Original Research Article

EFFECT OF FERMENTED *PENTACLETHRA MACROPHYLLA BENTH* (AFRICAN OIL BEAN) SEED EXTRACT ON PLASMA LIPID PROFILE IN HEALTHY RAT MODEL-A preliminary study

ABSTRACT

Background: African oil bean seed is one of several plant products commonly used in Nigeria as food. However, report as to whether or not it could predispose consumers to dyslipidemia is yet to be documented.

Aim: The study aim was to determine the effect of fermented *Pentaclethra Macrophylla Benth* (African oil bean) seed extract on lipid profile.

Methods: A total of twenty-five (25) male rats randomly divided into five groups of five rats per group were used. Each group received the crude methanol seed extract of *Pentaclethra Macrophylla Benth* (MEPB) once daily at the dose of 500, 1000, 1500 and 2000 mg kg^{-1} body weight respectively, for 14 days except the control group. Lipid profile parameters were determined according to enzymatic assay using a commercial kit from Randox Laboratories, United Kingdom and calculation using Friedewald's equation.

Results: A statistically significant increase in HDL and decrease in LDL content ($p < 0.05$) were obtained following the administration of MEPB in all dosed groups compared with the control group. Administration of MEPB in all dosed groups improved lipid metabolism and increased percentage protection against atherogenesis by a range between 61% - 90%.

Conclusion: Fermented African oil bean seed has a positive effect on lipid metabolism and showed an anti-atherogenic property. According to the result, African oil bean seeds at the level used in the study could protect against atherosclerosis.

Key Words: dyslipidemia, Ukpaka, Lipid profile, atherogenesis, *Pentaclethra Macrophylla Benth*

1. INTRODUCTION

Most people, at least to some extent, probably understood the overlap between nutrition and health [1, 2]. Many have explored the use of a large number of plant products in the treatment of diseases because dietary plant products can serve simultaneously as a source of food and therapy [1]. Documented evidence suggests that about 80% of primary healthcare provided in developing countries depend mostly on the use of plant products for the treatment of several diseases as they have been shown to be safe with no adverse effects [3,4]. In African, for instance, Nigeria, public promotion of medicinal plants underpins the normative basis for the use of plant products in the maintenance of health [4]. Besides important phytochemical components that adapt dietary plant products for pharmacological actions, every plant product including African oil bean seeds, possesses important nutrients and biochemicals such as protein, fat, vitamins, minerals and carbohydrate which are essential for metabolic functions [4,5].

The African Oil bean seed (*Pentaclethra macrophylla Benth*), is produced by a large woody plant (family Leguminosae Mimosoidae) which is native to tropical Africa [5]. The hard but smooth flat brown seeds are contained in a long flattened green pod. It becomes edible after processing and fermentation [5,6]. Among the South Eastern part of Nigeria where it is popularly

regarded as the “African Salad”, it is known as “Ugba” or Ukpaka” which is a very important delicacy in the life of South Eastern Nigerians. However, due to increase in integration and change in food habit, the “African Salad” as it is called is increasingly gaining popularity across every region in Nigeria [7]. African oil bean seed contains phytonutrients which include alkaloids, saponins, flavonoids, and tannins [5,8], as such have been reported to be effective in the treatment of diarrhea and anemia while the pod and leaf extracts are used in the treatment of convulsion [5-7]. Some medicinal plants have been shown to alter normal body chemistry which invariably affects the normal function of some organs in the body [9]. Fermented African oil bean seed (AOBS) also known as Ukpaka is one of several plants products commonly used in Nigeria as food. However, report as to whether or not it could predispose consumers to dyslipidemia which may lead to atherosclerosis and its associated coronary heart disease is yet to be documented. Given the paucity of the report on the effect of Ukpaka on lipid profile among consumers, the present study was undertaken to investigate the effect of crude methanol extract of the fermented African oil bean seeds (Ukpaka) on lipid profile using a healthy rat model.

2. MATERIALS AND METHODS

2.1.Collection and authentication of plant material

Fresh samples of fermented slices of *Pentaclethra Macrophylla Benth* seeds (Ukpaka) were purchased from Obeleagu Umana in Ezeagu, Enugu state, Nigeria. The plant material was authenticated by a consultant taxonomist at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka and a voucher specimen deposited at the herbarium for future reference.

2.2. Processing of *Pentaclethra Macrophylla Benth* seeds powder

The fermented slices of *Pentaclethra Macrophylla Benth* seeds were dried under the shade at room temperature. The dried seeds were milled with an electric blender and finally ground into powder using a hammer mill (500# grinder/Fuyu Metal, Linyi Fuyu Metal Products Co., Ltd, China) and after that, passed through 52 mm sieve (Turgens and Co., Germany).

