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Original article

Ameliorative Effect of Fermented *Pentaclethra macrophylla* (African oil bean seed) on High Fat Diet and Sucrose Drink Induced Metabolic Syndrome in Male New Zealand Rabbits.

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Abstract: The consumption of nutraceuticals has become progressively popular in improving health, as well as disease treatment and prevention. The aim of this study is to evaluate the ameliorative effect of fermented *Pentaclethra macrophylla* (African oil bean) seed on high fat diet and sucrose drink induced metabolic syndrome in male New Zealand rabbits. All experimental procedures were carried using standard methods. A total of twenty (20) rabbits were used for this study, the animals were randomly grouped into five (5) groups with four (4) animals each. Metabolic syndrome risk factors obesity, hypertension, dyslipidemia and hyperglycemia were determined. HFD+30%SUC had over 1500g body weight (bwt) and fasting blood sugar (FBS) 239.50±5.74 mg/dl relative to the normal control with 1000±6.73g and 77.00±5.94 respectively whereas ND+30%SUC had lower FBS (l172.50±2.52) compared to HFD+30%SUC. There was a significant $p<0.05$ increase in body weights in treated groups with 1274±11.19g, 1282.75±4.99g, 1285±4.76g and FBS levels in mg/dL198.25±4.79, 198.75±8.06, 200±3.74 in rabbits compared to untreated HFD+30%SUC. Glycated hemoglobin, and α-Amylase were significantly $p<0.05$ elevated, glucose-6-phosphate dehydrogenase levels were significantly $p<0.05$ reduced, lipid profile markers (HDL, TRIG & CHOL), electrolytes (sodium, potassium, calcium), urea and creatinine levels were significantly $p<0.05$ altered in HFD+30%SUC exposed group relative to the normal control and ND+30%SUC. However, dietary supplementation with fermented *Pentaclethra macrophylla* seeds caused weight, FBS, serum lipid reduction and potentiated electrolyte, urea and creatinine levels. Therefore, according to our results, fermented *Pentaclethra macrophylla* seed is an excellent nutraceutical and its consumption should be encouraged in modifying high fat and elevated intake of sucrose in rabbit

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INTRODUCTION

Metabolic syndrome is a constellation of cardiovascular risk factors, including hypertension, central obesity, dyslipidemia, and hyperglycemia (Lee et al., 2018). The contributing factors for the features of Metabolic Syndrome (MetS) can be hereditary or environmental such as food. Family history of type II diabetes, hypertension, insulin resistance and ethnic background are inevitable genetic factors that greatly increase the risk for developing metabolic syndrome (Wong et al., 2016; Ranasinghe et al., 2015). On the other hand, environmental risk factors for metabolic syndrome can be controllable. These include sedentary lifestyle, physical inactivity and eating habits (Wagner et al., 2012). It is estimated that around 20-25 percent of the world's adult population have the metabolic syndrome and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome (Alberti et al., 2006).

Metabolic syndrome, being a multifactorial condition with an alarming rate of prevalence and the accompanying deleterious effects, call for attention on the need to improve on research efforts in developing new interventions to reduce the burden of managing the disease on the healthcare system (Wong et al., 2016). Also experimental models of MetS are valuable tools in the study of its pathogenesis, prevention and treatment regimen (Gancheva et al., 2015). Due to its multifactorial nature, selecting an adequate experimental model that best represents the pathophysiology of MetS in humans can be rather challenging (Wong et al., 2016). Nonetheless, there has been various approaches used to induce MetS in rodents which include dietary manipulation, genetic modification and drugs (Wong et al., 2016). However, subsequent studies are encouraged using combination or modification of existing established methods in order to successfully develop an animal model of metabolic syndrome with the desired metabolic changes. Apart from pathophysiological similarity with human MetS, an excellent animal model should also be reproducible, simple, reliable, and affordable (Wong et al 2016; Gancheva et al.,

