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Effect of Aqueous Leaf Extract of *Ficus asperifolia* on Cardiac Enzymes and Lipid Profile in Male Albino Rats

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The effect of oral administration of aqueous leaf extract of *Ficus asperifolia* on cardiac enzymes and lipid profile in albino rats was checked in this study. *Ficus asperifolia* is a highly medicinal plant that has been used in folk medicine for the treatment of ailments ranging from wound healing to diabetes. This plant is used without the knowledge of its toxic potentials. This research therefore checks the cardiotoxicity of this plant. Sixty male albino rats were divided into 4 groups. The first group was administered with distilled water while the second, third and fourth groups were treated with 400, 800 and 1200 mg kg^{-1} b.wt. doses of the extract, respectively. The rats were sacrificed 24 h after treatment for 1, 7 and 14 days. Their serum was obtained and used for the analysis of the concentrations of Total cholesterol, Triglyceride, Low Density Lipoprotein, High Density Lipoprotein and Creatine Kinase activity. Alanine Aminotrasferase and Aspartate Aminotransferase activities were also assayed in the serum and heart homogenate. The result obtained revealed a significant decrease ($p<0.05$) in the concentrations of serum total cholesterol, triglyceride and low density lipoprotein while high density lipoprotein significantly increased ($p<0.05$). The activities of serum Creatine Kinase, Aspartate Aminotransferase and Alanine Aminotransferase increased significantly ($p<0.05$) while a concomitant significant increase ($p<0.05$) was observed in heart Aspartate Aminotransferase and Alanine Aminotrasferase activities. The available results suggest that *Ficus asperifolia* leaf extract possesses hypolipidemic properties but may be detrimental to heart cells at the doses tested.

Key words: Enzymes, *Ficus asperifolia*, cardiac, lipid, aminotransferase

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INTRODUCTION

Medicinal plants have been used in the management and treatment of various diseases (Yakubu *et al*., 2007). Their healing potentials have been attributed to the presence of phytochemicals found in them. A very high percentage of the estimated 250,000-500,000 plant species on earth are used for medicinal purposes while merely 1-10% are used as food by humans and other species (Jethro, 1999). According to a World Health Organization report, around 80% of the world's population use herbal medicine (Ghasemi, 2002). Since, a large percentage of the world population makes use of medicinal plants, it is therefore of paramount necessity to provide useful scientific information on the constituents, therapeutic dosage and the toxicity of these plants which are often overlooked by local herbalists.

Ficus asperifolia (Sand paper tree) is widely distributed across Africa. Nkafamiya *et al.* (2010) have reported its presence in Senegal, Cameron, Sudan, Central and East Africa. It is also found in Toro Local Government Area in Bauchi State, Michika, Hong and Song Local Government Areas in Adamawa State and Omala Local Government Area in Kogi State all in Nigeria (Nkafamiya *et al*., 2010; Omoniwa and Luka, 2012). *Ficus asperifolia* is reported to be highly medicinal and has been employed as analgesic, anti-tumors, anti-cancer, diuretic, abortificients, ecobolics and menstral cycle pain reliever (Adjanohoun, 1996; Arbonnier, 2004). Previous work done by Omoniwa and Luka (2012) on the aqueous stem extract of *Ficus asperifolia* revealed that it possesses Hypoglycemic and hypolipidemic properties on diabetic rats but significantly raised serum transaminases activities. Nkafamiya *et al.* (2010) also published that the leaves of *Ficus asperifolia* has a higher protein, crude fibre and mineral contents than some Nigerian vegetables.

The aim of this study however is to check the effect of this plant on lipid profile parameters and some heart marker enzymes and as a consequence ascertain any possible cardiotoxic effects.

MATERIALS AND METHODS

Sixty male albino rats of average weight 230±25.59 g were obtained from the animal holding unit of the University of Jos, Jos, Nigeria. The rats were housed in standard cages and allowed to acclimatize to the laboratory condition for seven days before commencement of extract administration. They were also allowed access to tap water and rat chow *ad libitum*. *Ficus asperifolia* leaf was obtained from Icheke, Omala Local Government Area, Kogi State, North Central Nigeria and was properly identified at the Forestry Department of the Kogi State Ministry of Agriculture and Natural Resources, Lokoja. Assay kits for Creatine Kinase, Alanine Aminotransferase and Aspartate aminotransferase were products of Randox Laboratory Ltd., United Kingdom. All other reagents used were of analytical grade and were prepared in all glass distilled water.