2.3 Chemicals and Reagents

The kits for, triglycerides, total cholesterol and HDL-Cholesterol were also purchased from Randox Laboratory United Kingdom. All the other reagents and chemicals of analytical grade were obtained from research laboratories in Enugu.

2.4 Preparation of the crude methanol extract

The powdered seeds (1500 g) of *pentaclethra macrophylla* Benth seeds powder was weighed out, placed in a gallon, and 2.5 liters of absolute methanol added and left for 48hrs. The mixture, intermittently, was agitated during the extraction process. After 48hrs, the mixture was sieved using a muslin cloth and filtered with a Whatman No. 1 filter paper and the filtrate then evaporated to dryness on a rotary evaporator (Model 349/2 Corning Ltd, England). The oily liquid residue obtained was stored in a refrigerator at $4 \pm 2^{o}C$ until required. This was labeled the methanol crude extract (MEPB).

2.5 Determination of Extractive Value for the crude methanol extract

The concentration of the crude methanol extract was determined by weighing the total oily liquid residue in electronic weighing balance and the density calculated which is expressed in mg/ml. The appropriate concentration then was calculated for the study.

2.5.1 Phytochemical Analysis

Standard procedures described in Bankole et al., [10] were employed to identify the bioactive chemical constituents present in Fermented *Pentaclethra Macrophylla Benth* (African Oil Bean).

2.6 Experimental Animals

2.6.1 Animal Housing and Management

Twenty -five (25) apparently healthy albino rats of same sex and age between 150g and 170g body weight were obtained from Animal House of the College of Medicine, University of Nigeria Teaching Hospital, Enugu. They were acclimatized for a period of two (2) weeks in clean gauzed cages in groups of five (5) according to their body weight under good laboratory conditions at the Animal House of the College of Medicine, University of Nigeria, Enugu campus. The rats had free access to food (commercial standard pellets, Topfeed^{R} Nigeria) and clean water daily.

2.6.2 Acute toxicity (Median Lethal Dose, LD50)

The median lethal dose (LD_{50}) of fermented African oil bean seeds [AOBS] was calculated to be > 6000 mg/kg body weight using the standard procedures described by Lorke [11].

2.6.3 Experimental Design

Animal Grouping and Extract Administration

A total of twenty–five (25) male rats were randomly grouped into five: A, B, C, D and E of 5 animals each per group after being allowed to acclimatize for 2 weeks. Rats in groups B, C, D, and E were administered with the methanol seed extract (MEPB) once daily at the dose of 500, 1000, 1500 and 2000 mg kg^{-1} body weight respectively, for 14 days. Group A (control), were treated just like the test groups except that the animals received only water instead of the seed extract. The methanol extract was administered to all animals in the different groups using oral gavage technique. The extract and distilled water were administered daily at the same point time throughout the duration of the experiment. The animals were allowed free access to rat pellets and tap water after the daily doses.

Sub-acute Study and Collection of Blood from Animals

The subacute study began with an oral administration of the extract every morning for 14 days. On the 15th day, following an overnight fast, the animals were bled through the medial canthus of the eye under ether anesthesia using capillary tubes. The blood sample was collected into plain tubes and separated from cells to assay for lipid profile.

2.7 Ethical approval

Handling, management and use of animals for the experiment were such that allowed minimal stress according to the international Guidelines on experiments involving the use of animals laid down in "Ethical and Scientific Considerations Regarding Animal Testing and Research"[12]. The study was approved by the Animal House of the College of Medicine, University of Nigeria Teaching Hospital, Enugu.

2.8 Biochemical Analyses

Measurement of serum lipid profile

Triglycerides and total cholesterol were estimated using enzymatic colorimetric methods as described by Fossati & Prencipe [13] and Fredrickson et al., [14] respectively. High-density lipoprotein (HDL) was measured enzymatically after all non-HDL lipoproteins were removed [15]. LDL-C was calculated using Friedewald's equation: LDL= total cholesterol – [HDL+ (TG/5)]. Atherogenic Index (AI) = (Total cholesterol — HDL cholesterol) / HDL cholesterol. Protection % = AI (control)-AI (treated)/AI (control) x 100 [16].

2.9 Data analysis

All data were analyzed using SPSS software (version 22) and results expressed as mean ± SEM. One way analysis of variance (ANOVA) followed by Post hoc multiple comparison tests was used to compare the difference in means among the groups. $P < 0.05$ was considered to be statistically significant value.

