Special Conference Edition, November, 2017
http://dx.doi.org/10.4314/bajopas.v10i1.59S

Bayero Journal of Pure and Applied Sciences, 10(1): 295 - 298
ISSN 2006 – 6996

PHYTOCHEMICAL SCREENING, LD_{50} DETERMINATION, AND SUB-CHRONIC TOXICITY STUDIES OF AQUEOUS LEAF EXTRACT OF *Ficus polita*

[*1]Nasir, A., [2]Alhassan, A.J., [2]Sule, M.S., [1]Mannir, A.R., [1]Yaradua, A.I., [2]Idi, A., [2]Muhammad, I.U. and [3]Kanadi, A.M.

[1]Department of Biochemistry, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, P.M.B. 2218, Katsina, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University Kano, P.M.B. 3011, Kano, Nigeria.
[3]National Agency for Food and Drug Administration and Control (NAFDAC), P.M.B. 2015, Katsina, Nigeria.
*Correspondence Author: amnuf01@yahoo.com Phone: +2348036851879

ABSTRACT
The young leaves of F. polita plant are edible and the bark and roots infusions are used in the treatment of infectious diseases, dyspepsia and diarrhoea like many other species of Moraceae family. Qualitative phytochemical screening of aqueous leaf extract of F. polita was determined. Lethal mean dose (LD_{50}) and sub-chronic oral toxicity studies of the extract of F. polita were evaluated in wistar albino rats. Phytochemical screening of the extract revealed the presence of alkaloids, polyphenols, flavonoids, flavonols, glycosides, anthraquinones, saponins, tannins, fats and oils, terpenes, triterpenoids, carbohydrates, starch, gums and mucilages, and proteins. The LD_{50} of this extract was estimated to be more than 5000 mg/kg. Oral administration of the extract at 1000, 2000, 3000, and 4000 mg/kg body weight revealed no significant difference ($P>0.05$) in haematological parameters like RBC, haemoglobin, PCV, MCH, and MCHC. There was significant increase ($P<0.05$) in MCV in 3000 mg/kg dose when compared with control. There were significant increases ($P<0.05$) in WBC, lymphocytes, and platelets, in some of the treated doses. Analyses of serum liver enzymes, total protein, albumin, electrolytes, and creatinine revealed no significant changes ($P>0.05$) in the treated doses compared to their controls. However, significant differences ($P<0.05$) were observed in urea, direct bilirubin, and total bilirubin of some treated doses when compared to their controls. These results suggest that the aqueous leaf extract of F. polita is rich in phytochemicals, and may be considered relatively safe at the tested doses.
Keywords:Ficuspolita, aqueous, leaf, extract.

INTRODUCTION

*Ficuspolita*Vahl is a tropical African evergreen shrub or small tree belonging to the family Moraceae, and usually growing up to 15 metres tall, and sometimes to 40 meters tall (SANBI, 2015). Leaf extracts had been demonstrated to exhibit antimalarial activity (Ayisi and Nyadedzor, 2003). For majority of herbal products in use, very little is known about their active and /or toxic constituents. Therefore, the present study was aimed at investigating the phytochemical composition, and determining the LD_{50} and sub-chronic toxicity of the aqueous leaf extract of *F. polita*. For the sub-chronic toxicity test, liver and kidney function parameters, as well as haematological indices, of the tested rats, were assessed.

MATERIALS AND METHODS

Collection and preparation of plant material:
F. polita leaves were obtained from KofarMarusa, New lay-out, Katsina, Nigeria. They were identified, and voucher specimen was deposited at the Herbarium, Department of Plant Biology, Bayero University Kano, Nigeria.Aqueous leaf extract of *F. polita* was prepared using the procedure of Hassan *et al*. (2007).

Phytochemical screening:
Phytochemical tests were carried out by using the standard methods (Velavan, 2015).

Lethal mean dose (LD_{50}) determination:
The limit test procedure described by Organisation for Economic Cooperation and Development (OECD) was adopted (OECD, 2001).Five rats, selected by random sampling, were used for the study.

The animals were fasted overnight, providing only water, after which the extract, was administered orally at a dose level of 5000mg/kg body weight to each rat at 48 hours interval. Each rat was kept under observation for the first 4 hour and within the period of the 48 hour. Behavioural changes, body weight, and mortality were observed for a period of 14 days.

Sub-chronic toxicity studies:
Twenty five (25) rats, divided into 5 groups of 5 rats each, were daily administered with different concentrations (based on LD_{50}) of the extract for 28 days. Control group received distilled water, while the other four groups received 1000, 2000, 3000, and 4000 mg/kg body weight of the extract. The rats were fasted overnight. On the 29th day, weight were taken, and the rats were humanely sacrificed. Blood samples were taken in EDTA containers and plane containers.

Haematological and Biochemical and Statistical analyses:
Haematological analyses were performed using automated Haematological Analyser. Spectrophotometric determination of biochemical parameters in the sera was performed using standard methods (Ukwuani *et al.*, 2012). Results were expressed as mean ± standard error. The data collected were subjected to one-way Analysis of Variance (ANOVA) using Graphad Instat.

