

Effect of *Achyranthes Aspera* Leaf Extract on Hematological Parameters of Swiss Albino Mice

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Abstract: Background/Objective: *Achyranthes aspera* is an erect flowering medicinal herb use in folkloric medicine. This study aims to examine the effect of methanolic leaf extract of *Achyranthes aspera* 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. *Achyranthes aspera* leaves was pulverized to coarse powder from where methanolic extract was obtained. 0.2ml of 50mg/kg, 100mg/kg, and 200mg/kg respectively of leaf extract was administered to mice for four days. After administration, 1ml of blood was collected from mice through cardiac puncture into EDTA bottle for full blood count estimation using Abacus-80 hematological analyzer. Results: The administration of 50mg/kg, 100mg/kg and 200mg/kg doses of the extract resulted in increased lymphocyte count from $29.4 \pm 7.35\%$ to $70.4 \pm 0.25\%$, $70.5 \pm 0.219\%$ and $81.3 \pm 0.282\%$ respectively at $P > 0.05$ while neutrophil value was reduced from $55.2 \pm 11.05 \times 10^9/l$ to $14.0 \pm 0.216 \times 10^9/l$, $11.2 \pm 0.109 \times 10^9/l$ and $12.3 \pm 0.148 \times 10^9/l$. The administration of 50mg/kg, 100mg/kg and 200mg/kg doses of the extract increases platelet count from $99.0 \pm 0.52 \times 10^3/ul$ to $529 \pm 0.01 \times 10^3/ul$, $332 \pm 0.523 \times 10^3/ul$ and $135 \pm 0.543 \times 10^3/ul$ respectively. Conclusion: Ingestion of methanolic leaf extract of *Achyranthes aspera* 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 cause by thrombocytopenia and lymphocytopenia in mammals. It is believed that information provided in this study can enhance appropriate usage of *Achyranthes aspera* in folkloric medicine in Nigeria.

Keywords: *Achyranthes Aspera*, Hematological Parameter, Albino Mice

1. Introduction

Achyranthes aspera is an annual, erect stiff flowering herb, usually about 0.3 to 1meter high [1, 2] and belong to Amaranthaceae family. It is commonly found as a way side weed in most tropical regions of the world. *Achyranthes aspera* have since been documented to have a wide application in folkloric medicine globally and it has been reported that various parts of this plant are used traditionally for treatment of various health problems such as: treatment of wounds, [3] fever, [4] dysentery, asthma, [5] gonorrhoea [6] and hydrated Paste of this plant's roots is used in ophthalmia and opacities of the cornea [7]. *A. aspera* leaves have also been shown to

have cancer preventive activity [8] and it has been shown that extracts of this plant exhibits antiarthritic, laxative, ecboic, immuno-stimulant, [9] antihelminthic, aphrodisiac, antiviral, mosquito larvicidal, [10] antitrypanosomal, [11] anticoagulant, diuretic [12, 13] and antiparasitic [14] characteristics including Hepatoprotective ability. [15] In addition, available medical literatures indicates that, various alcoholic extract of stem, leaves and flower of *Achyranthes aspera* possesses molluscicidal, [16] antimicrobial, [17] anti-inflammatory, [18] Anti-oxidant, [19] Anti-depressant, [6, 20] anti-allergic, [21] antiimplantation, abortifacient activities [22] as well as dose dependent contraceptive and antifertility properties in rats [23, 24] and also, extract of this plant have been known to improve

the haemostatic system of mammals. [25] The pharmacological effects of this plant is believed to be due to the present of certain phytochemicals such as: saponins, [26] β -D galactopyranosyl ester of D-Glucuronic Acid (also known as saponins B) and D-Glucuronic Acid (saponins A). [27] In male reproductive medicine model, *A. aspera* roots have been reported to possess spermicidal activity in both human and rat [28] and administration of *A. aspera* leaves extracts causes reduction in spermatogenesis, steroidogenesis, androgen production, and alteration of sexual behavior in Swiss albino mice. [29] 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 (tWBC), 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 [7] and Plants (including *A. aspera*) can still serve as possible sources for new drugs and therapeutic chemicals [28] but literature on the effect of *A. aspera* extract on hematological parameters of mammals is scanty, 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 *Achyranthes aspera* on the hematological parameter of mice in other 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 *Achyranthes aspera* in folkloric medicine in Nigeria.

2. 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 (12h light/dark cycle), fed with standard commercial pellet diet and given access to water *ad libitum*.

Fresh leaves of *Achyranthes aspera* were collected from bushes in Yola town. The leaves were washed, dried and pulverized to coarse powder. 100g of the powder was soaked in 500ml of 70% methanol for 72hours; 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:

$$\text{Dose} = \frac{\text{body weight} \times \text{required volume}}{100}$$

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 0.2ml of 50mg/kg, 100mg/kg, 200mg/kg body weight respectively of methanol leaf extract of *Achyranthes aspera* for four days per dose. The negative control group was administered with 0.2ml of distilled water, while the toxicity group was used for acute toxicity test. After administration, 1ml of blood was collected from the 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.

