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Effect of methanolic leaf extract of *Ficus thonningii* on hematological indices of Swiss albino mice

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Abstract

Background/Objective: *Ficus thonningii* is an evergreen medicinal tree. *Ficus thonningii* leaf is used in Nigerian folkloric medicine. This study aims to examine the effect of methanolic leaf extract of *F. thonningii* on hematological indices of albino mice.

Materials and Methods: 70 adult Swiss albino mice of both sexes with mean weight of 22 ± 5 g were used for this study. Leaves of *Ficus thonningii* was pulverized to coarse powder from which methanolic extract was obtained. Mice were divided into five groups comprising 3 experimental, 1 toxicity group and 1 negative control groups. The experimental group was administered with 0.2ml of 50mg/kg, 100mg/kg, and 200mg/kg of methanol leaf extract of *F. thonningii* respectively. The negative control group was administered with 1ml of distilled water while the toxicity group was used for acute toxicity test. 1.5ml of blood was collected from the mice into EDTA bottle through cardiac puncture. Full blood count was estimated in the blood using Abacus-80 hematological analyzer.

Result: The ingestion of 50mg/kg and 200mg/kg of the extract reduces Packed Cell Volume from $31.4 \pm 0.55\%$ to $26.3 \pm 0.077\%$ and $15.4 \pm 0.180\%$ respectively at $P < 0.05$. While ingestion of 200mg/kg of extract increases the lymphocyte value from 29.4 ± 7.35 to $83.4 \pm 0.47\%$. The ingestion of 50mg/kg of the extract increases platelet count value from $99.0 \pm 0.52 \times 10^9/l$ to $409 \pm 0.56 \times 10^9/l$ at $p < 0.05$.

Conclusion: Ingestion of methanolic leaf extract of *F. thonningii* alters the hematological indices and increases lymphocyte and platelet count. The extract therefore has the potential of improving immune and hemostasis system of mammals. Information provided in this study can enhance appropriate use of *F. thonningii* in folkloric medicine in Nigeria.

Keywords: *Ficus thonningii*, hematological indices, albino mice

Introduction

Ficus thonningii is an evergreen 6-12m fruit bearing tree with a rounded spreading dense crown leaves and aerial roots hanging down from branches. It belongs to the Moraceae family and it is widely distributed in upland, forests, open grasslands, savannahs, riverine and rocky areas [1]. Different parts of the plant are used in treatment of various diseases. The plant's leaf is used to treat stomach offset and many other disease conditions in Nigerian folkloric medicine [2, 3]. The inhibition of *E. coli* by *Ficus thonningii* has justified its use for the treatment of diarrhea and dysentery [4]. *Ficus thonningii* has been shown to possess hypoglycemic effects [5, 6] hence its use in the treatment of diabetes mellitus. Modern pharmacological studies have indicated that *F. thonningii* extracts have antimicrobial [7] antiprotozoal [8] antifungal [9] antihelminthic [10] antioxidant [11, 12] anti-inflammatory [13] and antipsychotic [14] effects. The therapeutic properties of *F. thonningii* is believed to result from the presence of a number of phytochemical compounds such as Flavonoids (orientin, vitexin and isovitexin) [15] Tannins, Alkaloids, Terpenes, Saponins and Cardiac Glycosides [1]. *F. thonningii* extracts also contain phytochemicals that mimic and/or enhance the action of regulatory peptides which increase the proliferation of parietal cells and exhibit trophic effects on the gut mucosa of rats [16]. High levels of saponins in *F. thonningii* aqueous extracts have been associated with haemolysis of red blood cells through interaction with membrane components such as proteins, phospholipids and cholesterol [17]. A significant increase in total leukocyte count and platelet values in rats [18] and ability to improve hematological parameters and protective ability on integrity of erythrocyte membrane has been reported to be associated with administration of

F. thonningii extract [19]. High levels of certain phytochemicals in *F. thonningii* aqueous extracts have been associated with red blood cells destruction through interaction with cell membrane components [16]. 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: PCV, total WBC, lymphocyte count, neutrophil count, monocyte count, hemoglobin level, platelet count, red blood cell count. Literature on the effect of *F. thonningii* extract on hematological parameters of albino mice is scanty, and effect of this plant's leaf extract on the hematological indices 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 *F. thonningii* on the hematological indices 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 *F. thonningii* 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 obtain from Nigerian National Institute of Veterinary Research, VOM, Plateau state were used for this study, 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 *Ficus thonningii* were collected from bushes in Yola town. The leaves were further washed, dried and pulverized to coarse powder. 100g of the powder was soaked in 500ml of 70% methanol for 72hours; which 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 [20-22].