3. RESULTS

There was significant increase ($p < 0.05$) in the serum High-Density Lipoprotein Cholesterol (HDL-C) content following the administration of the methanol seed extract in all the dose groups; B (56.60 ± 3.31 mg/dl), C (63.00 ± 5.03 mg/dl), D (56.50 ± 2.36 mg/dl), and E (32.40 ± 4.37 mg/dl) when compared with the control group A (13.20 ± 3.31 mg/dl) (Table 1). Serum Low-Density Lipoprotein Cholesterol (LDL-C) content following the administration of the methanol seed extract in all the dose groups; B (20.32 ± 7.55 mg/dl), C (18.16 ± 3.02 mg/dl), D (24.30 ± 6.02 mg/dl), and E (41.44 ± 8.43 mg/dl) when compared with the control group A (44.64 ± 6.71 mg/dl) showed a significant decrease($p < 0.05$), (Table 1). However, T.CHOL, T.G, and VLDL do not differ significantly when compared with those of control group respectively ($p > 0.05$). There were significant reductions in the atherogenic index in all dose

groups with a percentage protection between 61% - 90% as opposed to the control group A. (Table 2).

Table 1: Mean and standard error of mean of the biochemical parameters

GROUP	T.CHOL mg/dl)	T.G (mg/dl)	HDL (mg/dl)	VLDL(mg/dl)	LDL mg/dl)
A-CONTROL	81.00±9.38	92.60±6.86	13.20±3.31	18.52±1.37	44.64±6.71
B-500 mg/bwt	96.00±7.92	90.20±10.41	56.60±3.94[a,b]	19.08±2.94	20.32±7.55[a]
C-1000 mg/bwt	98.40±4.13	86.20±10.72	63.00±5.03[a,c]	17.24±2.14	18.16±3.02[a]
D- 1500 mg/bwt	96.25±5.28	89.75±8.59	56.50±2.36[a,d]	17.95±1.72	24.30±6.02[a]
E-2000 mg/bwt	91.80±8.74	89.20±3.39	32.40±4.37[a]	17.84±0.67	41.44±8.43
F-ratio	0.66	0.12	21.14	0.16	2.94
P-value	0.68	0.99	0.00*	0.99	0.03*

(*) significant difference, $P = .05$; bwt= body weight

a = ($P = .05$) when compared with the control group A.

b, c, d= ($P = .05$) when compared with group E.

Table 2: Atherogenic index (AI) of methanol seed extract of *Pentaclethra Macrophylla Benth*

GROUP	T.CHOL(mg/dl)	HDL(mg/dl)	AI	% Protection
A-CONTROL	81.00±9.38	13.20±3.31	5.2*	-
B-500 mg/bwt	96.00±7.92	56.60±3.94	0.7	87
C-1000 mg/bwt	98.40±4.13	63.00±5.03	0.5	90
D- 1500 mg/bwt	96.25±5.28	56.50±2.36	0.7	87
E-2000 mg/bwt	91.80±8.74	32.40±4.37	2.0	61

*$P = .05$ when control is compared with other groups

Table 3: The proximate analysis of the fermented African oil bean seed

Component	Percentage (%)
Moisture	18.384
Protein	13.397
Fats	52.820
Ash	2.966
Fibers	1.856
Carbohydrate	10.577

Table 4: The phytochemical analysis of the fermented African Oil Bean Seed

Component	Concentration
Carbohydrate	+++
Reducing sugar	++
Alkaloid	++
Glycoside	++
Saponins	++
Tannin	+++
Flavonoid	++
Resins	+++
Protein	++
Oil	++
Steroid	++
Terpenoids	+++
Acidic compound	-

Key. −: absent; +: low; ++: moderate; +++: abundant.

4. DISCUSSION

Both in the past and currently, phytochemical/nutritional constituents have been recognized as the basis for using plant or its products for herbal medicine [17] For instance, the intake of flavonoids in any plant products such as fruit and vegetable tends to decrease cancer risk [18,19]. Interestingly, this study has shown fermented African oil bean seed to be a good source of

dietary nutrients with carbohydrate (10.6%), protein (13.4%) and fat (52.8%)(Table 3) and a good source of important phytochemical components (Table 4). Fermented African oil bean (Ukpaka) contents about 2% of fiber (table 3). According to previous studies [20, 21], plant fiber exerts a physiological effect on the lipid metabolism, in that, it prevents the reabsorption of bile acids and absorption of dietary cholesterol in the intestine thereby leading to the reduction in the quantity of cholesterol entering the circulation. Apart from fiber, the presence of some phytosterols and phytostanols (e.g. steroids) found in many plant sources including fermented African oil bean seed (Ukpaka) can inhibit cholesterol absorption. The efficacy and safety of these phytochemicals as plasma cholesterol-lowering agents have been reported by many studies [22-24]. Any plant product possessing lipid-lowering and antioxidants properties plays a key role in the anti-atherosclerotic process [25]. In the present study, Ukpaka has emerged as one of many dietary herbal products with the potential to reduce cholesterol as well as enhance the safety profile by increasing HDL-C levels in plasma [26].