2.1. 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. 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 table 3.

2.2. Acute Toxicity

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

2.3. Full Blood Count

Using the Abacus-80 machine, 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.

3. Results

Table 1. Phytochemical constituents of the methanol plant extract.

Constituents	<i>A. aspera</i>
Saponins	+
Phenols	-
Tanins	-
Flavonoids	+
Quinolones	-
Glycosides	-
Terpenoids	+
Steroids	-
Alkaloids	+

KEY: - = Absent, + = Present

Chemical analysis of methanolic extract of leaf of *Achyranthes aspera* indicated the present of Saponins, Flavonoids, Alkaloids and Terpenoids. However, Phenols,

Tanins, Quinolones and Glycosides were not detected in the methanol leaf extract of this plant in Northeastern Nigeria as shown in Table 1.

Table 2. Acute oral toxicity of methanolic leaf extract of *Achyranthes aspera*.

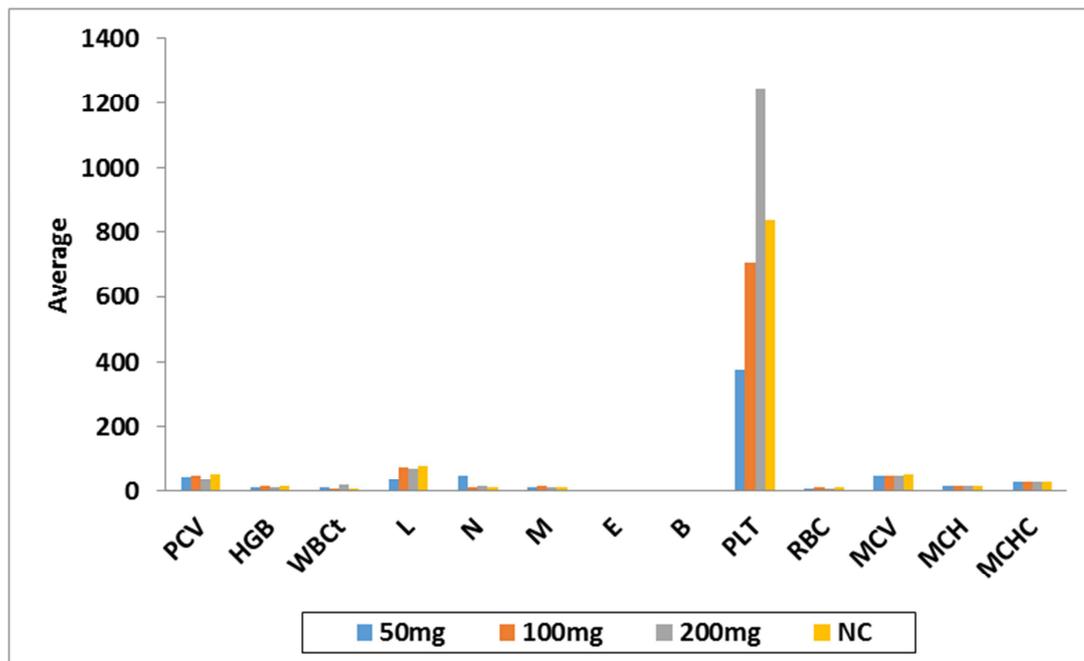
Dose (mg/kg)	Dead	Treated	Latency (hrs)	Toxicity symptoms
0	0	2	24	None
500	0	2	24	None
1500	0	2	24	None
3000	0	2	24	None
4000	0	2	24	None

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

Table 3. Effect of methanolic leaf extract of *Achyranthes aspera* on hematological parameters of swiss albino mice.

	DOSE (mg/kg)	*PCV	*HGB	*WBCT	*Lymphocyte	*Neutrophil	*Monocyte	*Platelet	*RBC
Achyranthes aspera	50	39.3±0.233	13.1±0.111	10.2±0.132	70.4±0.25	14.0±0.216	15.6±0.242	529±0.512	8.5±0.312
	100	41.6±0.356	12.6±0.121	7.7±0.081	70.5±0.219	11.2±0.109	14.3±0.187	332±0.523	7.6±0.219
	200	41.6±0.356	9.9±0.492	5.2±0.158	81.3±0.282	12.3±0.148	6.4±0.147	135±0.543	5.9±0.258
Negative control (DW)	1ml	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



NC = Negative Control = 1ml water

Figure 1. The chart of the effect of methanolic leaf extract of *Achyranthes aspera* on hematological indices of albino mice.