Experimental design: Sixty albino rats were divided into 4 groups (i.e., A, B, C and D) with each group containing 15 rats. Each of the groups was further divided into 3 subgroups of 5 animals that were treated for 1, 7 and 14 days. Group A served as the control and was orally administered with distilled water. Groups B, C and D were the experimental groups that received orally 400, 800 and 1200 mg kg^{-1} b.wt. doses of the extract, respectively. The animals were sacrificed 24 h after extract administration for 1, 7 and 14 days.

Preparation of plant extract: *Ficus asperifolia* leaves were air-dried to constant weight. The leaves were then pulverized into fine powder using mortar and pestle. Hundred grams of the powder was percolated in 300 cm^3 of distilled water, stirred properly and kept in the refrigerator for 48 h for proper extraction. The mixture was thereafter filtered using Whatman No.1 filter paper and the filtrate concentrated on a water bath at 60°C. The resulting concentrate was then used for preparing the needed doses of the extract.

Serum and heart collection: Rats were anaesthetized in a jar containing cotton wool soaked in diethyl ether, they were then sacrificed by jugular puncture and their blood collected in an unheparinized bottle and allowed to stand for 10 min to clot. Serum was then collected using a Pasteur pipette. The rats were dissected, their hearts removed and wiped clean of blood. The hearts were immediately kept in ice-cold 0.25 M sucrose solution. They were then homogenized and used for the various analyses.

Enzyme and lipid profile analysis: Aspartate Aminotransferase (AST) (EC 2.6.1.1) and Alanine Aminotransferase (ALT) (EC 2.6.1.2) activities were assayed at 546 nm (Schmidt and Schmidt, 1963). Creatine Kinase (CK) (EC 2.7.3.2) activity was assayed using the method as described by Duncan *et al.* (1995). Serum Total Cholesterol, Triglycerides and High Density Lipoproteins were estimated by enzymatic colorimetric end point methods using Span diagnostic reagent kit. LDL was

calculated using the formula provided in cholesterol diagnostic kit booklet. All measurements were done using spectronic 21 digital Spectrophotometer (Bausch and Lomb, Rochester NY).

Statistical analysis: Data are expressed as mean (of 5 replicates)±SD. The obtained data were subjected to statistical analysis using the IBM® Statistical Package for Social Sciences (SPSS) Software Version 20. All significant differences were determined by one way Analysis of Variance (ANOVA) and Post Hoc multiple comparisons was done using Duncan's multiple range test. The significance level was set at $p<0.05$.

RESULTS

Qualitative phytochemical screening of aqueous leaf extract of *Ficus asperifolia* (Table 1) revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, terpenes, steroids, balsam and phenol.

The effect of oral administration of aqueous leaf extract of *Ficus asperifolia* on serum Total Cholesterol and Triglyceride concentrations are presented in Table 2. Experimental animals that were treated with the various doses of the extract gave a dose and day-dependent significant decrease ($p<0.05$) in serum Total cholesterol concentrations when compared to the control animals. Animals that received the 400, 800 and 1200 mg kg^{-1} b.wt. doses for 1 day showed Total Cholesterol concentrations of 110.20±0.21, 108.50±0.19 and 102±0.21 mg dL^{-1}, respectively, values that are significantly lower ($p<0.05$) than the control (116.20±0.03). Similar results were obtained for animals that received the extract for 7 and 21 days. The observed decrease was also day-dependent. Rats that received the 400 mg kg^{-1} b.wt. dose of the extract showed significant decrease ($p<0.05$) in Total Cholesterol concentration with increase in extract administration days (Day 1-110.20±0.21, Day 7-102.50±0.09 and Day 14-97.30±0.38 mg dL^{-1}). Similar results were obtained for rats treated with the 800 and 1200 mg kg^{-1} b.wt. doses of the extract. Animals that received the 800 and 1200 mg kg^{-1} b.wt. doses for 1 day showed a significant decrease in serum triglyceride concentration (108.46±1.27 and 105.46±1.06 mg dL^{-1}, respectively). Similar results were obtained for rats that were treated for 7 days with the 800 and 1200 mg kg^{-1} b.wt. doses (103.93±0.66 and 105.46± 1.06, respectively) while all the animals treated for 14 days showed significantly reduced triglyceride concentrations (i.e., 107.29±1.23, 102.58±0.99 and 100.27±0.03 mg dL^{-1}) when compared to the control animals (112.83±2.37 mg dL^{-1}).