The extract was administered to the 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 *F. thonningii* for four days per dose. The negative control group was administered with 1ml of distilled water, while the

toxicity group was used for acute toxicity test. After ingestion, 1.5ml of blood was collected from the mice into EDTA bottle through cardiac puncture 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. 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.

Acute Toxicity

Acute toxicity was determined for the leaf extract for dose level of 0mg/kg, 500mg/kg, 1500mg/kg 2900mg/kg and 4000mg/kg respectively on mice using standard methods [23, 24]. The mice were monitored within 24hrs for signs of acute toxicity and mortality.

Full Blood Count

Using 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 five 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 this study.

Results

Table 1: Phytochemical constituents of the methanol leaf extract of *Ficus thonningii*

Phytochemical Constituents	Present/Absent
Saponins	-
Phenols	+
Tannins	+
Flavonoids	-
Quinolones	-
Glycosides	+
Terpenoids	-
Steroids	+
Alkaloids	+
Present = +, Absent = -	

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

Table 2: Acute oral toxicity of methanolic leaf extract of *Ficus thonningii*

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 *ficus thonningii* on hematological indices of swiss albino mice

	Extract Dose(mg/kg)	*PCV	*HGB	*WBCT	*Lymphocyte	*Neutrophil	*Monocyte	*Platelet	*RBC
	50	26.3±0.077	7.5±0.282	9.2±0.196	59.8±0.327	26.2±0.151	14.0±0.139	409±0.56	4.2±0.19
	100	36.3±0.065	10.1±0.151	9.8±0.025	77.1±0.30	12.3±0.220	10.8±0.11	1235±0.26	10.4±0.21
	200	15.4±0.180	4.6±0.170	20.9±0.03	83.4±0.47	10.5±0.23	6.0±0.18	183±0.93	2.6±0.33
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, * $P < 0.05$ # $P > 0.05$.

Table 3 indicates that varying doses of methanolic extract of the leaf of *F. thonningii* had multiple effects on hematological indices 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 and 200mg/kg of the extract reduces PCV (Packed Cell Volume) from 31.4±0.55% to 26.3±0.077% and 15.4±0.180% respectively at $P < 0.05$ while the administration of 100mg/kg of the same extract increases the PCV from 31.4±0.55 to 36.3±0.065 (Table 3) However, the administration of 50mg/kg and 200mg/kg reduces the hemoglobin (HGB) from 9.7±0.22 to 7.5±0.282g/dl and 4.6±0.170g/dl respectively. The dose of 200mg/kg had the strongest effect in reducing the PCV and hemoglobin level of albino mice at $P < 0.05$. On the other hand, the total white blood cell count (WBCT) was reduced to the lowest level (9.2±0.196x 10⁹/l) due to ingestion of 50mg/kg of the extract. On the contrary, the ingestion of the 200mg/kg dose of the extract resulted in the highest level of lymphocyte count of 83.4±0.47% at $P > 0.05$ while neutrophil was reduced to the lowest level of 10.5±0.23 x 10⁹/l with the administration of the highest dose (200mg/kg) of the extract. Monocyte level increases as the dosage of the ingested extract increases as shown in table 3. Platelet value was also affected by the dosage of the methanolic extract. The administration of 50mg/kg of the extract increases the platelet level in the blood of mice from 99.0±0.52 to 409±0.56 x 10³/ul but higher dose of 200mg and 100mg of the extract reduces the platelet level to 183±0.93 x 10³/ul and 1235±0.26 x 10³/ul respectively. The administration of 50mg/kg and 200mg/kg of the extract reduces the red blood level from 5.4±0.29 x 10¹²/l to 4.2±0.19 x 10¹²/l and 2.6±0.33 x 10¹²/l respectively while the dose of 100mg/kg increases the red cell level to 10.4±0.21 x 10¹²/l it appears that red cell level reduces when the concentration of the dose is below or above 100mg as shown in table 4 and in figure 1. In addition, of the eight hematological indices studied, lymphocyte and platelet counts values showed the highest increase after ingestion of the extract as shown in table 3 and figure 1 compare to other hematological parameters in the studied group.

