Prosopis africana Seeds Extract Affects Lipid Profiles and Antioxidant levels in Serum and Brain Tissue of Wistar Rats

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Abstract

*Prosopis africana (PA)*is a deciduous plant widely used in Nigeria as food flavouring condiments, but its medicinal benefits has not been fully established. We investigated the major phytochemical constituents and evaluated the effect of aqueous extract of *PA* seeds on lipids and antioxidants in the brain and serum of male Wistar rats. Three groups of rats (n = 5/group) orally received as follows: first group distilled water, second group 500 mg/kg and third group 1000 mg/kg of the aqueous extract respectively for 25 days. The phytochemical result revealed that *PA* is highly rich in terpenoids, flavonoids, saponins, steroids and alkaloids. Administration of *PA* (500 mg/kg) decreased serum total cholesterol (TC) and low-density-lipoprotein cholesterol (LDL-C) but raised serum triglycerides(TG), very-low-density-lipoprotein (VLDL), cardiovascular risk ratio II (CRRII) and atherogenic index of plasma (AIP). Besides, *PA*(500 mg/kg)increased brain content of TC, LDL-C and CRRs while both doses of *PA* decreased the serum and brain malondialdehyde (MDA), with no remarkable changes in the serum and brain content of glutathione peroxidase (GPx) and catalase (CAT). Meanwhile, both doses of *PA* raised serum content of superoxide dismutase (SOD) and *PA*(1000 mg/kg) alone increased the brain reduced glutathione (GSH). Conclusively, these findings show that *PA* exhibited antioxidant and anti-lipidaemic activities.

Keywords: Antioxidants, Atherosclerosis, Dyslipidemia, Lipid Peroxidation, *Prosopis africana seeds*

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Introduction

Prosopis africana (*PA*; commonly known as African mesquite, iron tree, *gele*, and *ayan*) is a multipurpose tree of great economic value among the rural communities in guinea savannah of Nigeria.[1] It is a tropical leguminous tree that is readily distinguished by its dark, pale drooping foliage with small pointed leaflets. The seeds of *PA*are used in Nigeria to prepare *daddawa, kpaye* or *ukpeye*, a fermented products that is used as food condiment among most tribes in Nigeria.[2] Non-polar lipid substances like cholesterol and triglycerides need to be transported in soluble form in the plasma or carried with various lipoprotein particles. Five major classes of plasma lipoproteins are chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) and they are separated by hydrated density; electrophoretic mobility; molecular weight (size) and their relative content of cholesterol, triglycerides and protein.[3]

Cholesterol is widely distributed in all cells of the body but particularly in nervous tissue. It is a major constituent of the plasma membrane and lipoproteins. Phospholipids are ubiquitous molecules that are important to the structural integrity of cells and lipoproteins. When oxidized, however, they can promote inflammation via production of autocoids. Prostaglandins, are taken up by scavenger receptors on macrophages, and are recognized by the innate immune system.[4,5]The fluidity and flexibility of cell membrane is increased by the presence of unsaturated fatty acids, cholesterol which prevent close packing of the hydrophobic tails of phospholipids. ***Cholesterol*** molecules present in the lipid bilayer in an almost 1:1 ratio with phospholipids. Cholesterol molecules themselves are amphiphilic and have a kinked conformation, thus preventing too crowded packing of the phospholipid fatty acid tails while at the same time stodgy the gaps between the 'kinks' of the unsaturated fatty acid tails. Cholesterol molecules thus stabilize and regulate the fluidity of the phospholipid bilayer.[6]

Despite the progress in conventional chemistry and pharmacology in the production of effective drugs, plants might provide a useful source of new medicines and may be used to replace or complement existing

drugs. This study was carried out to evaluate effect of the aqueous extract of *PA* seeds on the lipid profiles and oxidative status in the sera and the brain homogenates of adult male Wistar rats.

Materials and methods

Animal care

Fifteen (15) male Wistar rats (180±20 g) were purchased from trusted commercial breeder (Tunde's farm)in Ibadan, Nigeria. They were housed in plastic cages, maintained under standard conditions (12 hours light/dark cycle, ambient temperature (20-25ºC) and 50-80% relative humidity). The animals were acclimatized in the animal house for 2 weeks before the commencement of the study. The rats were fed with standard rodent pelleted diet (containing 60 % cornflour, 20 % fish meal, 10 % wheat flour, 7 % oil seed cake, 2 % bone meal and 1 % salt for 1 kg) purchased fromAce Feeds, Ilorin, Nigeria and portable water was supplied*ad libitum*. All the animals were in good care according to the recommended criteria outlined in the "Guide for the Care and Use of laboratory Animals" prepared by the National Academy of Science and approved by the Ethical Research Committee of the University of Ilorin, Nigeria(UERC/ASN/2018/1152)