2015). In this light, the present study induced metabolic syndrome with dietary manipulation compositing of high fat diet and sucrose drink.
Plants have always been an important source of drugs and the use of herbs as medicine is the oldest form of healthcare known to humanity (Althunibat et al., 2010; Anaka et al., 2013; Zeidan et al., 2013; Majali et al., 2015; Khleifat et al., 2019). A large number of the world's populations, especially in developing countries, depend upon medicinal plants as an alternative and complimentary therapy for various ailments (Althunibat et al., 2016; Lebeau et al., 2017). Ethno-pharmacological surveys indicated that more than 1,200 plants are used in traditional medicine systems (Khleifat et al., 2001; Nwozo et al., 2018). Besides, a numbers of researches have been carried out using medicinal plants to mitigate various disease conditions and the results have demonstrated that it provides efficient and cost effective therapeutic solution (Homady et al., 2002; Qaralleh et al., 2009; Tarawneh et al., 2010; Nwawuba and Okechukwu 2018).
The African Oil bean seed (*Pentaclethra Macrophylla Benth*), is produced by a large woody plant (family *Leguminosae Mimosoidae*) which is native to tropical Africa (Woo et al., 2002). The hard but smooth flat brown seeds are contained in a long flattened green pod. It becomes edible after processing and fermentation (Anioke, 2019; Woo et al., 2002). Among the South Eastern part of Nigeria where it is popularly known as "Ugba" or Ukpaka" which is used in preparing the popular dish regarded as the "African Salad" a very important delicacy in South Eastern Nigeria. African oil bean seed has been reported to be a good source of nutritional mineral elements (Igwenyi et al., 2015), contains phytonutrients (Anioke, 2019; Igwenyi et al., 2015), it is effective in the treatment of anemia (Nwanjo et al., 2006), improves lipid metabolism and increases percentage protection against atherogenesis by a range between 61% - 90% (Anioke, 2019), it also aids reduction of blood pressure (Okwuonu et al., 2013), it is rich in vitamins and the seeds can be considered as source of quality raw material for food and pharmaceutical industries (Okwu and Aluwuo, 2008). Although, the nutraceutical power of African oil bean seed has been evaluated and reported but, there are paucity of findings on its ameliorative effect on metabolic syndrome. Therefore, it was imperative to investigate the ameliorative effect of African oil bean seed on high fat diet and sucrose drink induced metabolic syndrome in Male New Zealand Rabbits.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Pentaclethra Macrophylla (African oil bean) seeds were obtained from Ojoo market, Ibadan, Oyo State. The plant materials were identified by Mr Donatus Esiemuokhai the Herbrium in the Department of Botany, University of Ibadan, Oyo state, Nigeria. The seeds were boiled for 12 hours at 105ºC. The seed coats were removed. The embryo was sliced into smaller pieces with sharp knife and boiled again for further 2 hours. The sliced seeds were washed thoroughly in water with four changes of water. The washed sliced seeds were wrapped with banana leaves and packed together in baskets and left to ferment for 3 days at room temperature (29-32ºC). The fermented sliced seeds were dried at room temperature, grounded into powder, then pelletized as feeds for the experimental animals and stored in an air tight container for further use.

Phytochemical screening

Phytochemical screening of *Pentaclethra Macrophylla* (African oil bean) seeds was performed by standard methods according to (Harbone, 1998).

Experimental animals

Twenty (20) male rabbits (New Zealand) with the average weight of 435g were obtained from Abayomi Farm at Ogbomosho. The animals were allowed to acclimatize for three (3) weeks under standard laboratory condition. During this period of acclimatization, they were fed with standard rabbit chow and allowed free access to clean drinking water.

Preparation of high fat diet

High fat diet was prepared according to method of (Yin *et al*., 2002) with slight modifications as previously described by (Nwozo et al., 2018).

Table 1: feed composition of normal and high fat diet

Composition	Normal diet (%)	High Fat diet (%)
Maize	20	18
Soya bean meal	10	9
Wheat Offal	30	27
Corn bran	15	-
Palm Kernel cake	20	18
Bone meal	3	3
Limestone	2	2
Salt	-	0.3
Grower premix	-	0.3
Rice bran	-	12.4
Pork Lard	-	10

Supplemented feed preparation

The *Pentaclethra Macrophylla* (African oil bean) seed sample was supplemented into normal diet at 20% concentrations. The 20%supplemented diet had 20g of *Pentaclethra Macrophylla* (African oil bean) mixed with 80g of normal diet. The supplemented diet was thoroughly mixed and made into pellets.

