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Anti-Obesity Potential of the Ethanolic Extract of *Alstonia boonei* Stem Bark on High Carbohydrate Diet Induced Obesity in Male *Wistar* Rats

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ABSTRACT: *Obesity increases the likelihood of various diseases, particularly dyslipidemia, type 2 diabetes, hypertension, heart disease and osteoarthritis. The aim of this study was to determine the anti-obesity potential of the ethanolic extract of Alstonia boonei stem bark on diet induced obesity (DIO) in male Wistar rats. Thirty male Wistar rats were divided into three groups (n=10): Animals in group 1 were fed normal control diet, Group 2 and 3rats were fed high carbohydrate diet for 14 weeks, which was the period of obesity induction. The treatment period lasted for the next 4 weeks in which the rats in Group 1 and 2 received the vehicle (normal saline) and only Group 3 received 500 mg/kg b.w Alstonia boonei extract.The results obtained showed that feeding rats with Alstonia booneiextract significantly decreased (p<0.05) food intake, body weight, fat mass, adiposity index, TC, LDL-C, leptin levels. It however had no significant effecton HDL-C, glucose and insulin levels when compared with the HCD obese control. The photomicrograph of HCD obese controls showed presence of numerous prominent fat deposits in the livers; this appeared reduced among the groups treated with the plant extract. Thus, the ethanolic extract of Alstonia boonei stem bark may have some potential in the management and/or treatment of obesity.*

Key words: Obesity, *Alstonia boonei*, Fat mass, Adiposity index and Diet.

Introduction

Obesity is reaching epidemic proportions worldwide; it is correlated with various comorbidities, among which the most relevant are dyslipidemia[1], diabetes mellitus type 2[2], fatty liver[3], and cardiovascular diseases such as heart failure and coronary heart disease[4].As of 2005 the WHO estimated that at least 400 million adults (9.8%) are obese, with higher rates among women than men[1].Almost 70% of adults in U.S.A. are overweight but, perhaps more alarmingly, 16% of juveniles are overweight[5].

Overweight and obesity are often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue[6]. While genetic influences are important to understanding obesity, they cannot explain the current dramatic increase seen within specific countries or globally[7].According to Slyper[8], in the United States, simple carbohydrate intake may be blamed for the rise in obesity, particularly in children who regularly consume fruit juices and snacks like potato chips. Foods high in fat or sugar (for example, fast food, fried food, and sweets) have high energy density. Carbohydrates increase blood glucose levels, which in turn stimulate insulin release by the pancreas, and insulin promotes the growth of fat tissue and can cause weight gain. Some scientists believe that simple carbohydrates (sugars, fructose, desserts, soft drinks, beer, wine, etc.) contribute to weight gain because they are more rapidly absorbed into the blood-stream than complex carbohydrates (pasta, brown rice, grains, vegetables, raw fruits, etc.) and thus cause a more pronounced insulin release after meals than complex carbohydrates. This higher insulin release isbelieved to contribute to weight gain.

It's easier to prevent obesity than to cure it. But in the event of obesity, its management and treatment includes dieting, exercising, surgery and medications such as Sibutramine and Orlistat. These drugs are more effective in people who make dietary changes and exercise more than in people who just take the drugs. As with almost all medications, weight-loss drugs have side effects, high cost and are not safe for everyone.

Traditional medicinal plants are often cheaper, locally available, and easily consumable (raw or as simple medicinal preparations). Some traditional healers have claimed that some medicinal plants in Nigeria, like *Alstonia boonei* could be used to treat obesity. The common name of Alstonia in English is Alstonia, cheesewood, timber trade — pattern wood, stoolwood and French: emien. In Nigeria, it is called égbū *or egbun* (Igbo), ahùn (Yoruba), Ukhu (Edo) and Ukpukunu (Urhobo). *A. boonei* De Wild is a tree of 30-40 m high by over 3 m in girth, bole cylindrical and long to 27 m, with high narrow buttresses.*Alstoni boonei* De Wild is a medicinal plant used extensively in West and Central Africa for the treatment of malaria, fever, intestinal helminthes, rheumatism, hypertension, etc[9, 10].This study is aimed to ascertain the anti-obesity potential of the ethanolic extract of *Alstonia boonei* De Wild stem bark on high carbohydrate diet induced obesity in male *Wistar* rats.