Preparation of the aqueous extract of *Prosopis africana seeds*

Prosopis africana was identified at the herbarium of Plant Biology University of Ilorin with the Voucher Number: UILH/001/472. The *Prosopis africana* pods were gathered from the vegetation within the University of Ilorin premises. The pods were broken up and seeds were harvested. Thereafter, the seeds were ground to powder (500 g). The powdered seeds were then soaked in 1000 ml distilled water for 72 hours and filtered. The filtered solution was then oven dried at a temperature of 60°C to 80°C for about 5 to 6 hours daily for 2 weeks. The extract obtained was in a paste form and it weighed 256.1 g. Distilled water was added to prepare10% solution. The solution was kept in a deep freezer to avoid fermentation and deterioration.

Preliminary Phytochemical Screening

A preliminary screening for secondary metabolites in the crude extract *Prosopis africana* seeds was evaluated. The test was carried out for alkaloids, tannins, flavaniods, terpenoids, phenols, saponins and steroids using the procedure described by Mendonça-Filho[7]

Experimental design

The rats were randomly divided into three groups (n = 5 per group). Group 1 was given 1 ml distilled water and serves as control; Groups 2 and 3 underwent 500 mg/kg body weight (bw) and1000 mg/kg bw of *Prosopis africana respectively*. All treatments were given via intragastric gavage, once daily for a period of 25 days.

Animal sacrifice and sample collection

At the end of the experiments, the animals were anesthetized with intramuscular ketamine of 20 mg/kg bw. Blood samples were collected by intracardial puncture into plain bottles, allowed to clot, and then centrifuged at 3000 rpm for 15 min to obtain serum. The brain tissues were excised, weighed and processed for homogenization with mechanical homogenizer in 30% chilled sucrose, centrifuged at 4000 rpm for 15 min to sediment nuclei and cell debris, then the resulting supernatant and the serum were used for biochemical parameters.

Biochemical assays

Assay for lipid profiles in the serum and brain homogenates was estimated by using reagent kits. Lipids were extracted from serum and brain homogenatesas described by Folch*etal*. (1957)[8]. After washing with 0.05 MKCl solution, aliquots of the chloroform-methanol extract were then used for the determination of total cholesterol (TC), triglycerides (TG) and HDL-C concentrations using Labkit®,a diagnostic kit reagents, as described by the manufacturer. While VLDL-C was estimated as one-fifth of TG and LDL-C was calculated using Friedward's formula:[LDL-C= TC – (HDL-C + VLDL) mg/dl].[9]

Estimated lipid indices/ratios were:
Cardiovascular risk ratio CRR: CRRI= TC/HDL-C ratio; CRRII= LDL-C/HDL-C ratio,
Atherogenic index of plasma (AIP): Log TG/HDL-C.[10]
Lipid peroxidation was evaluated by measuring thiobarbituric reactive oxygen species (TBARS) in form of malondialdehyde(MDA).[11] The activities of superoxide dismutase (SOD),glutathione peroxidase (GPx), reduced glutathione (GSH), and catalase (CAT)were done using spectrophotometer according to the manufacturers' instructions.[12-15]

Data Analysis

Data are represented as the mean ± standard error of mean (SEM). Comparisons between means were carried out using a One-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test using SPSS version 20.0. P-values less than 0.05 were considered statistically significant.

Results

Preliminary phytochemical screening

Qualitative tests for the presence of bioactive compounds in the crude extract of *Prosopis africana* seeds revealed the following secondary metabolites

Table 1. Qualitative phytochemical screening of *Prosopis africana* seeds crude extract

S/N	Phytochemicals	Test	Observations	Inference
1	Terpenoids	Salkowski	Formation Reddish brown precipitate	Present
2	Flavonoids	Alkaline reagent	Formation of yellow color	Present
3	Saponins	Froth	Formation of persistent foaming layer	Present
4	Steroids	Liebermann-Burchard	Appearance of green color	Present
5	Alkaloids	Dragendorff	Formation of creamy precipitate	Present
6	Anthraquinones	Borntrager	No dark brown precipitate	Absent

Table 2. Effects of *Prosopis africana*seeds aqueous extract on lipid profiles in the serum and brain of male Wistar rats

Parameters	Serum			Brain		
(mg/dl)	Control	PA 500 (mg/kg)	PA 1000 (mg/kg)	Control	PA 500 (mg/kg)	PA 1000 (mg/kg)
TG	80.56 ±1.05	132.39±10.15[a]	86.87±1.92	106.06±10.92	99.61±2.71	98.34±5.00
VLDL	16.11±0.21	26.48±2.03[a]	17.37±0.38	21.21±2.18	19.92±0.54	19.68±1.00
TC	245.63±14.09	218.13±10.03	208.59±6.48[a]	200.45±5.83	252.65±11.05[a]	177.75±15.08
HDL-C	28.12±0.30	27.85±0.66	26.59±0.56	24.63±0.47	25.75±0.28	24.30±0.43
LDL-C	201.40±13.73	163.81±11.58[a]	164.62±6.86[a]	154.60±4.91	206.98±10.44[a]	133.77±14.39

