

PHYTOCHEMICAL SCREENING AND BIOCHEMICAL EVALUATION OF *Ficus* platyphylla STEM BARK EXTRACT OF NUPE LAND ORIGIN IN ALBINO RATS

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ABSTRACT

Extracts of Ficus platyphylla (Moraceae) have been used by medicinal herbalist in Nigerian for the treatment of pain, psychosis, epilepsy, depression, and inflammation for decades. Phytochemical screening and biochemical evaluation of F. platyphylla stem bark extract of Nupe land origin in albino rats were carried out to ascertain its safety and complement earlier efficacy studies on this widely used medicinal plant. Extraction of 80 g of the grand *F. platyphylla* stem bark was performed in 500 mL of cold acetone and the filtrate was evaporated in a rotary evaporator, the paste collected was reconstituted separately in distilled water to give the required doses of 1000, 3000 and 5000 mg/kg as working concentrations. The healthy rats (n=6 per group) were treated orally with three graded doses of F. platyphylla stem bark extracts, separately, for 28 days. Phytochemical screening, lipid parameters, liver enzyme activities, antioxidant enzyme, and hematological indices were examined following 28 days of daily oral administration of graded doses of the extract in rats. Administration of the three graded doses of extracts in healthy rats demonstrated high potentials against hyperglycemia, atherogenicity and oxidative stress in rats upon 28th days of treatments. The atherogenicity and coronary risk indices were drastically decreased, which is in support of the antihyperglycemic and antiatherogenic effects in treated rats. These findings revealed that all doses of F. platyphylla stem bark extracts have exhibited high protective mechanisms against hyperlipidemia, cardiovascular disorder and oxidative stress in healthy rats. Therefore, the doses of extracts used showed a toxic free mechanism and may be a good promising in developing food supplements targeting main complications associated with various human disorders.

Keywords: antioxidants enzymes, atherogenicity, *Ficus platyphylla*, hematology, lipid indices, liver enzymes, rats.

INTRODUCTION

About 80 % of people in developing countries depend relatively on traditional medicines to manage their health problems (Ajoku *et al*; 2004). Recently, interest has increased in plant value and traditional medicines in drug development and their healthy lifestyles (Aniagu *et al.*, 2004; Mustapha et al., 2012). Successful treatments of various diseases using different medicinal plants



with no proper scientific evidence have been established by traditional herbalists. Hence, proper evidence with well-established claims of these medicinal plants and their pharmacological and toxicological properties should be ascertained to confirm their biological potential and safety. Human chronic diseases are associated with deleterious damage, dysfunction of various organs and oxidative stress are suggested to be their main contributory factors leading to cardiovascular diseases (Muruga and Pari, 2006; Anoja *et al.*, 2018). A lot of medicinal plant extracts have been used widely, in Ayurveda medicine in Nupe lands against vascular complications (Ediriweera and Ratnasooriya, 2009; Anoja *et al.*, 2018). However, most of these plant extracts have not been properly scrutinized in appropriate model. Hence, the assessment of their efficacy against vascular disorders in animal models is of urgent importance.

F. platyphylla belongS to the family Moraceae. It is a deciduous plant locally identified by Hausas and Nupes as "Gamji and Gbagun", respectively, and widely distributed throughout the savannah region of West African coast (Chindo et al; 2003). Preparations of the plant have been used in the Nigerian traditional medicine for the treatment of insomnia, epilepsy, psychosis, depression, pain, inflammation and central nervous disorders (Audu, 1989, Chindo et al., 2003). F. platyphylla aqueous stem or root bark extract, powder/decoction is orally taken, the powder is sometimes taken with food, or placed in burning charcoal and inhaled to enhance fertility (Audu, 1989). Chindo et al. (2008) his reported that the plant contains certain biologically active compounds that have antinociceptive, anti-inflammatory, and gastrointestinal effects in rodents. Mustapha et al. (2012) have reported that crude doses of F. platyphylla stem bark methanol extract administered orally for 28 days are safe in rats. Preliminary phytochemical tests of the plant part methanol extract showed the presence of some bioactive compounds (Amos et al., 2001). The selection of plant part was based on the frequent uses of it in Ayurveda preparations in the management of cardiovascular diseases. Due to the different utilizations, well established and proven effectiveness of F. platyphylla parts extract in Nigerian traditional medicine and lack of optimum effective doses in the management of human diseases has necessitated this study. Hence, the study is aimed at evaluating the phytochemical components and effects of different



concentrations of acetone bark extract of *F. platyphylla* on some selected biochemical parameters in rats.