Materials and Methods

Plant Material: The fresh stem bark of *Alstonia boonei* De Wild was collected from a farm land located in Umuekwune community, Imo State, Nigeria. The authentication of the plants was done by a taxonomist at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. A voucher specimen of the plant was deposited in the Herbarium, FRIN.

Preparation of Plant Extract: The fresh parts of the stem bark of *A.boonei* was washed, chopped into pieces and air-dried in the laboratory at room temperature. The dried plant part was milled into powder and weighed. 250g of the plant powder was soaked in 500 ml of absolute ethanol in bottles for 72 hours with intermittent shaking. Then, it was filtered through Whatman No. 1 filter paper. The resulting filtrates were evaporated under reduced pressure using a rotary evaporator and there after freeze dried to a powder. The yield was stored in a refrigerator (4 ^{o}C) until required.

Phytochemical Screening: Phytochemical screening of the stem bark of *A.boonei* De Wild was done according to methods described by Treatise and Evans[11] and Sofowora[12].

Acute Toxicity (LD_{50}) of Plants: The acute toxicity of the ethanolic extract of the plant was carried out as described by Shah *et. al.*[13] and Burger *et. al.*[14].

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Composition of Experimental Diet:
The food/supplement used to compose the diet include: carbohydrate (processed cassava locally called *garri*), protein (dried *bonga*fish), fat (butter), fibre, vitamins and mineral mixture were obtained from a retail goods manufactured by SuperMax Nig. Ltd (Table 1).
Table 1: Composition of Experimental Diet

Composition	Food /Supplements	NPD (%)	HCD (%)
Carbohydrates	Garri	60.0	60.0
	Sucrose	-	20.0
Fat	Butter	5.0	5.0
Proteins	Bonga Fish	30.0	10.0
Fiber		1.5	1.5
Mineral mixture	Multi-minerals	2.5	2.5
Vitamin mixture	Multi-vitamins	1.0	1.0
Energy (KCal/g)		4.095	4.095

Note: NPD-normal pellet diet; HCD- high carbohydrate diet
Experimental Design and Animal Grouping: Thirty male Wistar rats (average weight 80-90 g) were divided into 3 groups containing 10 rats per group. Group 1 was fed on normal pelleted diet, while obesity was induced in Groups 2 and 3 by feeding high carbohydrate diet (HCD) for 14 weeks (Table 1). Thereafter, only the rats in group 3 were treated with 500mg/kg b.w *Alstonia boonei* extract for the next 4 weeks. The rats had free access to their diets and water.
Food Intake and Body Weight Measurement: Daily food intake and weekly body weight was measured in grams (g).
Adipose Tissue Dissection and Fat Mass Determination: After abdominal incision, five different white adipose depots (two subcutaneous and three intra-abdominal) and interscapular brown adipose tissue were harvested from each rat and referred to as fat mass.
Adiposity Index: Adiposity index was determined by the sum of epididymal, visceral and retroperitoneal fat weights divided by body weight × 100, and expressed as adiposity percentage[15].
Blood Sample Preparation and Bioassays: At the end of the experiment, rats were fasted for 12 to 14 hours. Blood was collected by cardiac puncture into plain sample bottles, allowed to stand for 15mins for the blood to retract, and then centrifuged at 3000 rpm for 5mins. The serum was separated and used for the assay of the following parameters: total cholesterol[16], triglycerides[17], high density lipoprotein cholesterol[18], very low density lipoprotein cholesterol (triglycerides/5), low density lipoprotein cholesterol[19], glucose[16], insulin (DRG Insulin ELISA kit, USA) and leptin levels (DRG Leptin ELISA kit, USA).

Histopathology
The parts of the liver and heart were dissected out and placed in formal saline for histopathology studies. Sections of the liver and heart were prepared and stained with haematoxylin and eosin following fixation. Permanent mounts were examined by light microscopy and the results obtained were compared with control[20].
Statistical Analysis: The experimental results was expressed as the Mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by One way ANOVA followed by Duncan's multiple range test. $P<0.05$ was considered to be significant.

