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

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ABSTRACT
Preparations of *Ficus platyphylla* (Moraceae) have been used in the Nigerian traditional medicine for the management of epilepsy, psychosis, depression, pain and inflammation for many years. Phytochemical screening and biochemical evaluation of *Ficus platyphylla* stem bark extract of Nupe land origin in albino rats was carried out to ascertain its safety and complement earlier efficacy studies on this widely used medicinal plant. The healthy rats (n=6 per group) were treated orally with three graded doses of *F. platyphylla* stem bark extracts, separately, for 28days. Phytochemical screening, lipid parameters, liver enzyme activities, antioxidant enzyme and hematological indices were examined following 28days 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 treatment 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 human various disorders.

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

INTRODUCTION

In developing countries, about 80% of its people depend solidly on traditional medicines to manage their health problems. Recently, in developed countries a lot of interest has increased for plant value and traditional medicines in drug development and their healthy lifestyles (Ajoku *et al*., 2004; Watkins *et al.*, 2006; Ben A. *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 evidences with well-established claims of these medicinal plants on their pharmacological and toxicological properties to ascertain their biological potential and safety is indeed necessary.

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 plants 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, its efficacy assessment against vascular disorders in animal model is of urgently important.

Ficus platyphylla is belonging to a 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 the West African coast. 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 are orally taken, the powder is sometimes taken with food, or placed in burning charcoal and inhaled to enhance fertility (Audu, 1989, Chinenye *et al*., 2011). Chindo *et al*. (2008) have reported that the plant contain certain biological active compounds that have antinociceptive, anti-inflammatory, and gastro-intestinal effects in rodents. Ben *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 the Nigerian traditional medicine and lack of optimum effective doses in the management of human diseases, this study is aimed to evaluate the phytochemical components and effects of different concentrations (1000, 3000 and

5000 mg/kg) 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

Stem bark as selected part 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 was confirmed in the Biological Department, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, Lapai. The debris on the stem bark was removed and air dried at room temperature. Dried samples were pounded in to fine powder using mortar and pestle and stored in a labeled air-tight container for future use.

Preparation of Crude Extract

The extractant used was acetone due to its selective property of extracting tannin and flavonoid (Iyambo, 1991). Eight (80) grams of *Ficus 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 with intermittent shaking for five days. The mixture was filtered using muslin cloth and the filtrate was evaporated in a rotary evaporator to give a yield of 12.47g. the paste was poured in to beaker and placed in a water bath for 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 160g 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 ±1°C; photoperiod: 12h natural light and 12h dark: humidity: 45–50%), and also allowed free access to Balanced Trusty Chunks and tap water freed of contaminants before and during the experiment. The rats were acclimatized after randomized in to different groups for a period of seven (7) days in standard environmental conditions before the experiments. The protocols used were in accordance with that of Organization for Economic Development (OECD) guidelines on good laboratory practice (OECD).

Experimental Animal group design

The rats were grouped in to 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 extract for 28 days

Group3: Healthy rats treated with 3000 mg/kg of extract for 28 days

Group 4: Healthy rats treated with 5000 mg/kg of extract for 28 days

The animals were anaesthetized and sacrificed after the 28^{th} day of treatments and blood were collected by cardiac puncture. The collected blood from each group were pulled, dispensed in to plain bottles and allowed to clot and centrifuged at 3500 rpm for 10 min. The sera were separated, stored at $-4^{o}C$ 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 find out the presence of the active chemical constituents that included; 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 spectro-photometric enzyme assay kits (Bergmeyer *et al*., 1978; Boners 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). Cardioprotective indix (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 enzymes activities, alanine aminotransferase (ALT, aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were analyzed using spectrophoto-metric enzyme assay kits (Bergmeyer *et al*., Bowers Jr and McComb, 1966). The serum activities of reduce 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 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 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 comparisons test. Data were considered significant at $p<0.05$.

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 present of steroids were not detected (Table 1).

