

Effect of *Luffa cylindrica* Leaf Extract on Hematological Parameters of Swiss Albino Mice

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Abstract

Background: *Luffa cylindrica* is a tropical fibrous flowering plant used in folkloric medicine. This study aims to examine the effect of methanolic leaf extract of *Luffa cylindrica* on hematological parameters of Swiss albino mice.

Materials and methods: 70 adult mice of both sexes with mean weight of 22 ± 5 g were used for this study. *Luffa cylindrica* leaves was pulverized to coarse powder from where methanolic extract was obtained. 0.2 ml of 50 mg/kg, 100 mg/kg, and 200 mg/kg respectively of leaf extract was administered to mice for four days. After administration, 1 ml of blood was collected from each mice through cardiac puncture into EDTA bottle for full blood count estimation using Abacus-80 hematological analyzer.

Results: The administration of 50 mg/kg, 100 mg/kg and 200 mg/kg doses of the extract resulted in increased lymphocyte count from $29.4 \pm 7.35\%$ to $82.6 \pm 0.30\%$, $81.9 \pm 0.25\%$ and $60.0 \pm 0.35\%$ respectively at $P > 0.05$ while neutrophil value was reduced from $55.2 \pm 11.05 \times 10^9/l$ to $11.0 \pm 0.12 \times 10^9/l$, $04.8 \pm 0.13 \times 10^9/l$ and $17.0 \pm 0.11 \times 10^9/l$. The administration of 50 mg/kg, 100 mg/kg and 200 mg/kg doses of the extract increases platelet count from $99.0 \pm 0.52 \times 10^3/\mu l$ to $979 \pm 1.03 \times 10^3/\mu l$, $286 \pm 0.32 \times 10^3/\mu l$ and $913 \pm 0.70 \times 10^3/\mu l$ respectively.

Conclusion: Ingestion of methanolic leaf extract of *Luffa cylindrica* resulted in a dose dependent changes in hematological parameter of albino mice including significant increase in lymphocyte and platelet counts. This plant's leaf extract may therefore have the potentials of being effective in the treatment of diseases caused by thrombocytopenia and lymphocytopenia in mammals. It is believed that information provided in this study can enhance appropriate usage of *Luffa cylindrica* in folkloric medicine in Nigeria.

Keywords: *Luffa cylindrica*; Hematological parameter; Albino mice

Introduction

Luffa cylindrica is a tropical fibrous flowering plant [1] with green cylindrical edible fruits (i.e., gourd) from where its name *Luffa cylindrica* was derived, it belongs to the Cucurbitaceae family. Luffa gourds have usefulness in medicinal, industrial and cosmetic needs of man and the Use of Luffa sponges for personal hygiene and household cleaning is common in many countries [2]. It has been reported that, dry fiber of Luffa gourds is use as bathroom sponge, component of shock absorbers, sound proof linings, utensils cleaning sponge, packing materials, making crafts [3,4] filters in factories, [5] bio diesel [6] biosorption of metals [7] and chemical extractions in industries [8]. Extracts from stem and seed of *Luffa cylindrica* has emetic action [9] and Available medical literature indicates that alcoholic and aqueous extract of various parts of *Luffa cylindrica* possess expectorant [10], analgesic sedative [11], antiinflammatory [12], antifungal [13], antihyperglycemic [14], antiparasitic [15], antimicrobial [16], antidiabetics [8], antiprotozoal [17], anthelmintic, stomachic, antipyretic [18], Anti-myocardial ischemia, hepatoprotective [19] and antioxidant [4] activities. It has also been reported that, *Luffa cylindrica* extract have been known to possess anti-leukemic cell properties [20] and fruit of *Luffa cylindrica* has been known to be

effective in treatment of bowel and bladder hemorrhage, hemorrhoids, jaundice, menorrhagia, haematuria, leprosy, spleenopathy and a good carrier for immobilization of microorganisms, biological cells [21-24].