Table 3 indicates that varying doses of methanolic extract of leaf of *Achyranthes aspera* had multiple effects on hematological parameters of albino mice. The alteration in the hematological indices was observed to be dependent on the dosage of the extract. The administration of 50mg/kg of the extract increases the PCV value from 31.4±0.55% to 39.3±0.233% while the administration of 200mg/kg and 100mg/kg increases the PCV (Packed Cell Volume) value from 31.4±0.55% to 41.6±0.356% at P< 0.05 (Table 3)

However, the administration of 50mg/kg and 100mg/kg of the extract increases the hemoglobin (HGB) value from 9.7±0.22 to 13.1±0.111g/dl and 12.6±0.121g/dl respectively but when 200mg/kg dose was ingested, the hemoglobin value did not show significant change. On the other hand, total white blood cell count (WBCT) was reduced from 34.9±8.72x10⁹/l to 10.2±0.132x10⁹/l, 7.7±0.081x10⁹/l and 5.2±0.158x 10⁹/l after ingestion of 50mg/kg, 100mg/kg, and 200mg/kg of the extract respectively. On the contrary, the

ingestion of the 200mg/kg dose of the extract resulted in very high level of lymphocyte count up to $81.3 \pm 0.282\%$ and the dose of 50mg/kg and 100mg/kg resulted in increase of lymphocyte value from $29.4 \pm 7.35\%$ to $70.4 \pm 0.25\%$ and $70.5 \pm 0.219\%$ at $P > 0.05$ while neutrophil value was reduced from $55.2 \pm 11.05 \times 10^9/l$ to $14.0 \pm 0.216 \times 10^9/l$, $11.2 \pm 0.109 \times 10^9/l$ and $12.3 \pm 0.148 \times 10^9/l$ after the administration of 50mg/kg, 100mg/kg and 200mg/kg of the extract respectively at $P > 0.05$. The ingestion of 50mg/kg and 100mg/kg doses of the extract increases the value of monocyte from $8.4 \pm 0.41 \times 10^9/l$ to $15.6 \pm 0.242 \times 10^9/l$ and $14.3 \pm 0.187 \times 10^9/l$ respectively but ingestion of 200mg/kg of the extract reduces the monocyte level from $8.4 \pm 0.41 \times 10^9/l$ to $6.4 \pm 0.147 \times 10^9/l$.

Platelet value was also affected by dosage of the methanolic extract. Platelet and red blood cell value increases inversely as the dosage of the ingested extract increases as shown in table 3. The administration of 50mg/kg, 100mg/kg and 200mg/kg doses of the extract increases platelet level in the blood of mice from 99.0 ± 0.52 to $529 \pm 0.512 \times 10^3/ul$, $332 \pm 0.523 \times 10^3/ul$ and $135 \pm 0.543 \times 10^3/ul$ respectively. Moreover, the value of red blood cell increases from $5.4 \pm 0.29 \times 10^{12}/l$ to $8.5 \pm 0.312 \times 10^{12}/l$, $7.6 \pm 0.219 \times 10^{12}/l$ and $5.9 \pm 0.258 \times 10^{12}/l$ after ingestion of 50mg/kg, 100mg/kg and 200mg/kg of methanolic leaf extract of *Achyranthes aspera* 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.

4. Discussion

Effect of methanolic leaf extract of *Achyranthes aspera* on the blood of Swiss albino mice have been investigated and from this study, it was observed that, the ingestion of 500mg/kg, 1500mg/kg, 3000mg/kg, and 4000mg/kg doses of methanolic extract of the leaf of *Achyranthes aspera* 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 4000mg/kg may be toxically and clinically safe for intake by mammals and mice.

Chemical analysis of methanolic extract of *Achyranthes aspera* leaf indicated the present of certain phytochemicals such as: Saponins, Flavonoids, Alkaloids and Terpenoids. However, Phenols, Tanins, Quinolones and Glycosides 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 that are absent in this plant may be found in aqueous [35] not in methanolic medium that was used for the extract in this study and the present of Saponins, Flavonoids, Alkaloids and Terpenoids phytochemicals in *Achyranthes aspera* have long been demonstrated by various researchers. [25, 26]

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 present of Saponins phytochemicals in this plant because Saponins have been shown to increase cytosolic calcium concentration which results in calcium permeable cation channels [36] and calcium play an important role in lymphocyte kinetics and proliferation [37] therefore, the present 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 table 3 is also believed to be due to the presence of alkaloids as a phytochemical compound in the leaf extract of *Achyranthes aspera* since alkaloid fraction stimulate defense system by improving and modulating several immunological parameters including lymphocyte proliferation. [38]

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 cause by lymphocytopenia in mammals and in addition, there was a significant increase in platelet count value after ingestion of methanolic leaf extract of *Achyranthes aspera* 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 *Achyranthes aspera* 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.

5. Conclusion

The ingestion of methanolic leaf extract of *Achyranthes aspera* results in a dose dependent changes in hematological parameter of albino mice. The ingestion of 50mg/kg, 100mg/kg and 200mg/kg doses of methanolic extract of the leaf of *Achyranthes aspera* extract causes a significant increase in lymphocyte and platelet 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 and lymphocytopenia in mammals. It is believed that the information provided in this study will enhance the appropriate use of *Achyranthes aspera* in folkloric medicine in Nigeria.

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The views expressed in this article are that of the authors not an official position of any hospital or institution.

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Conflict of Interest

There is no conflict of interest regarding this work among the authors.

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