Values are mean±S.E.M; [a]p<0.05 vs. control;TG, triglycerides; VLDL, very low density lipoprotein; TC,total cholesterol; HDL-C, high density lipoprotein; LDL-C, lowdensity lipoprotein. Post hoc LSD

Table 3. Effects of *Prosopisafricana* seeds aqueous extract on lipid indices in the serum and brain homogenate of male Wistar rats

Parameters	Serum			Brain		
	Control	PA 500 (mg/kg)	PA 1000 (mg/kg)	Control	PA 500 (mg/kg)	PA 1000 (mg/kg)
CRR I	8.73±0.44	7.85±0.42	7.85±0.30	8.13±0.15	9.81±0.42	7.32±0.62
CRR II	7.15±0.44	5.90±0.46[a]	6.20±0.31	6.38±0.17	8.04±0.40[a]	6.51±0.59
AIP	0.46±0.00	0.06±0.31[a]	0.51±0.01	0.62±0.04	0.59±0.01	0.59±0.01

Values are mean±S.E.M; [a]p<0.05 vs. control; CRR I denotes cardiovascular risk ratio I; CRR II denotes cardiovascular risk ratio II; AIP denotes atherogenic index of plasma

such as: terpenoids, saponins, alkaloids, flavonoids, tannins and phenols as shown in Table 1.

Effect of *Prosopis Africana* seeds aqueous extract on lipid profiles

Prosopis africana (500 mg/kg) significantly (p<0.05) increased the serum TG and VLDL level but decreased TC and LDL-C without a change in HDL-C level when compared with control. However, *PA* (1000 mg/kg) significantly (*p<0.05*) decreased the serum TG, VLDL, TC and LDL-C levels when compared to control. In the brain, *PA* (500 mg/kg) significantly (*p<0.05*) increased TC and LDL-C level but insignificantly (*p>0.05*) decreased TG, VLDL and HDL-C level while insignificant(p>0.05) decrease in TG,VLDL, HDL-C,TC and LDL-C were observed at *PA* (1000 mg/kg) when compared to control group (Table 2).

Effect of *Prosopis africana* seeds aqueous extract on lipid indices

Treatment with *PA*(500 mg/kg)decreased

Table 4. Effects of *Prosopis africana* seeds aqueous extract on pro-oxidant /anti-oxidant potential in the serum and brain of male Wistar rats

Parameters	Serum			Brain		
(U/L)	Control	PA 500 (mg/kg)	PA 1000 (mg/kg)	Control	PA 500 (mg/kg)	PA 1000 (mg/kg)
SOD	16.05±0.44	23.77±2.99[a]	22.87±2.99[a]	34.4±2.64	31.47±5.28	35.82±2.50
GPx	37.04±1.27	39.87±2.23	31.15±0.95	64.47±6.56	29.18±2.97	48.15±3.30
CAT (µmol/L)	10.80±0.90	10.54±0.04	10.59±0.05	10.50±0.73	10.49±0.03	10.40±0.02
GSH	3.13 ±0.4	2.65±0.32	3.22±0.13	1.29±0.11	1.45±0.24	2.23±0.58[a]
MDA	6.76±0.31	3.45±0.56[a]	3.62±0.08[a]	44.30±1.50	11.09±2.95[a]	36.44±1.32[a]

Values are mean±S.E.M; [a]p<0.05 vs. control; SOD denotes superoxide dismutase; GPxdenotes glutathione peroxidise; CAT denotes catalase; GSH denotes reduced glutathione; MDA denotes malondialdehyde

significantly (*p<0.05*) CRR II and AIR when compared to control. However, *PA* produced no significant change in CRR I, CRR II and AIP in the brain homogenates at both doses when compared to the control group (Table 3).

Effect of *Prosopis africana* aqueous extract on prooxidant/antioxidant system

Both doses of *PA* decreased MDA level in the serum and brain significantly (*p<0.05*) without significant difference in GPx, and CAT when compared to the control group. Meanwhile, *PA*(1000 mg/kg)alone decreased MDA level in the brain significantly (*p<0.05*) when compared to control. Again, both doses significantly (*p<0.05*) increased serum SOD however, *PA*(1000 mg/kg)alone increases SOD in the brain while *PA*(500 mg/kg) decreases it in the brain when compared to the control group. Furthermore, *PA*(1000 mg/kg) alone increased the brain GSH when compared to control group (Table 4).