Methanolic and various extract of *Luffa cylindrica* have been reported to contain phytochemicals such as: alkaloids, tannins, saponins, Sterols, Glycosides, carbohydrates, flavinoids and Triterpenes [14,25]. The bioactive characters of this plant can be trace to the present of this phytochemicals.

Albino mice are one of the two white-colored varieties of the domesticated house mouse, *Mus musculus*, and according to scientific American report, Albinism in mice is the result of a single genetic color factor being lost, which leads to a loss of pigmentation in the skin and eyes. The hematological parameters of mice considered in this study includes: Packed Cell Volume (PCV), Total White Cell Count (WBCt), lymphocyte count, neutrophil count, monocyte count, hemoglobin level, platelet count and red blood cell count.

Herbal drugs constitute a major part of many traditional medical systems globally [26] and Plants still serve as possible sources for new drugs and therapeutic chemicals [27]. Herbal medicine is still the mainstay of about 75-80% of the world population [28] and up to 60% of Nigerian rural population depends on use of herbal plants including *Luffa cylindrica* for folkloric medical care [29] but literature on the

effect of *Luffa cylindrica* extract on hematological parameters of mammals is not readily available, and effect of this plant's leaf extract on the hematological parameter of albino mice in Northeastern Nigeria have not yet been fully studied therefore, this study aims to examine the effect of methanolic leaf extract of *Luffa cylindrica* on the hematological parameter of mice in order to elucidate the patho-hematological effects and hematotoxicity of this plant in traditional medicinal care. It is believed that the information obtained in this study will enhance safe and appropriate use of *Luffa cylindrica* in folkloric medicine in Nigeria.

Materials and Methods

This study was carried out in Adamawa Hospital Yola, Adamawa state in Nigeria. 70 adult Swiss albino mice of both sexes with mean weight of 22 ± 5 g were used for this study. The mice were obtained from Nigerian National Institute of Veterinary Research, VOM, Plateau state, and the mice were acclimatized for six weeks in colony cages (5 mice per cage) under standard laboratory conditions (12 h light/dark cycle), fed with standard commercial pellet diet and given access to water ad libitum.

Fresh leaves of *Luffa cylindrica* were collected from bushes in Yola town. The leaves were washed, dried and pulverized to coarse powder. 100 g of the powder was soaked in 500 ml of 70% methanol for 72 hours; the mixture was further filtered using filter paper (Whatman No. 1) to obtain methanolic leaf extract. Chemical tests were carried out on the methanolic extracts to identify its phytochemical constituents using standard procedures [30-32].

The extract was administered to mice through intragastric route using the stomach tube to ensure adequate ingestion. The dose level of the extract was calculated based on the weight of mice using the formula: $\text{Dose} = (\text{body weight} \times \text{required volume}) / 100$

The mice were divided into five groups comprising of three experimental, one toxicity (further divided into five) and one negative control groups. The experimental group was administered with 0.2 ml of 50 mg/kg, 100 mg/kg, 200 mg/kg body weight respectively of methanol leaf extract of *Luffa cylindrica* for four days per dose. The negative control group was administered with 0.2 ml of distilled water, while the toxicity group was used for acute toxicity test. After administration, 1 ml of blood was collected from mice through cardiac puncture into EDTA bottle and used for hematological analysis. Full blood count was estimated in the blood using Abacus-80 hematological analyzer.

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) 20.0 software (Chicago IL). Descriptive values were given as mean standard error of mean (mean \pm SEM). Categorical variables were expressed as the number of cases and the percentage value. One-way ANOVA was used to compare mean results among and within groups. All data was analyzed at confidence interval of $p < 0.05$ and $p > 0.05$ as indicated in given tables.

Acute toxicity

Acute toxicity was determined on the leaf extract for dose level of 0 mg/kg, 500 mg/kg, 1500 mg/kg 2900 mg/kg and 4000 mg/kg respectively on mice using standard methods [33,34]. The mice were monitored within 24 hrs for signs of acute toxicity and mortality.