MATERIALS AND METHODS

Chemicals and Assay Kits.

Acetone was purchased from Sigma-Aldrich Company (st. Louise, MO, United State). Albumin, alkaline phosphatase (ALP), alanine and aspartate aminotransferase (ALT and AST), antioxidant enzyme assay kits were obtained from Roche Diagnostic GmbH, Mannhein, Germany.

Sample Collection

The stem bark of *F. platyphylla* was collected in March 2016 from Lapai in Lapai Local Government Area of Niger State, Nigeria. The botanical identification and authentication of the selected plant wre confirmed by Researchers in Biology Department, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, Lapai. The debris on the stem bark washed away using cleaned water and air dried at room temperature. Dried samples were pounded into a fine powder using mortar and pestle and stored in a labeled airtight bottle.

Preparation of Crude Extract

Briefly, exactly 80 g of *F. platyphylla* stem bark powder in one (1) litre capacity conical flask containing 500 ml of cold acetone (Sigma-Aldrich, Europe) was prepared, kept in a cupboard on a Shaker to ensure intermittent shaking for five days. The mixture was filtered using a muslin cloth and the filtrate was evaporated in a rotary evaporator to give a yield of 12.47 g, the paste was poured into the beaker and placed in a water bath to ensure complete evaporation of the solvent. This was reconstituted separately in distilled water to give the required doses of 1000, 3000 and 5000 mg/kg of the extract used in this study.

Experimental animals

Apparently, healthy twenty four (24) adult male rats of Wister strain weighing between 125 and 160 g body weight were obtained from the Animal House of the Biochemistry Department, Federal University of Technology, Minna, Niger State. They were housed in clean metabolic



cages placed in well-ventilated house conditions (temperature $23 \pm 1^{\circ}$ C; photoperiod: 12:12 h L:D: Relative humidity: 45–50%), and also allowed free access to Balanced Trusty Chunks and distilled water freed of contaminants before and during the experiment. The rats were acclimatized after randomized into different groups for seven (7) days in standard environmental conditions before the experiments. The methods used were by following that of the Organization for Economic Development (OECD) guidelines on good laboratory practice (OECD).

Experimental Animal group design

The rats were grouped into four with six rats per group, and orally administered as follows: Group1: Healthy control rats administered with distilled water for 28 days Group2: Healthy rats treated with 1000 mg/kg of the extract for 28 days Group3: Healthy rats treated with 3000 mg/kg of the extract for 28 days Group4: Healthy rats treated with 5000 mg/kg of the extract for 28 days The animals were anesthetized and sacrificed after the 28th day of treatments and blood samples were collected by cardiac puncture. The collected blood from each group was pulled, dispensed in plain bottles and allowed to clot and centrifuged at 3500 rpm for 10 min. The sera were separated, stored at -4 °C and used for evaluation of biochemical indices.

METHODS

Phytochemical analysis: The qualitative analysis of the plant constituents was assessed by the methods described by Trease and Evans, (1999), El-Olemmy *et al.* (1994) and Harbone (1993). The tests were carried out to determine out the presence of active chemical constituents which include; Saponins, tannins, flavonoids, and glycosides.

Lipid parameters: The serum total cholesterol (TC), high density lipoprotein (HDL) and triglyceride (TG) concentrations in rats were analyzed using spectrophotometric enzyme assay kits (Bergmeyer *et al.*, 1978; Bowers and McComb, 1966). The serum Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) concentrations were calculated following the Friedewald formulae (Friedwald et al., 1972). The cardioprotective index (CPI), atherogenic (AI) and coronary risk indices (CRI) were calculated using the formulae;



CPI = HDL/LDL; AI = TC- HDL/HDL and CRI = TC/HDL.