Induction of Metabolic syndrome (Overweight, dyslipidemia and hyperglycemia)

The experimental animals were fed with the formulated high fat diet (Overweight and dyslipidemia) and 30% sucrose solution (hyperglycemia and overweight) for the period of six (6) weeks.

Study Design

A total of twenty (20) rabbits were used for this study, the animals were randomly grouped into five (5) groups with four (4) animals in each group.

Group A: Normal control fed with normal diet

Group B: Negative control fed with high fat diet and 30% sucrose solution for 6 weeks and remained untreated.

Group C: Fed normal diet and 30% sucrose solution for 6 weeks and remained untreated.

Group D: Positive control fed with high fat diet and 30% sucrose solution for 6 weeks and treated with 20mg/kg Simvastatin for a period of three (3) weeks

Group E: Fed with high fat diet, 30% sucrose solution for 6 weeks and treated with 20% supplemented with 20% *Pentaclethra Macrophylla* (African oil bean) seed for 3 weeks

Biochemical analysis

Blood glucose level was measured using ACCU-CHEK glucometer, glycated hemoglobin (HBA1c) was measured by high performance liquid chromatography (HPLC), Randox Laboratory assay kit was used to determine Glucose 6 phosphate, α-Amylase, triglyceride, total cholesterol and high density lipoprotein-cholesterol concentrations. Serum level of alanine and aspartate aminotransferases (ALT and AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) were quantified by spectroscopy using Randox commercial assay kits. Indices used in hypertension diagnosis were selected as recommended in Harrison's Principles of Internal Medicine (Loscalzo et al., 2008). Serum urea, creatinine and electrolytes were assayed as kidney and endocrine function tests.

Statistical analysis

Data were treated by ANOVA (analysis of variance) and mean separation was done using Duncan multiple range test and Turkey. Paired T-test was used to establish difference in timely events. $p<0.05$ were considered significant. Data was expressed as means±standard deviation and pictorially presented in form of charts. All statistical analysis was done using IBM SPSS Version 22 and Microsoft Excel.

RESULTS

Bodyweight

Figure 1 depicts the effect of fermented *Pentaclethra Macrophylla* seed feeding on body weight of male New Zealand Rabbits. Prior to commencement of experiment, following three (3) weeks of acclimatization, the rabbits body weights were recorded and reported as baseline, and there was no observed significant difference $p>0.05$ between all the groups. However, after the experimental groups were fed a high fat diet (HFD) and 30% sucrose solution, the body weights were recorded and they was an observed significant $p<0.05$ increase in the body weights relative to the normal control. Treatment followed, and the treated groups HFD+30%Sucrose+Simvasttin and *Pentaclethra Macrophylla* seed supplementation HFD+30%Sucrose showed a reduction in body weights 1307.50±9.84g and 138.50±6.30g respectively, in comparison to the untreated HFD+30%Sucrose group with 1593.25±5.38g.

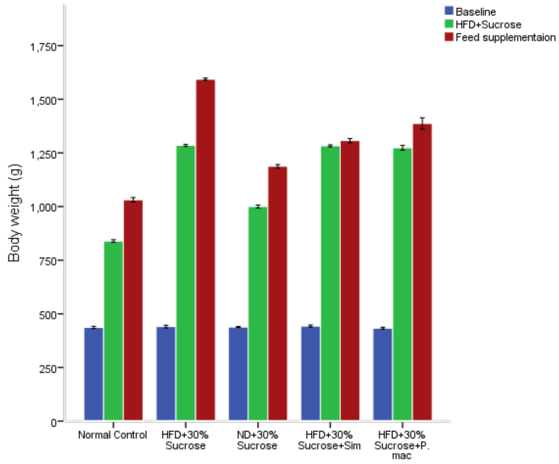

Figure 1: Effect of *Pentaclethra Macrophylla* seed on body weight of male New Zealand Rabbits

Diabetes (hyperglycemia)

In Figure 2, baseline sugar level was recorded and reported after three (3) weeks of acclimation and they was no significant difference $p>0.05$ between all the groups. However, following high fat diet and 30% sucrose feeding, FBS levels was significantly $p<0.05$ elevated (HFD+30%Sucrose 200.00±3.74), (HFD+30%Sucrose+sim 198.75±8.06) and (HFD+30%Sucrose+p.mac 198.25±4.79) as compared to the normal control (77.00±5.94) and normal diet + 30% sucrose fed group (172.50±2.52). Treatment followed and the treated groups showed significant $p<0.05$ reduction in the FBS level HFD+30%Sucrose+sim (178.00±4.32), HFD+Sucrose+p.mac (177.75±8.65) relative to the negative control HFD+30%Sucrose (212.50±5.26) and similar trend was observed for glycated hemoglobin (HBAIC), glucose 6 phosphate dehydrogenase (G6PDH), and α-Amylase as shown on Table 2.

