leaf extract of *Solanum incanum* may have a potential stimulatory effect on platelet production or function. However, there were no clear changes in platelet indices among the different groups, indicating that the extract may influence platelet count, it does not have a significant impact on other platelet parameters such as mean platelet volume, platelet distribution width or Plateletcrit. However, this is in contrast to a report by Muriithi *et al. (*2015) who found an improvement in platelet parameters after oral administration of methanolic seed extract of *Solanum incanum*. This may be due to the different part of the plant used.

Conclusion

In conclusion, the findings from this study demonstrate the potential effectiveness of methanolic leaf extract of *Solanum incanum* in significantly increasing white blood cells in the treated groups. The observed increase in WBC, Neutrophil suggests a positive effect on blood parameters. These effects may be attributed to the presence of phytochemicals such as alkaloids, saponin, tannin, flavonoids, and phenol. The RBC and platelets were slightly increased though not statistical significance difference was observed.

Recommendation

1. Further study should be carried out on *Solanum incanum* leaf to assess its effectiveness on Full blood count and to validate its therapeutic composition on blood related conditions.

Conflict of interest: Authors declare no conflict of interest

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