Discussion

The current study revealed some bioactive compounds in *Prosopis africana* seeds such as alkaloids, flavonoids, terpenes, and saponnins compounds which was similar to finding of Gurib-Fakim.[16] Studies have shown that alkaloids, terpenoids, saponin, and phenolic compounds that are detected in *Prosopis africana* exhibit remarkable biological/pharmacological activities such as antioxidant, antidiabetic, hypocholesterolemic, antitumor, anti-inflammatory and antimicrobial activities.[17]Alkaloids from *Prosopisspp* have a strong ability to capture free oxygen radicals.[18] Free radicals, the most naturally destructive agents in the living body system, cause oxidative damage upon interaction with the long chain polyunsaturated fatty acids (LC-PUFAs) in the cell membrane and membrane-bound organelles; persistent contact may lead to disequilibrium between the rate of generation and mopping up or scavenging capacity of endogenous antioxidant system.

Lipid profile is a panel of blood/fluid tests that serves as an initial screening tool for abnormalities in lipid metabolism and its associated clinical risk, especially for cardiovascular risk prediction.[19] A lipid profiles usually comprised the levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and the calculated very low density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C).

Lipid indices or ratio such as the Castelli's risk indices: CRR-l and CRR-ll, atherogenic coefficient (AC) and atherogenic index of plasma(AIP) are the diagnostic alternative that have been reported in predicting the risk of developing cardiovascular events [20-22] and effectiveness of therapy.[23]

Clinical conditions like diabetes mellitus, hypertension, atherosclerosis, arthritis and neoplasm are associated with changes in serum lipid and lipoprotein profiles with an increased risk in coronary heart diseases.[24] Hyperlipidemia is characterized by elevated serum levels of cholesterol, triglycerides and phospholipids as well as changes in lipoprotein composition. [25] In the present study, aqueous extract of *PA* produces alteration in lipid profile and the effects were dose dependent. The observed isolated hypertriglyceridemia may be due to stimulating effect of *PA* in the lipid synthetic pathway. The high level of serum lipids may be primarily due to uninhibited actions of lipolytic proteins (hormones and enzymes) in the adipose tissues (fat depot).

The serum hypocholesterolemic effect may be due in part to cholesterol- reducing activities of saponins, which bind with bile salt and cholesterol in the intestinal tract.[26] Usually, bile salts form small micelles with cholesterol facilitating its absorption following emulsification but saponins cause a reduction of blood cholesterol by preventing its re-absorption from

enterohepatic circulation.[27] Phenols (polyphenols) may protect against the development of atherosclerosis possibly by modulating cellular lipid metabolism, thereby mitigating atherosclerotic plaque formation. Although, this beneficial activity have been previously linked mainly to antioxidant or anti-inflammatory properties.[25]

In the brain, lipid profiles were down-regulated but up-regulation of TC and its lipoprotein LDL-C was observed in PA 500 mg/kg treated rats. This may support the report that said nervous tissues required more cholesterol content for myelination, maintenance of cellular fluidity and subsequent electrical insulation that facilitate conduction of neural transmissions.[28]Also the cholesterol maintains the integrity of cell membranes, here the neuronal membranes. Furthermore, LDL-C is a small cholesterol-rich lipoprotein containing only apoB, it represent about 70% of the total circulating cholesterol concentration.[29] LDL-C can be taken up by most cells but primarily by the liver via LDL-receptor; within the cell (nerve cells), the LDL particles are broken down by lysosomes, releasing cholesterol for cellular processes.[26] Moreover, nervous tissue cholesterol serves as precursor for the biosynthesis of neurosteriods by glial cells.[30]

Oxidative reactions lead to formation of free-radicals. The most common compounds which are attacked by oxidation are unsaturated fatty acid side chain in the PUFAs. These reactions are often enhanced with ferrous or copper ions. Degradation due to oxidative reactions can cause adverse effects on all biomolecules. Antioxidants protect the biological systems physiologically and against the potentially harmful effects of several biochemical reactions causing excessive oxidation[31], thereby preventing oxidative stress. Oxidative stress is often consequent to imbalance ROS production and /or inactivation and scavenging systems and may predispose to malnutrition, cancer, atherosclerosis, cardiovascular and/or neurodegenerative disorder.[32] In this study, the secondary metabolites, flavonoids, which are a group of structurally similar compounds containing two spatially separate aromatic rings have been hypothesized to contribute to our free radical defenses in a number of ways. Some flavonoids inhibit enzymes responsible for superoxide anion production, such as xanthine oxidase and NADPH oxidase thereby restricting initiation of free radical generation.[33] Others efficiently chelate iron(Fe) and copper (Cu), making it difficult for these metals to participate in the Fenton reaction and prevent propagation of free radical production.[34]

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