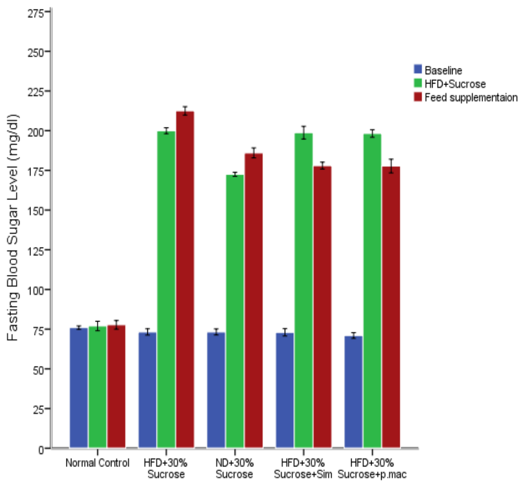

Figure 2: Effect of *Pentaclethra Macrophylla* seed on fasting blood sugar level of male New Zealand Rabbits

Lipid profile

At the end of the experimentation, blood was collected and plasma concentration of high density lipoprotein (HDL), Triglyceride (TRIG) and cholesterol (CHOL) was determined and reported in Fig 3. HDL was significantly $p<0.05$ elevated in the normal control 47.25±2.57, followed by the standard drug treated group 35.74±3.30, then *Pentaclethra Macrophylla* seed supplemented group 34.50±3.30 and the untreated group significantly had the least value of HDL 25.00±2.94 relative the other groups. Contrarily, the levels of CHOL and TRIG were significantly $p<0.05$ increased in the untreated group (HFD+30%SUC) relative to other groups.

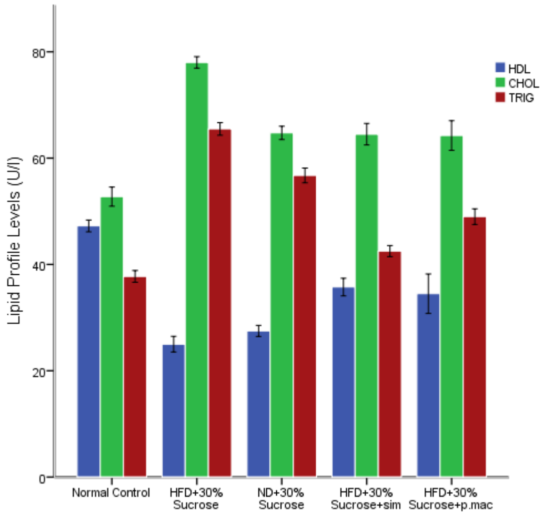

Figure 3: Effect of *Pentaclethra Macrophylla* seed on lipid profile level of male New Zealand Rabbits

Electrolyte, urea and creatinine (Hypertension)

At the end of the study, serum levels of electrolytes (sodium, potassium and calcium) and also urea and creatinine were recorded and reported as revealed on Table 3. *Pentaclethra Macrophylla* seed supplemented fed group (HFD+30%Sucrose+p.mac) and simvastatin treated group (HFD+30%Sucrose+sim) similarly showed a significant $p<0.05$ increase in the levels of sodium and potassium and a significant $p<0.05$ decrease in the levels of urea and creatinine relative to the negative group (HFD+30%Sucrose). However, no significant difference $p>0.05$ was observed between the experimental groups for calcium levels.

DISCUSSION

The consumption of nutraceuticals, natural plant foods, and the use of nutrition as therapy and medicinal plants for treatment has become progressively popular to improve health conditions generally as well as in the prevention and management of diseases (Olaiya et al., 2013). Sequel to this, the nutraceutical power of fermented *Pentaclethra Macrophylla* seed on metabolic syndrome was evaluated. Metabolic syndrome rises from a combination of cardiovascular risk factors such as hypertension, obesity, elevated serum sugar and lipid levels (Lee et al., 2018). The present study induced MetS related risk factors overweight, hyperglycemia, dyslipidemia and hypertension using dietary manipulation approach (high fat diet + 30% sucrose) in rabbits. An approach/model previously adopted by (Yang et al., 2012) in mice and (Gancheva et al., 2015) in rats.