RESULTS AND DISCUSSION

Table 1: Phytochemical Screening of the aqueous leaf extract of *F. polita*

Phytochemicals	**Inference**
Alkaloids	+
Polyphenols	+
Flavonoids	+
Flavonols	+
Glycosides	+
Cardiac Glycosides	+
Anthraquinones	+
Saponins	+
Tannins	+
Phlobatannins	-
Fats and Oils	+
Terpenes	+
Triterpenoids	+
Steroids	-
Phytosterols	-
Anthocyanins	-
Leucoanthocyanins	-
Emodins	-
Coumarins	-
Chalcones	-
Carbohydrates	+
Starch	+
Gums and Mucilages	+
Proteins	+

Key: (+) present (-) absent

Many of these natural products have been shown to demonstrate interesting biological and pharmacological activities and are used as chemotherapeutic agents or a starting point in the development of modern medicine. However, some plant extracts could be inherently dangerous, containing naturally occurring toxins, which may be cytotoxic or carcinogenic (Humphrey and McKenna, 1997).

The $LD_{50}$of the aqueous leaf extract of *F. polita* was estimated to be more than 5000 mg/kg. The dose produced no mortality after 72 hours of observation and up to 14 days period. It also had no adverse effects on the behavioural responses of the tested rats after 14 days of observation. Neither mortality nor weight loss occurred.

Table 2: Effect of aqueous leaf extract of *F. polita* on haematological parameters

Parameters	Control	Aqueous Leaf Extract Of *F. Polita* (mg/kg)			
		1000	2000	3000	4000
RBC ($X10^6$cell/mm^3)	7.20±0.31	7.01±0.54	6.94±0.30	6.90±0.22	6.83±0.14
Hemoglobin (g/dl)	12.12±1.12	11.00±0.20	11.12±0.34	12.55±0.32	11.68±0.57
PCV (%)	41.12±1.54	41.01±0.12	43.45±0.85	43.22±1.33	41.05±0.12
MCV(μM^3/redcell)	67.52±1.55	68.10±1.03	69.50±2.54	75.75±1.20*	68.93±2.03
MCH (pg/red cell)	17.03±1.10	17.02±0.40	17.50±0.63	18.06±0.32	17.33±0.53
MCHC (g/dl RBC)	25.21±1.10	25.30±0.14	24.80±0.55	24.53±0.21	24.25±1.01
WBC($X10^6$cells/mm^3)	4.70±1.11	5.30±0.22	7.50±0.64*	5.03±0.32	4.77±0.08
Neutrophils (%)	14.32±4.92	12.32±6.36	16.32±2.31	15.32±2.76	13.23±4.31
Lymphocytes (%)	78.55±6.10	80.44±3.43	81.75±4.76*	81.25±0.33*	79.00±1.80
Monocytes (%)	5.06±1.32	5.36±1.36	6.31±0.32	7.01±0.03	5.45±2.31
Platelet($X10^3$cells/mm^3)	561.50±84.63	642.75±42.02*	749.00±52.50*	562.04±20.93	561.75±19.91
PDW (%)	10.15±0.10	10.12±0.11	10.58±0.34	12.04±0.12	12.10±0.04

Values are mean ± SEM (n=5), *= Significant different (P<0.05), RBC= Red blood cells, PCV= Packed cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, WBC= White blood cells, PDW= Platelets distribution width.

The results from this study revealed that the aqueous leaf extract of *F. polita* did not alter red blood cells and its related indices. The non-toxic effect of the extract on the parameters (RBC, Hb, PCV, MCH, and MCHC) suggests that it neither modulate the incorporation of haemoglobin into red cells, nor alter the morphology and osmotic fragility of the red blood cells (Adebayo *et al.*, 2005). These observations also implied that the extract did not affect the oxygen-carrying capacity of the red cells. However, elevation of MCV (3000 mg/kg dose) may be associated with folic acid and/or vitamin B_{12} deficiency but not due to the extract.

The rise in WBC may be due to enhancement of white cell production, increase in its entrance into the blood, and a reduction in its removal from the circulation (Yakubu and Afolayan, 2009). The increased lymphocytes seen in the study demonstrated that the extract boosted the immune system of the affected rats.

The rise in platelets count seen, in the rats administered with 1000 and 2000 mg/kg doses,may not be associated with the extract.