Table 3 shows the effect of the extract on serum Low Density Lipoprotein and High Density Lipoprotein concentrations. There was a significant reduction ($p<0.05$) in serum Low Density Lipoprotein-Cholesterol concentration in all the animals treated with the various doses of the extract. Animals treated with the 400, 800 and 1200 mg kg^{-1} b.wt. doses for 1 day gave 47.80±0.04, 45.27±0.35 and 40.33±0.33 mg dL^{-1}, respectively, values significantly lower ($p<0.05$) than the control group (55.50±0.46 mg dL^{-1}). Similar results were observed in animals treated with the various doses for 7 and 14 days. On the other hand, Animals that received the 400, 800 and 1200 mg kg^{-1} doses for 1 day showed a significant increase ($p<0.05$) in High Density Lipoprotein-Cholesterol (70.40±0.46, 75.60±0.52 and 73.40±0.42 mg dL^{-1}, respectively) than the control animals (40.26±0.46 mg dL^{-1}). Similar results were obtained for experimental animals that were administered with the various doses of the extract for 7 and 14 days.

Table 1: Qualitative phytochemical screening of aqueous leaf extract of *Ficus asperifolia*

Phytochemicals	Status
Alkaloids	+
Saponins	+
Tannins	+
Cardiac glycosides	+
Terpenes	+
Steroids	+
Balsam	+
Phenol	+

+: Present

Table 2: Effect of aqueous leaf extract of *Ficus asperifolia* on serum total cholesterol and triglyceride concentration in male albino rats

Administration days	Control	400 (mg kg^{-1})	800 (mg kg^{-1})	1200 (mg kg^{-1})
Serum total cholesterol				
1	116.20±0.03^a	110.20±0.21^b	108.50±0.19^c	102.10±0.21^d
7	116.20±0.03^a	102.50±0.09^b	101.80±0.08^c	98.50±0.11^d
14	116.20±0.03^a	97.30±0.38^b	98.51±0.58^c	97.81±0.51^b
Serum Triglyceride				
1	112.83±2.37^a	112.46±1.37^a	108.46±1.27^b	105.46±1.06^c
7	112.83±2.37^a	113.63±1.06^a	103.93±0.66^b	105.46±1.06^b
14	112.83±2.37^a	107.29±1.23^b	102.58±0.99^c	100.27±0.03^d

Mean±SD (n = 5), Values carrying superscripts different from the control are significantly different at $p<0.05$. Concentrations are expressed in mg dL^{-1}

Table 3: Effect of aqueous leaf extract of *Ficus asperifolia* on serum low density lipoprotein and high density lipoprotein concentration in male albino rats

Administration days	Control	400 (mg kg^{-1})	800 (mg kg^{-1})	1200 (mg kg^{-1})
Serum low density lipoprotein-cholesterol				
1	55.60±0.46^a	47.80±0.04^b	45.27±0.35^c	40.33±0.33^d
7	55.60±0.46^a	47.10±0.72^b	43.50±0.31^c	42.51±0.15^d
14	55.60±0.46^a	36.00±0.46^b	40.40±0.42^c	38.90±0.51^d
Serum high density lipoprotein-cholesterol				
1	40.26±0.46^a	74.40±0.46^b	75.60±0.52^c	73.40±0.42^d
7	40.26±0.46^a	57.40±0.51^b	63.20±0.46^c	40.40±0.46^d
14	40.26±0.46^a	73.87±0.58^b	75.60±0.46^c	73.73±0.58^b

Mean±SD (n = 5), Values carrying superscripts different from the control are significantly different at $p<0.05$, Concentrations are expressed in mg dL^{-1}

Table 4: Effect of aqueous leaf extract of *Ficus asperifolia* on serum and heart alanine aminotransferase activities in male albino rats