Overweight been one of the risk factors of metabolic syndrome, the present study revealed that the dietary manipulation with a high fat diet and 30% sucrose drink elicited a significant $p<0.05$ increase in body weight of the rabbits in relation to the baseline. They rabbits overweight was clearly revealed, in that, they groups that were fed high fat diet and 30% sucrose drink had a significant $p<0.05$ increase in bodyweight vis-à-vis the normal control and the group fed a normal diet and 30% sucrose drink as revealed in Fig 1. This finding is consistent with the report of (Li et al., 2015) which indicated that high-fat diet feeding caused an increased body weight although in mice, and (Aguilera et al., 2004) that 30% sucrose in drinking water led to the development of MetS in male Wistar rats with increase in body weight. However, supplementation with fermented *Pentaclethra macrophylla* seed significantly $p<0.05$ caused a decrease in body weight of the rabbits in relation to the negative control group and showed a similar weight reduction capacity when compared to standard drug (simvastatin) treated group. Although, numerous medicinal properties have been attributed to the seeds of Pentaclethra macrophylla (Ufelle et al., 2015), but its weight reduction ability has received little attention.

Table 2: Effect of *Pentaclethra Macrophylla* seed on markers of diabetes (hyperglycemia) of male New Zealand Rabbits

Groups	HBAIC (%)	G6PDH (U/I)	α-Amylase (U/I)
Normal Control	4.52±0.30[a]	98.50±2.89[c]	83.00±2.58[a]
HFD+30%SUC	10.72±0.38[d]	55.50±2.65[a]	158.25±3.40[c]
ND+30%SUC	9.05±0.13[c]	57.50±2.38[a]	154.00±2.94[c]
HFD+30%SUC+SIM	7.95±0.21[b]	83.00±2.58[b]	134.00±3.92[b]
HFD+30%SUC+P.Mac	7.90±0.22[b]	85.75±3.30[b]	134.75±4.99[b]

Data are expressed as means±SD: Means with different alphabet as superscript within each column variable are significantly (p<0.05) different from each other. The abbreviations denotes, HBA1C: Glycated hemoglobin, G6PDH: Glucose-6-phosphate dehydrogenase, HFD: high fat diet, ND: normal diet, SIM: Simvastatin and P.Mac: *Pentaclethra Macrophylla*

Table 3: Effect of *Pentaclethra Macrophylla* seed on electrolytes, Urea and Creatinine of male New Zealand Rabbits

Groups	Sodium	Potassium	Calcium	Urea	Creatinine
Normal Control	131.25±2.27c	5.10±0.48c	0.95±0.13b	18.75±1.50a	48.75±2.00a
HFD+30%SUC	98.50±5.51a	2.95±0.26a	0.71±0.02a	32.50±3.87c	71.50±2.65c
ND+30%SUC	118.50±3.42b	4.10±0.18b	0.70±0.01a	27.25±1.71b	66.50±3.70bc
HFD+30%SUC+SIM	120.75±3.59bc	4.07±0.53b	0.68±0.04a	24.25±1.71b	56.50±3.70ab
HFD+30%SUC+P.Mac	118.00±13.56b	4.22±0.27b	0.70±0.05a	24.50±1.29b	58.00±2.94ab

Data are expressed as means±SD: Means with different alphabet as superscript within each column variable are significantly (p<0.05) different from each other. The abbreviations denotes, HFD: high fat diet, ND: normal diet, SIM: Simvastatin and P.Mac: *Pentaclethra Macrophylla*