Table 3: Effects of aqueous leaf extract of *F.polita* on biochemical parameters

Parameters	Control	AqueousLeaf Extract of*F.Polita* (mg/kg)			
		1000	2000	3000	40000
AST(u/l)	109.83±3.42	113.20±1.57	107.44±2.75	114.33±1.58	105.44±1.27
ALT(u/l)	45.38±0.77	45.65±0.66	47.34±1.10	47.36±1.23	47.32±2.57
Total Bilirubin(μmol/l)	1.12±0.09	1.02±0.21	0.63±0.42*	0.53±0.43*	0.78±3.48
Direct Bilirubin(μmol/l)	1.14±0.15	0.88±0.27	0.99±0.11	0.73±0.32*	0.98±0.08
Total Protein(g/dl)	8.11±0.14	8.19±0.14	7.71±0.26	8.63±0.29	8.21±0.11
Albumin(g/dl)	3.90±0.17	3.85±0.10	3.95±0.03	3.99±0.04	3.98±0.12
Creatinine(mg/dl)	1.27±0.06	0.94±0.06	1.01±0.05	0.96±0.05	0.95±0.06
Urea(mg/dl)	54.58±1.78	45.06±1.52*	53.05±1.98	53.99±1.83	53.98±1.09
Sodium(mEqL/L)	144.00±1.86	144.80±1.54	142.99±3.32	141.86±3.98	144.69±0.88
Potassium(mEqL/L)	7.43±0.46	6.65±0.32	8.32±0.51	6.79±0.72	6.83±0.38
Chloride(mEqL/L)	106.87±0.45	102.45±2.03	101.96±4.32	100.53±4.98	101.09±3.59

Values are mean ± SEM (n=5), * = Significant different (P<0.05), AST= Aspartate amino transferase, ALT= Alanine amino transferase.

Serum ALT and AST are always found to increase in liver cell damage and the greater the degree of the liver damage the higher the activities of both enzymes (Cheesbrough, 1991). The result (Table 3) showed no significant effects on ALT and AST at all treatment doses. This suggests that sub-chronic administration of aqueous leaf extract of *F. polita* has no hepatotoxic effects in rats.

Raised serum levels of direct and total bilirubin is an indication of an impaired hepatic excretion (Moyer and Balistreri, 2011). In the present study, the extract did not cause a significant increase, rather a significant decrease, in the total bilirubin (2000 and 3000 mg/kg doses) and direct bilirubin (3000 mg/kg dose). In the present study, the extract did not cause a significant change in total protein and albumin. The nontoxic effect of the extract at all the doses investigated on the renal function indices may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected. However, a significant decrease in urea (2000 mg/kg dose) could be as a result of water overload.

CONCLUSION

In view of the findings of this research, it may be apparent to suggest that the aqueous leaf extract of *F. polita* is rich in phytochemicals that may be responsible for the reported pharmacological activities of this plant. It is demonstrated that it will take more than 5000 mg/kg dose of aqueous leaf extract of *F. polita* to kill 50% of the tested albino rats. It may be considered that the aqueous leaf extract of *F. polita* is safe, at the tested sub-chronic doses, and well tolerated for the 28 days study period. The little or no toxicity observed in these studies may be connected to the fact that the secondary metabolites that my likely cause toxic effects may be absent from the extract, or if present, may be in a very negligible nontoxic levels.

REFERENCES

Adebayo, J.O., Adesokan, A.A., Olatunji, L.A., Buoro, D.O. and Soladoye, A.O. (2005). Effect of Ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Biokemistri*, **17**: 4-49.

Ayisi, N.K. and Nyadedzor, C. (2003). Comparative in vitro effects of AZT and extracts of *Ocimum gratissimum, Ficus polita, Clausena anisata, Alchornea cordifolia, and Chrysophyllum albidum seed*. *EwJ Herb Chem Pharmacol Res*., 1(1):**6**-12.

Cheesbrough, M. (1991). Medical Laboratory Manual for Tropical Countries. 2 ed. ELBS., 1: 508-511, 605.

Hassan, S.W., Ladan, M.J., Dogondaji, R.A., Umar, R.A., Bilbis, L.S., Hassan, L.G., Ebbo, A.A. andMatazu, I.K. (2007). Phytochemical and toxicological studies of aqueous leaves extract of *Erythrophleum africanum*. *Pakistan Journal of Biological Sciences*, **10(21)**: 3815-3821.

Humphrey, S.I. and McKenna, D.J. (1997). Herbs and breastfeeding. *Breastfeeding Abstracts*, **17**: 11-12.

Moyer, K.D. and Balistreri, W.F. (2011). Liver Disease Associated with Systemic Disorders. In: Robert, M.; Stanton, B.F.; St. Geme, J.W.; Schor, N.F.; Richard, E.N. Textbook of Pediatrics. Saunders. P. 1405.

OECD (2001). 423. Adopted by the council on 17 December. Organization for Economic Co-operation and Development (OECD) guideline for the testing of chemicals, Acute oral toxicity –Acute toxic class method.[Internet]Available from: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf

SANBI (2015). Observations in the Species: Ficuspolita subsp. Polita. Available at: http://www.ispotnature.org/speciesdictionaries/sanbi/Ficus%20polita%20subsp.%20polita. [Accessed 17.11.2015].

Ukwuani, A.N., Abubakar, M.G., Hassan, S.W. and Agaie, B.M. (2012). Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*. *International Journal of Pharmaceutical Sciences and Drug Research*, **4(4)**: 245-249.

Velavan, S. (2015). Phytochemical techniques. A review. *World Journal of Science and Research*, **1(2)**: 80-91.

Yakubu, M.T. and Afolayan, A.J. (2009). Effect of aqueous extract of *Bulbinenata lensis* Baker stem on haematological and serum lipid profile of male Wistar rats. *Indian J Exp Biol.*, **47**: 283-288.