Results
The bioactive compounds in the ethanolic stem bark extract of *Alstonia boonei* include alkaloids, saponins, tannins, steroids, flavonoids, cardiac glycosides and terpenoids. The acute toxicity (LD_{50})study of *A.boonei* revealed general weakness and sluggishness as the major behavioral changes observed in the rats at 6400 mg/kg b.w oral dose. These behavioural changes disappeared after 1 hour of observation. No death was recorded at any of the doses administered. Oral LD_{50} was therefore not determined because mortality was not observed.

The food intake, body weight, fat mass and adiposity index of the HCD obese control was increased significantly ($P<0.05$) compared to the normal control at the 18th week. The group treated with *A.boonei* extract had significant decrease ($P<0.05$) in the food intake, body weight, fat mass and adiposity index compared to the obese control (Table 2).

Table 2: Food intake, body weight, fat mass and adiposity index of rats treated with *A. boonei* extract.

GROUP	Food Intake (g)	Body Weight (g)	Total Fat Mass (g)	Adiposity Index
Normal Control	63.13 ± 2.11^{b}	293.67 ± 9.17^{c}	20.58 ± 0.50^{c}	3.29 ± 0.18^{b}
HCD Obese Control	76.72 ± 1.64^{a}	399.33 ± 7.06^{a}	29.43 ± 0.68^{a}	4.63 ± 0.11^{a}
HCD + *A. boonei*	40.66 ± 2.73^{c}	357.00 ± 7.37^{b}	22.67 ± 0.72^{b}	3.27 ± 0.20^{b}

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

Table 3 shows the lipid profile of rats treated with A.boonei extracts. The HCD obese rats had significantly increased ($P<0.05$) serum triglycerides, total cholesterol, VLDL cholesterol and LDL cholesterol, with a decrease in HDL cholesterol which was not significant ($P<0.05$).All the parameters with exception of LDL-C of the treated group 3 had no significant decrease when compared to HCD obese control.

Table 3: Lipid profile of rats treated with the *A. boonei* extract

GROUP	TG (mg/dl)	TC (mg/dl)	HDL-C(mg/dl)	VLDL-C(mg/dl)	LDL-C (mg/dl)
Normal Control	45.58 ± 0.91^{b}	43.78 ± 1.45^{c}	29.97 ± 1.70^{c}	9.12 ± 0.11^{b}	4.68 ± 0.50^{b}
HCD Obese Control	83.57 ± 5.00^{a}	54.50 ± 2.09^{ab}	23.98 ± 1.51^{ab}	16.71 ± 1.00^{a}	13.82 ± 1.39^{a}
HCD + *A. boonei*	83.87 ± 3.56^{a}	46.39 ± 4.83^{b}	22.48 ± 3.81^{b}	16.77 ± 0.63^{a}	7.13 ± 0.7^{b}

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

Serum glucose and insulin concentrations were significantly higher ($P<0.05$) in HCD obese control, while no significant increase ($P<0.05$) was observed in leptin level when compared to the normal control. On the other hand, the HCD obese rats treated with *A. boonei* had no significant

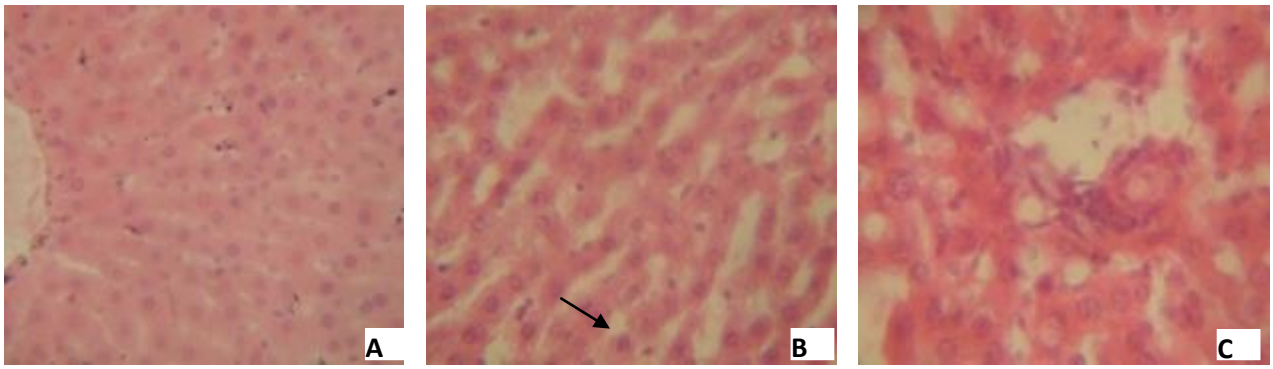
difference(P<0.05) in glucose and insulin concentration, but showed significant decrease (P<0.05) in leptin compared to the normal and HCD obese control (Table 4).