Administration days	Control	400 (mg kg^{-1})	800 (mg kg^{-1})	1200 (mg kg^{-1})
Serum alanine aminotransferase				
1	22.00±1.15^{a}	398.00±0.15^{b}	111.00±1.15^{c}	127.00±0.66^{d}
7	22.00±1.15^{a}	121.00±1.15^{b}	125.66±2.60^{c}	138.66±1.20^{d}
14	22.00±1.15^{a}	138.66±0.88^{b}	162.00±1.15^{c}	155.00±1.15^{d}
Heart alanine aminotransferase				
1	32.9±0.11^{a}	35.80±0.11^{b}	44.60±0.11^{c}	57.30±0.10^{d}
7	32.9±0.11^{a}	39.20±0.11^{b}	65.30±1.12^{c}	89.90±1.13^{d}
14	32.9±0.11^{a}	36.35±1.33^{b}	39.35±0.01^{c}	35.86±0.08^{d}

Mean±SD (n = 5), Values carrying superscripts different from the control are significantly different at p<0.05. Enzyme activities are expressed in UI

Table 5: Effect of aqueous leaf extract of *Ficus asperifolia* on serum and heart aspartate aminotransferase activities in male albino rats

Administration days	Control	400 (mg kg^{-1})	800 (mg kg^{-1})	1200 (mg kg^{-1})
Serum aspartate aminotransferase				
1	32.00±0.03^{a}	141.00±0.15^{b}	167.00±1.15^{c}	178.00±1.15^{d}
7	32.00±0.03^{a}	163.00±1.15^{b}	180.00±1.15^{c}	176.00±1.15^{d}
14	32.00±0.03^{a}	187.00±1.15^{b}	192.00±1.15^{c}	193.30±0.66^{c}
Heart aspartate aminotransferase				
1	55.8±0.01^{a}	746.00±0.02^{b}	681.00±0.12^{c}	417.00±0.21^{d}
7	55.8±0.01^{a}	331.00±0.02^{b}	321.00±3.10^{c}	216.00±1.02^{d}
14	55.8±0.01^{a}	241.00±0.01^{b}	222.00±0.21^{c}	212.00±0.41^{d}

Mean±SD (n = 5), Values carrying superscripts different from the control are significantly different at p<0.05, Enzyme activities are expressed in UI

The effect of the extract on Serum and Heart Alanine Aminotransferase activity is shown in Table 4. Day 1 animals that received the 400, 800 and 1200 mg kg^{-1} b.wt. doses of the extract gave 398.00±0.15, 111.00±1.15 and 127.00±0.66 UI, respectively which are significantly higher (p<0.05) than the control group (22.00±1.15 UI). Similar results were obtained for rats that were treated for 7 and 14 days. Heart Alanine aminotransferase activities also increased significantly (p<0.05) for all the experimental animals in a dose-dependent manner. Day 1 animals administered with 400, 800 and 1200 mg kg^{-1} doses had significantly increased (p<0.05) Heart Alanine aminotransferase activities (i.e., 55.80±0.11, 44.60±0.11 and 57.30±0.10 UI, respectively) than the control group (32.90±0.11 UI). Similar results were obtained for the day 7 and 14 animals.

In Table 5, Serum Aspartate aminotransferase significantly increased after administration of 400, 800 and 1200 mg kg^{-1} b.wt. doses of the extract (141.00±0.15, 167.00±1.15 and 178.00±1.15 UI, respectively) when compared to the control group (32.00±0.03 UI). Similar results were obtained for the animals treated for 7 and 14 days with various doses of the extract. Result obtained for heart Aspartate aminotransferase was similar to what was observed in the serum. A drastic significant increase (p<0.05) aws observed for rats treated for 1 day with 400, 800 and 1200 mg kg^{-1} doses (746.00±0.02, 681.00±0.12 and 417.00±0.21 UI, respectively) when compared to the control group (55.80±0.01 UI). Similar results were obtained for animals treated for 7 and 14 days.