Liver and antioxidant Markers: Liver enzyme activities, alanine aminotransferase (ALT, aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were analyzed using spectrophotometric enzyme assay kits (Bergmeyer *et al.*, 1978, Bowers and McComb, 1966). The serum activities of reducing glutathione (GSH), glutathione reductase (GR), glutathione peroxide (GPx), and glutathione S-transferase (GST) were estimated using reported procedures (Sedlak and Lindsay, 1968; Habig *et al.*, 1974). Also, the total protein and lipid peroxidation concentrations were analyzed by malondialdehyde (MDA) formation following Lowry and thiobarbituric acid methods, respectively (Ohkawa *et al.*, 1979; Lory *et al.*, 1951).

Hematological indices: EDTA-added whole blood samples were used for hematological examination. Hematological parameters like the number of red blood cell (RBC), white blood cell (WBC), lymphocytes (LYM), platelets (PLT), hemoglobin (Hb) concentration and hematocrit (Ht) values were determined by standard methods using an automated hematology analyzer (Abacus 360, Japan).

Statistical analysis: Data were presented as mean±SEM. Analysis variance (ANOVA) was used to analyze the data using Dunnett's multiple comparison test. Data were considered significant at $p \square 0.05$.

RESULTS

The results of phytochemical studies of *F. platyphylla* stem bark are presented in Table1. It shows the presence of Saponins, flavonoids, tannins, volatile oils and phenols. However, the presence of steroids was not detected (Table 1).



Constituent	Qualitative					
	analysis					
Saponins	+					
Flavonoids	+					
Tannins	+					
Volatile	+					
oils						
Glycosides	+					
Steroids	-					
Phenols	+					
	+ = present, - = absent					

Table 1. The phytochemical analyses of acetone extract of *F. platyphylla* stem bark

The results of lipid, antioxidant and hematological indices in all studied rats are presented in Table 2 and 3 and 4, respectively. As presented in Table 2, all rats treated with 1000, 3000 and 5000 mg/kg of extracts displayed a significant reduction (p<0.05) in serum concentrations of TC (3.2, 3.4 and 3.1 mmol/L), TG (1.1, 0.8 and 1.2 mmol/L), VLDL (0.5, 0.7 and 1.1 mmol/L), AI (0.1, 0.03 and -0.1), and CRI (1.1, 1.0 and 1.9), and significant elevation in HDL (3.0, 3.3 and 3.5 mmol/L) and calculated CPI (1.4, 1.5 and 1.8), respectively, when compared to HDL (1.3 mmol/L) and CPI (1.2) of untreated rats after 28 days of treatment (Table 2).

Parameter	TC	HDL	TG	LDL	VLDL	AI	CRI	CPI
s tested	(mmol/	(mmol/	(mmol/	(mmol/	(mmol/			
	L	L	L	L	L			
Control rats 1000	3.7±0.2	1.3±0.1	1.3±0.1	1.1±0.1	2.3±0.1	1.8±0.3	2.8±0. 2	1.2±0. 4
mg/kg treated rats 3000	3.2±0.3	3.0±0.1*	$1.1 \pm 0.1^{*}$	2.1±0.5*	0.5±0.3*	$0.1 \pm 0.2^{*}$	1.1±0. 5*	1.4±0. 2*
mg/kg treated rats 5000	3.4±0.2	3.3±0.1*	0.8±0.3*	2.2±0.3*	0.7±0.1*	0.03±0.1*	1.0±0. 1*	1.5±02 *
mg/kg treated rats	3.1±0.1 *	3.5±0.2*	1.2±0.4*	2.0±0.2*	1.1±0.2*	- 0.1.3±0.3*	1.9±0. 2*	1.8±0. 4*

Table 2: Effect of plant extract on lipid indices in rats after 28 days of treatment



TC: total cholesterol, HDL: high density lipoprotein, TG: triglyceride, LDL: low density lipoprotein, AI: atherogenic index, CRI: coronary risk index, CPI: Cardioprotective index. The data are presented in mean \pm SEM (n = 6/group) and * means statistically significant from healthy control rats at p<0.05 by ANOVA and Dunnett's test.