Following the administration of African oil bean seeds (AOBS) extract, the present study showed a significant increase in HDL-C. Although, the mechanism by which HDL-C increased is not completely understood; however, AOBS may have influenced a variety of molecules involved in HDL metabolism and the Reverse cholesterol transport (RCT) system. The first speculation involved in HDL increase may be attributed to the increase in the amount of ApoA-1 level in the liver which is the main component protein of HDL [27-31]. ATP-binding cassette transporter A1 (ABCAI) in the hepatocytes, which transports cholesterol within cells to Apo A-1 forming pre-β HDL [32, 33], may have leveraged on those phytonutrients of AOBS to drive the increase in HDL fraction. Secondly, HDL containing a reduced level of phospholipids is prone to decomposition and is easily metabolized by endothelial lipase (EL). EL is one of the factors

196 promoting HDL catabolism due to its phospholipase activity and the ability to hydrolyze
197 phospholipid in HDL particles [34-36]. Thus, it is speculative that the extract may have
198 decreased the serum endothelial lipase (EL) mass or activity thereby decreasing the HDL
199 catabolism [37]. Therefore, inhibition of EL activity by the extract may have resulted in the
200 elevated level of HDL. The result is in agreement with previous studies done by other
201 researchers [38-40], who reported an increase in HDL-C levels with dietary plants products.
202 Inhibition of cholesteryl ester transfer protein (CETP) which regulates the transfer of cholesteryl
203 ester from HDL to other fractions of plasma cholesterol [41] is another mechanism that could
204 explain this. As such, HDL fraction may have been elevated through the inhibition of CETP by
205 AOBS extract.
206 The increase in HDL fraction is clinically significant in the maintenance of good cardiovascular
207 health, in that increase in the concentration of HDL-C have been demonstrated to correlate
208 inversely with coronary heart diseases [42-45]. HDL-C transports cholesterol from peripheral
209 tissues to the liver for metabolism and excretion thereby decreasing the amount stored in the
210 tissue and the possibility of developing atherosclerotic plaques. As such, HDL-Cholesterol is
211 considered to possess anti-atherogenic properties and hence regarded as the good cholesterol
212 [46,47].

213 Following the administration of AOBS extract, the result also showed a significant
214 decrease in low-density lipoprotein cholesterol (LDL-C) level. African oil bean seeds contain a
215 moderate and abundant amount of saponins and tannin respectively, (Table 4). These
216 photochemical components have been reported to reduce cholesterol levels [48]. The precise
217 mechanism of action of the extract, in relation to reduction in LDL-C fraction, was not
218 elucidated in this work. However, AOBS may have contributed to the inhibition of lipid

absorption from the gut due to the presence of saponins and tannins in the extract [48]. The significant decrease in serum LDL-C is quite understandable since an increase in serum total cholesterol could be an indirect effect of the increase in serum HDL-C [5]. LDL-C acts as the primary transporter of plasma cholesterol to the peripheral tissue through the arterial walls. It is, therefore considered the bad cholesterol as it may build up, forming plaques with progression to atherosclerosis and increasing the risk of high blood pressure and stroke [49]. The decreased LDL fraction observed in the study suggests that consumption of AOBS is not associated with dyslipidemia, which constitutes a major risk factor for the development of cardiovascular diseases, particularly atherosclerosis [50]. This finding is incongruent with work done by Ferdowsian et al.,[51] which demonstrated that plant-based dietary interventions are effective in lowering plasma low-density lipoprotein cholesterol concentrations. Atherogenic index (AI) [ratio of LDL-cholesterol to HDL-cholesterol] is a normative predicator of cardiovascular risk [41] with an index of greater than 5 set as the cut-offs for high risk of atherosclerosis [16]. Following the extract administration, the values for AI for all dosed groups were less than 5. This indicates that the extract improved lipid metabolism and increased percentage protection against atherogenesis by a range of 61% - 87%. This is suggestive that fermented African oil beans seed (AOBS) is not linked with any positive risk for atherogenesis, hence may not predispose to heart diseases.

5. CONCLUSION

Taken together, the study demonstrated that fermented African oil beans seed (Ukpaka) has a good anti-atherogenic potential evidenced by the reduction in Atherogenic index as shown from the increase in the concentrations of HDL-C fraction and a decrease in LDL-C fractions.

Therefore, consumption of fermented African oil bean seeds could potentially reduce cardiovascular risk and prevent atherosclerotic process because of elevated HDL content of the serum lipid observed.

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