Original Research Article

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

ABSTRACT

Background: African oil bean seed is one of several plants products commonly used in Nigeria as food. However, report as to whether or not it could predispose consumers to atherosclerosis as evidenced by alteration in the lipid profile 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 profiles.

Methods: A total of twenty-five (25) male rats randomly divided into five groups of 5 rats each 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% - 87%.

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

Among all people of all places and times, the overlap between nutrition and health has been probably understood, at least to some extent [1, 2]. Many have explored the use of large number of plants products in treatment of diseases as 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 use of plants 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 in Nigeria, public promotion of medicinal plants underpins the normative basis for use of plant products in 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 biochemical such as protein, fat, vitamins, minerals and carbohydrate which are essential for metabolic functions [4,36].

The African Oil bean seed (*Pentaclethra macrophylla Benth*), is produced by large woody plant (family Leguminosae Mimosoidae) which is native to tropical Africa [36]. The hard but smooth flat brown seeds are contained in a long flattened green pod. It becomes edible after processing and fermentation [5,36]. Locally, particularly 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 [6]. African oil bean seed contains phyto-nutrients, including alkaloids, saponins, flavonoids, and tannins [7,36], as such have been reported to be effective in the treatment of diarrhoea and anaemia while the pod and leaf extracts are used in the treatment of convulsion [5,6,36]. Some dietary/medicinal plants have been shown to alter normal body chemistry which invariably affects normal function of some organs in the body [8]. Fermented African oil bean seed (AOBS) is one of several plants products commonly used in Nigeria as food. However, report as to whether or not it could predispose consumers to atherosclerosis and its associated coronary heart disease evidenced by alteration in the lipid profile is yet to be documented. Given the paucity of report on its effect on lipid profile in consumers of AOBS (ukpaka), 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 thereafter, 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 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 labelled 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.

2.6 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 as described by Lorke [9].

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 sub- acute study began with an oral administration of the extract every morning for 14 days. On the 15th day, following overnight fast, the animals was bled through the median canthus of the eye under ether anaesthesia using capillary tubes. 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 was 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"[42]. 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 [10] and Fredrickson et al., [40] respectively. High density

lipoprotein (HDL) was measured enzymatic ally after all non HDL lipoproteins were removed [41]. 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 [11].

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 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% - 87% 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

4. DISCUSSION

This study showed a significant increase in HDL-C following the administration of African oil bean seeds (AOBS) extract. 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 increase in the amount of ApoA-1 level in the liver which is a main component protein of HDL [12-16]. ATP-binding cassette transporter A1 (ABCAI) in the hepatocytes which transports cholesterol within cells to Apo A-1 forming pre-β HDL [17, 18], may have leveraged on the phyto-nutrients of AOBS to drive the increase in HDL fraction. Secondly, HDL containing reduced level of phospholipids is prone to decomposition and is easily metabolized by endothelial lipase (EL). EL is one of the factors promoting HDL catabolism due to its phospholipase activity and the ability to hydrolyse phospholipid in HDL particles [19-21]. Thus, it is speculative that the extract may have decreased the serum endothelial lipase (EL) mass or activity thereby decreasing the HDL catabolism [22]. Therefore, inhibition of EL activity by the extract may have resulted in the elevated level of HDL. The result is in agreement with previous studies done by other researchers [23-28], who reported increase in HDL-C levels with dietary plants products. Inhibition of cholesteryl ester transfer protein (CETP) which regulates the transfer of cholesterol ester from HDL to other fractions of plasma cholesterol [39] is another mechanism that could explain this. As such, HDL fraction may have been elevated through the inhibition of CETP by AOBS extract.

The increase in HDL fraction is clinically significant in maintenance of good cardiovascular health, in that increase in the concentration of HDL-C have been demonstrated to

correlate inversely with coronary heart diseases [29-32]. HDL-C transports cholesterol from peripheral tissues to the liver for metabolism and excretion thereby decreasing the amount stored in the tissue and the possibility of developing atherosclerotic plagues. As such, HDL-Cholesterol is considered to posses anti-atherogenic properties, and hence regarded as the good cholesterol [33,34].

Following the administration of AOBS extract, result also showed a significant decrease in low-density lipoprotein cholesterol (LDL-C) level. African oil bean seeds contain phytochemical components such as saponins that can reduce cholesterol levels [35]. The precise mechanism of action of the extract, in relation to reduction in LDL-C fraction, was not 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 [35]. 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 [36]. LDL-C act as the primary transporter of plasma cholesterol to peripheral tissue through the arterial walls which may build up, forming plagues with progression to atherosclerosis and increasing the risk of high blood pressure and stroke [37], therefore is considered the bad cholesterol. The decreased LDL fraction observed in the study suggests that, consumption of AOBS is not associated with dyslipidemia, which constitute a major risk factor for development of cardiovascular diseases, particularly atherosclerosis [38]. Atherogenic index (AI) [ratio of LDL-cholesterol to HDL-cholesterol] is a normative predicator of cardiovascular risk [39] with an index of greater than 5 set as the cut-offs for high risk of atherosclerosis [11]. 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 between 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 AI as shown from the increase in the concentrations of HDL-C fraction and 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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