In addition, a general reduction of liver enzymes was experienced in all rats treated with 1000, 3000 and 5000 mg/kg of extracts when compared to those of untreated rats (Table 3). Furthermore, a significant increase in serum concentrations of GSH (761. 2, 810.1and 825.3 μ g/g), GR (8.8, 10.5 and 10.7 nmol/min/mg), GPx (11.5, 12.1 and 12.6 nmol/min/mg), and GST (10.5, 10.8, and 11.2 nmol/min/mg) were observed in rats treated with 1000, 3000 and 5000 mg/kg, respectively (p<0.05) when compared to the untreated rats after 28 days of study (Table 3). In contrast, MDA concentrations in rats treated with all levels of extract were decreased significantly (p<0.05) when compared to untreated rats (Table 3).

Paramete	ALT	AST	ALP	GSH	GR	GPx	GST	MDA
rs tested	(U/L)	(U/L)	(U/L)	$(\mu g/g)$	(nmol/min	(nmol/min	(nmol/min	(nmol/mi
					/mg of	/mg of	/mg of	n/mg of
					protein)	protein)	protein)	protein)
Control	$12.3\pm$	$40.1\pm$	$58.3\pm$	711.7±	8.5 ± 0.6	10.5 ± 0.5	9.6 ± 0.7	10.8 ± 0.3
Rats	0.1	0.2	0.5	0.4				
1000								
mg/kg	10.6±	$30.2\pm$	$48.5\pm$	761.2±	$8.8{\pm}0.7^*$	$11.5 \pm 0.2^{*}$	$10.5 \pm 0.3^{*}$	$9.5{\pm}0.5^{*}$
treated	0.2^{*}	0.3^{*}	0.2^{*}	0.5^{*}				
rats								
3000								
mg/kg	11.2±	$29.8\pm$	$50.2\pm$	$810.1\pm$	$10.5{\pm}0.2^{*}$	$12.1\pm0.2^{*}$	$10.8{\pm}0.5^*$	$8.6{\pm}0.7^*$
treated	0.5^*	0.7^{*}	0.3^{*}	0.7^{*}				
rats								
3000								
mg/kg	9.8±0.	33.0±	$51.0\pm$	$825.3\pm$	$10.7{\pm}0.3^{*}$	$12.6 \pm 0.3^*$	$11.2 \pm 0.3^{*}$	$10.2{\pm}0.5^{*}$
treated	6^*	0.6^{*}	0.5^{*}	0.5^{*}				
rats								

Table 3: Effect of plant extract on antioxidant parameters in rats after 28 days of treatment

ALT: alkaline aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GSH: reduce glutathione, GR: glutathione reductase, GPx: glutathione peroxidase, GST:



glutathione S-transferase, MDA: malondialdehyde. The data are presented in mean \pm SEM (n = 6/group) and * means statistically significant from healthy control rats at p<0.05 by ANOVA and Dunnett's test.

Subsequently, significant elevation of all hematological indices was observed in all extract treated rats (p<0.05) when compared to untreated rats (Table 4)

Table 4. Effect of plant extract on hematological parameters in fats after 28 days of freatment								
Parameters	RBC	WBC	LYM	PLT	Hb (g/dl)	Ht (PVC		
tested	$(10^{12}/L)$	$(10^{9}/L)$	$(10^{9}/L)$	$(10^{9}/L)$		%)		
Control	6.1±0.2	3.4±0.2	1.8 ± 0.4	92.1±0.3	10.5±0.5	35.1±0.6		
rats								
1000								
mg/kg	$9.2{\pm}0.1^{*}$	$3.9{\pm}0.3^{*}$	$2.1{\pm}0.2^{*}$	$411.1 \pm 0.5^{*}$	$16.5 \pm 0.2^{*}$	$55.1 \pm 0.3^*$		
treated rats								
3000								
mg/kg	$8.3{\pm}0.5^{*}$	$3.8{\pm}0.5^{*}$	$2.1{\pm}0.1^{*}$	$292.3 \pm 0.6^*$	$15.3 \pm 0.2^{*}$	$52.3 \pm 0.5^*$		
treated rats								
5000								
mg/kg	$6.8{\pm}0.3^{*}$	$3.5{\pm}0.2^{*}$	$1.8{\pm}0.1^{*}$	$152.3 \pm 01^{*}$	$10.9{\pm}0.5^{*}$	$29.2{\pm}0.3^{*}$		
treated rats								