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

Constituent	Qualitative analysis
Saponins	+
Flavonoids	+
Tannins	+
Volatile oils	+
Glycosides	+
Steroids	-
Phenols	+

+ = present, - = absent

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 3.1mmol/L), TG (1.1, 0.8 and 1.2 mmol/L), VLDL (0.5, 0.7 and 1.1mmol/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).

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

Parameters tested	TC (mmol/L	HDL (mmol/L	TG (mmol/L	LDL (mmol/L	VLDL (mmol/L	AI	CRI	CPI
Control rats	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
1000 mg/kg treated rats	3.2±0.3*	3.0±0.1*	1.1±0.1*	2.1±0.5*	0.5±0.3*	0.1±0.2*	1.1±0.5*	1.4±0.2*
3000 mg/kg treated rats	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*
5000 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*

*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 ± SEM (n = 6/group) and * means statistically significant from healthy control rats at p<0.05 by ANOVA and Dunnett's test.*

In addition, general reduction of liver enzymes were 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 µg/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).

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

Parameters tested	ALT (U/L)	AST (U/L)	ALP (U/L)	GSH (µg/g)	GR (nmol/min/ mg of protein)	GPx (nmol /min/mg of protein)	GST (nmol/min/mg of protein)	MDA (nmol/ min/mg of protein)
Control rats	12.3±0.1	40.1±0.2	58.3±0.5	711.7±0.4	8.5±0.6	10.5±0.5	9.6±0.7	10.8±0.3
1000 mg/kg treated rats	10.6±0.2*	30.2±0.3*	48.5±0.2*	761.2±0.5*	8.8±0.7*	11.5±0.2*	10.5±0.3*	9.5±0.5*
3000 mg/kg treated rats	11.2±0.5*	29.8±0.7*	50.2±0.3*	810.1±0.7*	10.5±0.2*	12.1±0.2*	10.8±0.5*	8.6±0.7*
3000 mg/kg treated rats	9.8±0.6*	33.0±0.6*	51.0±0.5*	825.3±0.5*	10.7±0.3*	12.6±0.3*	11.2±0.3*	10.2±0.5*

*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 ± 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 were 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 rats after 28 days of treatment

Parameters tested	RBC (10^{12}/L)	WBC (10^9/L)	LYM (10^9/L	PLT (10^9/L)	Hb (g/dl)	Ht (PVC %)
Control rats	6.1±0.2	3.4±0.2	1.8±0.4	92.1±0.3	10.5±0.5	35.1±0.6
1000 mg/kg treated rats	9.2±0.1*	3.9±0.3*	2.1±0.2*	411.1±0.5*	16.5±0.2*	55.1±0.3*
3000 mg/kg treated rats	8.3±0.5*	3.8±0.5*	2.1±0.1*	292.3±0.6*	15.3±0.2*	52.3±0.5*
5000 mg/kg treated rats	6.8±0.3*	3.5±0.2*	1.8±0.1*	152.3±01*	10.9±0.5*	29.2±0.3*

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

DISCUSSION

This study was conducted evaluate the phytochemical components in acetone bark extract of *F. platyphylla* and its effects at different dose concentrations on some selected biochemical indices in rats. The medicinal property or effect of various plants extracts in the treatment and managements of various ailments is ascribed to their bioactive substances. 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 signified 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 significantly 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 risks the formation of atherosclerotic plaques (Adaramoye and Akanni, 2014; Anoja *et al*., 2017). The high levels of HDL associated with high risk of cardiovascular disorders have been established by epidemiological and clinical studies (Tan, 2016; Anoja *et al*., 2018 and paper). High levels of HDL demonstrated in all extract treatments indicated that all doses of extracts exhibit a protective role against cardiovascular disorders by 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). More again, low levels of calculated 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 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 reported literature, 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 extracts 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.

CONCLUSSION

This study reveals that the bark extracts of *F. platyphylla* are rich phytochemicals and all doses extracts may 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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