Full blood count

Using the Abacus-80 hematological analyzer, the procedure for blood cell (full blood count) determination was performed as follows: EDTA samples were placed in a hematology blood mixer for three minutes and the blood cells were automatically counted through a probe fitted in the Abacus-80 machine. After one minutes, the results of the blood cell count were displayed on the color LCD screen of the machine. The hematological indices values of the negative control group were used as the reference range for the estimated parameters in this study.

Results

Chemical analysis of methanolic extract of leaf of *Luffa cylindrica* indicated the present of Saponins, Flavonoids, Glycosides, Alkaloids and Terpenoids. However, Phenols, Tanins, Quinolones and Steroids were not detected in the methanol leaf extract of this plant in Northeastern Nigeria as shown in Table 1.

Phytochemicals	<i>Luffa cylindrica</i>
Saponins	+
Phenols	-
Tanins	-
Flavonoids	+
Quinolones	-
Glycosides	+
Terpenoids	+
Steroids	-
Alkaloids	+
Key: - = Absent, + = Present	

Table 1: Phytochemical constituents of the methanol plant extract.

Toxicity test indicated that none of the mice shows symptoms of acute toxicity for the period under observation as shown on Table 2.

Dose (mg/kg)	Dead	Treated	Latency (hrs)	Toxicity symptoms
0	0	2	24	None
500	0	2	24	None
1500	0	2	24	None
3000	0	2	24	None
4000	0	2	24	None

Table 2: Acute oral toxicity of methanolic leaf extract of *Luffa cylindrica*.

Table 3 and Figure 1 indicates that varying doses of methanolic extract of leaf of *Luffa cylindrica* had multiple effects on hematological parameters of albino mice.

	DOSE (mg/kg)	*PCV	*HGB	*WBCT	*Lymphocyte	*Neutrophil	*Monocyte	*Platelet	*RBC
<i>Luffa cylindrica</i>	50	49.1 ± 0.30	14.1 ± 0.15	14.9 ± 0.02	82.6 ± 0.30	11.0 ± 0.12	6.3 ± 0.09	979 ± 1.03	8.5 ± 0.17
	100	37.7 ± 0.29	10.9 ± 0.04	22.6 ± 0.07	81.9 ± 0.25	4.8 ± 0.13	13.3 ± 0.14	286 ± 0.32	6.7 ± 0.17
	200	44.6 ± 0.15	13.0 ± 0.30	17.2 ± 0.34	60.0 ± 0.35	17.0 ± 0.11	23.1 ± 0.12	913 ± 0.70	8.0 ± 0.25
Negative control (DW)	1 ml	31.4 ± 0.55	9.7 ± 0.22	34.9 ± 8.72	29.4 ± 7.35*	55.2 ± 11.05*	8.4 ± 0.41	99.0 ± 0.52	5.4 ± 0.29

Data are expressed as mean ± SEM, *P<0.05 *P>0.05

Table 3: Effect of methanolic leaf extract of *Luffa cylindrica* on hematological parameters of swiss albino mice.