Table 4: Effect of plant extract on hematological parameters in rats after 28 days of treatment

RBC: red blood cells, WBC: white blood cells, LYM: lymphocyte, PLT: platelets, Hb: hemoglobin, Ht: hematocrit. The data are presented in mean \pm SEM (n = 6/group) and * means statistically significant from healthy control rats at p < 0.05 by ANOVA and Dunnett's test.

DISCUSSION

The medicinal property or effect of various plant extracts in the treatment and management of various ailments is ascribed to their bioactive substances (Babalola, 1993). Different portion extract of *F. platyphylla* could be used to manage diseases such as Anaemia, gonorrhoea, dysentery, jaundice and liver disorder (Babalola, 1993; James *et al.*, 2010). The presence of these Phytochemicals in *F. platyphylla* can be attributed to its various medicinal properties as it is being used traditionally. The Phytochemicals detected in *F. platyphylla* stem bark extracts signify that it contains bioactive chemical compounds such as Flavonoids, Alkaloids, phenols, tannins, Saponins, and glycosides etc. These Phytochemicals were reported severally for their



medicinal values on ailments such as diabetes, microbial diseases, atherosclerosis, cancer and inflammatory diseases (Chukwuka *et al.*, 2011).

A significant reduction in the serum concentrations of TC, TG, LDL, VLDL and increased concentration of HDL recorded in all extract treated rats as compared to untreated rats may signify extract's high protective effect against hypercholesterolemia and hypertriglyceridemia at all doses of extract in rats. This could be due to rate-limiting enzyme HMG-CoA reductase of cholesterol biosynthesis inhibition and activation of lipolysis by reducing the activity of hormone sensitive lipase, respectively. High levels of LDL may risk the formation of atherosclerotic plaques (Adaramoye and Akanni, 2014; Anoja et al., 2017). Higher levels of HDL associated with a high risk of cardiovascular disorders have been established by epidemiological and clinical studies (Tan, 2016; Anoja et al., 2018). High levels of HDL demonstrated in all extract treatments indicated that all doses of extracts exhibit a protective role against cardiovascular disorders in counteracting LDL oxidation, leading to inhibition of cholesterol transport pathway by preventing the formation of modified LDL oxidative particle (Yokozawa et al., 2006); Anoja et al., 2018). Furthermore, low levels of calculating indices like CRI and AI in all doses of extract treated rats when compared to untreated rats could be a demonstration of their high protective potency against hyperlipidaemia. In contrast, increased levels of CPI in terms of the HDL/LDL ratio in all extract treated rats may further strengthen the potency of the plant extracts against atherogenicity. In clinical trials and animal models, relationships between lipid peroxidation and hypercholesterolemia have been established (Perry et al., 2014; Samarghandian et al., 2013). In agreement with foregoing references, this study demonstrates low levels of serum concentrations of MDA in all doses of extract treated rats when compared with that of untreated rats, which may indicate extract's potency in hepatoprotection. In addition, high serum concentrations of GR, GPx, and GST were observed in all rats treated with different doses of extracts as compared to untreated rats. This observation could be due to the extract's protective effect against oxidative mechanisms. The antioxidant preservative mechanism signifies a system protective effect against chronic diseases (Ndatsu and Umaru, 2016). The presence of bioactive



components (phytochemicals) as stated earlier in this study may also contribute to antioxidant mechanism protection by all doses of medicinal plant extracts.

CONCLUSION

This study reveals that the bark extracts of *F. platyphylla* are rich in phytochemicals and all doses of extracts could possess a protective potency against various human disorders. Therefore, all doses of extract in this study are toxic free and may be an excellent promising in the management of diseases as acclaimed by traditional herbalists.

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