Table 6: Effect of aqueous leaf extract of *Ficus asperifolia* on serum creatine kinase activities in male albino rats

	Serum creatine kinase			
Administration days	Control	400 (mg kg^{-1})	800 (mg kg^{-1})	1200 (mg kg^{-1})
1	0.52±0.03^{a}	0.56±0.01^{b}	0.50±0.01^{a}	0.57±0.01^{b}
7	0.52±0.03^{a}	0.76±0.01^{b}	0.65±0.01^{c}	0.88±0.01^{d}
14	0.52±0.03^{a}	1.01±0.01^{b}	1.47±0.01^{c}	1.73±0.01^{d}

Mean±SD (n = 5), Values carrying superscripts different from the control are significantly different at p<0.05. Enzyme activities are expressed in UI

Table 6 represents the effect of oral administration of aqueous leaf extract of *Ficus asperifolia* on Serum Creatine Kinase in male albino rats. Experimental animals that received 400 and 800 mg kg^{-1} for 1 day showed a significant increase (p<0.05) in Creatine Kinase activity (0.56±0.01 and 0.57±0.01 UI, respectively) when compared to the control animals (0.52±0.03). All the day 7 and 21 animals showed a significant increase in Creatine Kinase activities when compared to the control animals.

DISCUSSION

Qualitative phytochemical screening of aqueous leaf extract of *Ficus asperifolia* revealed the presence of Alkaloids, Saponin, Tannin, Cardiac glycosides, Terpenes, Steroids, Balsam and Phenols. Plants possess therapeutic or toxic effects due to the presence of phytochemicals present in them. Phytochemicals like polyphenols, saponins, tannin, alkaloids and flavonoids have been linked to the lipid lowering effect observed in many plants (Dineshkumar *et al*., 2010; Owolabi *et al*., 2010; Price *et al*., 1987; Singh *et al*., 2011; Taoying *et al*., 2009).

Cardiovascular diseases like coronary heart diseases, stroke and hypertension represent some of the major health problems across the globe today (Owolabi *et al*., 2010). Elevated plasma lipids are risk factors in cardiovascular problems. A rise in blood Low Density Lipoprotein-Cholesterol, triacylglycerols and total cholesterol with a reduced High Density Lipoprotein-Cholesterol enhance the development of atherosclerosis and other related cerebrovascular disorders (Nwanjo, 2004). In this study, there was a significant decrease in serum concentrations of total cholesterol, triglyceride and Low density lipoprotein while the concentration of high density lipoprotein significantly increased on administration of the extract. This may be attributed to the hypolipidemic effect of phytochemicals like tannin, saponin, alkaloids which are present in the plant extract. The result obtained in this study agrees with those obtained in previous similar experiments (Dineshkumar *et al*., 2010; Owolabi *et al*., 2010; Price *et al*., 1987; Singh *et al*., 2011; Taoying *et al*., 2009).

Aspartate aminotransferase, Lactate dehydrogenase and Creatine kinase amongst other enzymes are present in the myocardium in abundant concentration. These enzymes are released into the extracellular fluid once metabolic damage to the myocardium occurs (Sharma *et al*., 2001). Serum creatine kinase activity is a more sensitive indicator in early stage of myocardial ischemia while peak rises in Lactate dehydrogenase is roughly proportional to the extent of the myocardial tissue (Chatterjea and Shinde, 2002). Assessment of serum levels of Aspartate aminotransferase, Lactate dehydrogenase and Creatine kinase could be used in the evaluation of the integrity of the cardiac apparatus in drug biotransformation and metabolism (Alnahdi, 2012). Alanine aminotransferase though a marker of non-alcoholic fatty liver disease has recently been shown to also be associated with endothelial dysfunction and carotid atherosclerosis (Schindhelm *et al*., 2007).

In this study, administration of the plant extract caused a significant increase in the serum and heart activity of Alanine aminotransferase and Aspartate aminotransferase. This result can be attributed to the ability of the extract to induce not only the overproduction of these enzymes beyond normal levels but also bring about permeability changes leading to the leakage of the enzymes into the serum (Yakubu and Omoniwa, 2012). It would have been expected that following the leakage, heart concentrations of the enzymes should reduce. This may mean that the rate of enzyme leakage is much slower than the rate of its induction in the heart (Yakubu *et al*., 2001). The administration of the extract also led to an increase in serum Creatine kinase activity. This results further confirms that the membrane of cardiac cells was compromised leading to leakage of the enzyme into the serum.

CONCLUSION

The available results are suggestive of the cardiotoxic effect of aqueous leaf extract of *Ficus asperifolia* at the dose tested. The fractionation of the phytochemicals of this plant should be researched into so that its medicinal benefits can be maximized.

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