The alteration in the hematological indices was observed to be dependent on the dosage of the extract. The administration of 50 mg/kg dose of the extract increases the PCV value from $31.4 \pm 0.55\%$ to $49.1 \pm 0.30\%$ while the administration of 100 mg/kg and 200 mg/kg increases the PCV (Packed Cell Volume) value from $31.4 \pm 0.55\%$ to $37.7 \pm 0.29\%$ and $44.6 \pm 0.15\%$ respectively at $P < 0.05$ (Table 3). However, the administration of 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract increases the hemoglobin (HGB) value from 9.7 ± 0.22 to 14.1 ± 0.15 g/dl, 10.9 ± 0.04 g/dl and 13.0 ± 0.30 g/dl respectively. On the other hand, total white blood cell count (WBCT) was reduced from $34.9 \pm 8.72 \times 10^9/l$ to $14.9 \pm 0.02 \times 10^9/l$, $22.6 \pm 0.071 \times 10^9/l$ and $17.2 \pm 0.34 \times 10^9/l$ after ingestion of 50 mg/kg, 100 mg/kg, and 200 mg/kg of the extract respectively. On the contrary, the ingestion of the 50 mg/kg dose of the extract resulted in very high level of lymphocyte count from $29.4 \pm 7.35\%$ to $82.6 \pm 0.30\%$ and the dose of 100 mg/kg and 200 mg/kg resulted in increase of lymphocyte value from $29.4 \pm 7.35\%$ to $81.9 \pm 0.25\%$ and $60.0 \pm 0.35\%$ at $P > 0.05$. while neutrophil value was reduced from $55.2 \pm 11.05 \times 10^9/l$ to $11.0 \pm 0.12 \times 10^9/l$, $4.82 \pm 0.13 \times 10^9/l$ and $17.0 \pm 0.11 \times 10^9/l$ after the administration of 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract respectively at $P > 0.05$. The ingestion of 100 mg/kg and 200 mg/kg doses of the extract increases the value of monocyte from $8.4 \pm 0.41 \times 10^9/l$ to $13.3 \pm 0.14 \times 10^9/l$ and $23.1 \pm 0.12 \times 10^9/l$ respectively but ingestion of 50 mg/kg of the extract reduces the monocyte count from $8.4 \pm 0.41 \times 10^9/l$ to $6.3 \pm 0.09 \times 10^9/l$. Platelet and red cell value was also affected by dosage of methanolic extract of *Luffa cylindrica*. Platelet value increases from $99.0 \pm 0.52 \times 10^3/ \mu l$, to $979 \pm 1.03 \times 10^3/ \mu l$, $286 \pm 0.32 \times 10^3/ \mu l$, and $913 \pm 0.70 \times 10^3/ \mu l$ respectively after the administration of 50 mg/kg, 100 mg/kg and 200 mg/kg of the methanolic extract.

Moreover, the value of red blood cell increases from $5.4 \pm 0.29 \times 10^{12}/l$ to $8.5 \pm 0.17 \times 10^{12}/l$, $6.7 \pm 0.17 \times 10^{12}/l$ and $8.0 \pm 0.25 \times 10^{12}/l$ after ingestion of 50 mg/kg, 100 mg/kg and 200 mg/kg of methanolic leaf extract of *Luffa cylindrica* respectively. In addition, of the eight hematological indices studied, lymphocyte and platelet counts values showed the highest increase value change after ingestion of the extract as shown in Table 3 and Figure 1 compare to other hematological parameters in the studied group.

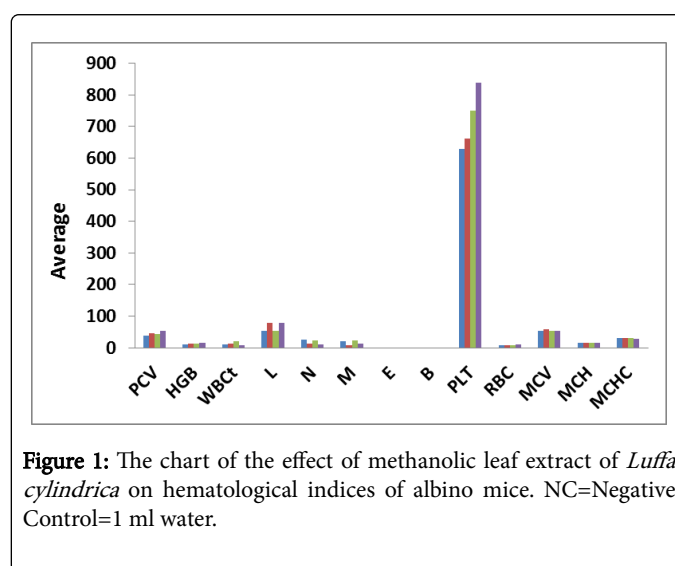


Figure 1: The chart of the effect of methanolic leaf extract of *Luffa cylindrica* on hematological indices of albino mice. NC=Negative Control=1 ml water.