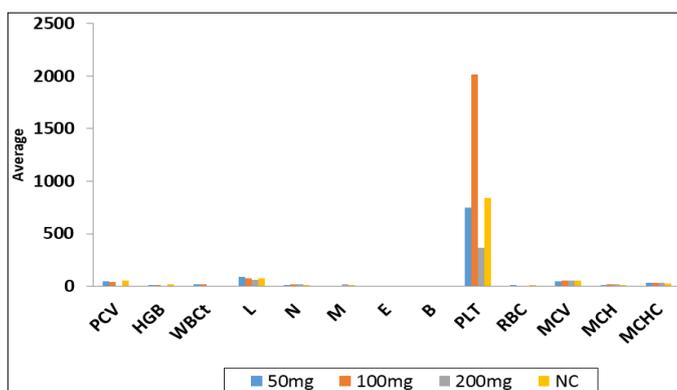


Fig 1: the chart of the effect of methanolic leaf extract of *F. thonningii* on hematological indices of albino mice
NC = Negative Control = 1ml water

Discussion

Effect of methanolic leaf extract of *Ficus thonningii* on Swiss albino mice have been investigated and from this study, it is observed that, the ingestion of 500, 1500, 3000, 4000mg/kg doses of methanolic extract of the leaf of *Ficus thonningii* did not produce observable acute toxicity within the stipulated period as shown in table 1. This implies that methanolic extract of this plant's leaf is clinically safe for consumption by mammals and mice.

Chemical analysis of the extract of leaf of *F. thonningii* indicated the present of phytochemicals such as: phenols, Tannins, Glycosides, Steroids, and Alkaloids, while saponins, flavonoids, quinolones, terpenoids were not detected in the extract as shown in table 1. This is because some phytochemicals of this plant's leaf (like saponins) can be found in aqueous^[17] not in methanolic medium. The ingestion of leaf extract of the plant resulted in increase in the platelet count in blood as shown in table 3, this similar result was also observed by Aniagu SO in 2008^[18] this increase in platelet count is believed to be as a result of the presence of phenols and tannins in the extract (table 1) because phenols and some tannins have been known to increase clotting process by enhancing platelet proliferation and platelet factor activities^[25]. However, the present of tannin in the extract is believed to be responsible for the reduction in the red blood cell count of mice ingested with 50mg/kg and 200mg/kg of methanolic extract of the leaf of *F. thonningii* compare with the negative control group because since in the 1970s tannins have been reported to combine with hemoglobin to increase hemolysis of blood red blood cell.^[26] There was a reduction in the PCV of mice's blood after ingestion of the extract this was also observed among rats by Dangarembizi in 2014^[27]. The reduction in PCV, hemoglobin, red blood cell counts and total white count in the blood of mice in the experimental group as shown in table 3 is believed to be due to the present of certain glycosides in the plant's leaf extract because glycosides inhibits and alter the functioning of the active membrane pumps of hematological cells^[28], resulting in untimely destructions and eventual reduction of these cells in the blood stream. In addition, the total white blood cell (WBCt) was reduced after ingestion 50mg/kg, 100mg/kg and 200mg/kg of the extract this is believed to be due to the presence of steroid on the leaf extract since some steroids inhibits the kinetics and proliferation of white bloods. On the other hand, lymphocyte count in the blood of mice was increased after ingestion of various doses of the extract and the higher the dose, the higher was the peripheral blood lymphocyte count (table 3) this is because of the presence of alkaloids as a phytochemical compound in the leaf extract of *Ficus thonningii* since alkaloid fraction stimulate defense system by improving and modulating several immunological parameters including lymphocyte^[29]. The increase in lymphocyte count implies that leaf extract of this plant could have the ability to improve the immune and defense system in the folkloric medical care for immune dysfunction arising from lymphocytopenia. Also, there was a significant increase in platelet count value after

ingestion of methanolic leaf extract of *Ficus thonningii* this indicates that this plant's leaf extract has the potency to improve the haemostasis system in the management of bleeding disorders caused by thrombocytopenia in mammals.

Conclusion

The ingestion of methanolic leaf extract of *Ficus thonningii* results in changes in hematological indices of albino mice. The variations of these indices were dependent on dosages of the ingested extract. The ingestion of 50mg/kg, 100mg/kg and 200mg/kg doses of methanolic extract of the leaf of *Ficus thonningii* extract causes a significant increase in the lymphocyte and platelet counts in blood of mice in the experimental group compare with the control at $P < 0.05$. This plant leaf extract therefore has the potentials of improving the haemostasis and immune defense systems. Ingestion of this leaf extract at the considered doses upto 4000mg/kg did not produce acute toxicity in the animals. It is believed that the information provided in this study will enhance the appropriate use of *Ficus thonningii* in folkloric medicine in Nigeria.

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