Diabetes is a metabolic disorder characterized by hyperglycemia which is also a component of metabolic syndrome. Hyperglycemia was induced by adding 30% sucrose drink and a high fat manipulated diet in this study, thus and they two key players (sucrose and high fat diet) have been correlated with the development of hyperglycemia. Figure 2 corroborates this claim as previously reported by Aguilera et al., 2004; and Li et al., 2015. FBS levels were significantly $p<0.05$ elevated after high fat diet and 30% sucrose feeding when compared to the baseline and the normal control group. Also, other indicators of diabetes (hyperglycemia) which includes; glycated hemoglobin (HBA1C) glucose-6-phosphate dehydrogenase (G6PDH), and α-Amylase levels were investigated as shown on Table 2. Glycated hemoglobin (HbA1C) is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high blood glucose level, Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first step in the hexose monophosphate (HMP) shunt an alternative pathway for the catabolism of glucose to yield pentose sugar and alpha-amylase, is an enzymes involved in the digestion of carbohydrates (Nwozo and Nwawuba, 2019; Antonoi et al., 2013; Tundis et al., 2010). However, supplementation with fermented *Pentaclethra macrophylla* seed demonstrated a significant $p<0.05$ reduction in FBS levels, HBA1C, α-Amylase and a significant increase $p<0.05$ in G6PDH when compared to the negative control. Therefore, this finding is in agreement with the report numerous medicinal properties that has been attributed to the seeds of *Pentaclethra macrophylla* (Ufelle et al., 2015).

High levels of blood lipids such as triglyceride, total cholesterol and LDL-cholesterol culminating in dyslipidemia is another component of metabolic syndrome and it is closely related to coronary heart disease. As previously reported by (Nwozo et al., 2019), among the established risk factors for coronary heart disease (CHD), the lipid triad (elevated triglyceride, LDL-cholesterol levels and decreased HDL-cholesterol concentrations) are there major predisposing factor for atherosclerosis. Also, there is an established positive relationship between lipid profile and hypertension (Olaiya et al., 2013). Based on above stated facts, the obtained result (Fig 3) revealed that high fat diet and 30% sucrose feeding resulted in the alteration in lipid metabolism, as there was a significant $p<0.05$ increase in the levels of triglycerides, total cholesterol, LDL-cholesterol and a significant decrease in HDL-cholesterol in negative control relative to the normal control. However, dietary supplementation with fermented *Pentaclethra macrophylla* seeds elicited a restoration of lipid metabolism balance, as there was a significant $p<0.05$ decrease in the levels of triglyceride, total cholesterol and a significant $p<0.05$ increase of HDL vis-à-vis the negative control and similar to the positive control (simvastatin) treated group. Our finding corresponds with the report that fermented *Pentaclethra macrophylla* seed has a positive effect on lipid metabolism and showed an anti-atherogenic property (Anioke et al., 2019).

Balance of electrolytes is essential for normal function of cells and organs and electrolyte tests are commonly used to monitor treatment of certain health problems, including high blood pressure (hypertension) (Olaiya et al., 2013). Electrolytes such as chloride, potassium, calcium and sodium also tend to show slight modulations in hypertensive conditions and significant independent relationships have been found to exist between blood pressure and several serum cations (Olaiya et al., 2013; Agada and Braide, 2009). Hypertension is a risk factor of MetS, thus some ions, urea and creatinine levels were measured, in order to ascertain the hypertensive condition of the animals following induction of metabolic syndrome and treatment. The result as shown on Table 3 revealed a significant alteration in electrolyte, urea and creatinine levels in the experimental groups relative to the normal control. However, following dietary supplementation with fermented *Pentaclethra macrophylla* seed, there was a significant $p<0.05$ increase in the levels of sodium, potassium and a significant decrease $p<0.05$ in urea and creatinine relative to the negative control. Conversely, there was no observable significant difference $p>0.05$ in the levels of calcium. Our result is consistent with finding of Okwuonu et al., 2013, who reported that fermented *Pentaclethra macrophylla* seed possesses blood pressure reduction capability.

CONCLUSION

Components of metabolic syndrome (overweight, hyperglycemia, dyslipidemia and hypertension) were established in New Zealand rabbits using a high fat diet and 30% sucrose drink. Following the establishment of metabolic syndrome, dietary supplementation with fermented *Pentaclethra macrophylla* seeds demonstrated a weight reduction capacity, hypoglycemic ability, altered lipid metabolism and potentiated electrolyte, urea and creatinine levels which characterized hypertension. Therefore, according to our results, fermented *Pentaclethra macrophylla* seed at twenty percent supplemental in rabbit chow is an excellent nutraceutical.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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