Discussion

Effect of methanolic leaf extract of *Luffa cylindrica* on the hematological parameters of Swiss albino mice have been investigated and from this study, it was observed that, the ingestion of 500 mg/kg, 1500 mg/kg, 3000 mg/kg, and 4000 mg/kg doses of methanolic extract of the leaf of *Luffa cylindrica* did not produce observable acute toxicity within the stipulated period of observation as shown in Table 1. This demonstrates the level of toxically safety of this plant's extract and implies that methanolic extract of this plant's leaf at dosage below 4000 mg/kg may be toxically and clinically safe for intake by mammals and mice.

Chemical analysis of methanolic extract of leaf of *Luffa cylindrica* indicated the present of Saponins, Flavonoids, Glycosides, Alkaloids and Terpenoids. However, Phenols, Tanins, Quinolones and Steroids were not detected in the methanol leaf extract of this plant in Northeastern Nigeria as shown in Table 1. This is because some of the phytochemicals may be found in aqueous not in methanolic medium that was used for the extract in this study. The present of phytochemicals like saponins, flavonoids, glycosides, and alkaloids in this plant have also been demonstrated by earlier researchers [14,25]. The bioactive and pharmacomedical characteristics of this plant is believed to be due to the presence of these phytochemicals in alcoholic extract of this plant. The ingestion of 50 mg/kg, 100 mg/kg, and 200

mg/kg doses of this plant's leaf extract resulted in changes in hematological indices including increase in red blood cell count of mice as shown in Table 3 and Figure 1. It is not yet clear which phytochemical mechanism resulted in the observed dose dependent alterations in hematological parameter of Swiss Albino mice, but increase value of lymphocyte after ingestion of the extract is believed to be due to the presence of Saponins phytochemicals in this plant because Saponins have been shown to increase cytosolic calcium concentration which results in calcium permeable cation channels [35] and calcium play an important role in lymphocyte kinetics and proliferation [36] therefore, the presence of Saponins may through the calcium activation mechanism improve lymphocytes proliferation in the blood. In addition, the dose dependent increase of blood lymphocyte counts as shown Figure 1 and Table 3 is also believed to be due to the presence of alkaloids as a phytochemical compound in the leaf extract of *Luffa cylindrica* since alkaloid fraction stimulate defense system by improving and modulating several immunological parameters including lymphocyte proliferation [37].

The strong increase of lymphocyte count in the experimental group after ingestion of the provided dosage of methanolic leaf extract of this plant demonstrates that, this plant may have the potentials of improving the cellular immune system and may be effective in treatment of disease conditions caused by lymphocytopenia in mammals and in addition, there was a significant increase in platelet count value after ingestion of methanolic leaf extract of *Luffa cylindrica* although it is not yet clear of which mechanism resulted in increased thrombocyte in mice's blood but the dose-dependent increase in platelet count as a result of administration of extract of *Luffa cylindrica* leaf indicates that this plant's leaf extract may also have the potency to improve the hemostasis system in the management of bleeding disorders caused by thrombocytopenia in mammals.

Conclusion

The ingestion of methanolic leaf extract of *Luffa cylindrica* results in a dose dependent changes in hematological parameter of albino mice. The administration of 50 mg/kg, 100 mg/kg and 200 mg/kg doses of methanolic extract of the leaf of *Luffa cylindrica* extract causes a significant increase in lymphocyte, platelet counts, PCV, red blood cell counts in blood of mice in the experimental group at $P < 0.05$. This plant leaf extract therefore has the potentials of being effective in the treatment of diseases cause by both thrombocytopenia, anemia and lymphocytopenia in mammals. It is believed that the information provided in this study will enhance the appropriate use of *Luffa cylindrica* in folkloric medicine in Nigeria.

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