Table 4: Blood glucose, insulin and leptinof rats treated with the *A. boonei* extract

GROUP	GLUCOSE (mg/dl)	INSULIN (µlU/ml)	LEPTIN (ng/ml)
Normal Control	65.00 ± 2.89 [b]	2.33 ± 0.03 [b]	2.63 ± 0.15 [b]
HCD Obese Control	89.67 ± 3.18 [a]	2.50 ± 0.06 [a]	2.86 ± 0.09 [a b]
HCD + *A. boonei*	89.67 ± 6.06 [a]	2.40 ± 0.00 [a b]	0.80 ± 0.12 [c]

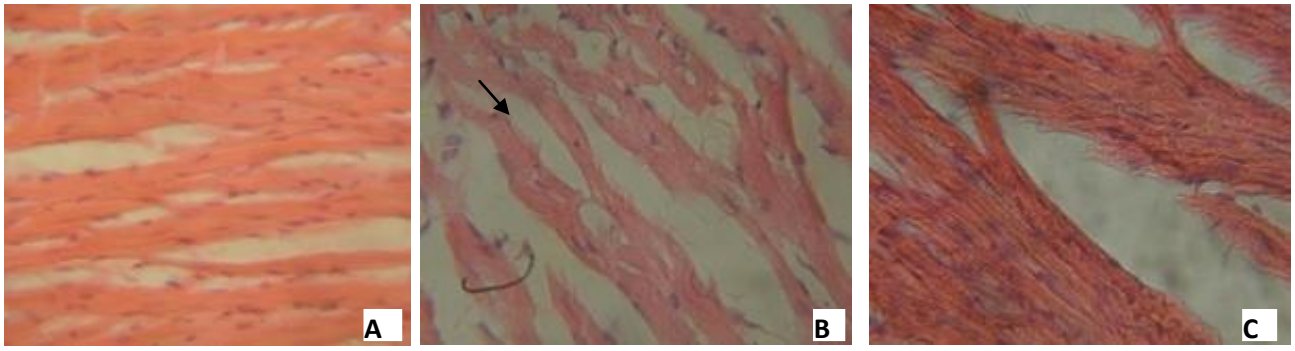
Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

In the histopathology of the liver, the cells of normal control (A) were compact; hepatocytes appeared distinct with relatively few tiny fats deposits scattered around the sinusoidal space and the central vein appeared distinct. The HCD obese control (B) had some fat deposits scattered around the sinusoidal space. Also, there was a little distortion in the architecture of the cells. The liver of *A. boonei* treated rats looked coarse as though a reaction was taking place and the fat deposits appeared reduced compared to the HCD obese control. In the histopathology of the heart, the cardiac muscle fibre of normal control (A) showed the myocardium separated by intercalated disc with no fat deposits. Similarly, no fat deposits were seen in the HCD obese and treated groups although there were signs of mild myocardial infarction.



Normal Control HCD Obese Control **HCD + *A. boonei***

Plate 1: Sections of liver of normal control (A) without fat deposits, HCD obese control (B) with fat deposits and treated animals (C) showed reduced fat deposits (X40).



Normal Control HCD Obese Control HCD + *A. boonei*

Plate 2: Sections of heart of normal control (A), HCD obese control and treated animals (C) showed no fat deposits (X40).

Discussion and Conclusion

The phytochemical screening of the ethanolic extract of *Alstonia boonei* stem bark shows the presence of alkaloids, saponins, tannins, steroids and flavonoids. This is consistent with the earlier reports of Akinmoladun *et al.*[21], Amole and Ilori[22] and Onyeneke and Anyanwu[23].

The increase in food intake in the HCD groups may be due to the palatability of the diet as refined sugar (sucrose) was part of its ingredients and sucrose is a combination of glucose and fructose. Sugar metabolism occurs primarily in the liver, where a high fructose flux leads to enhanced accumulation of hepatic triglycerides, resulting in impaired glucose and lipid metabolism[24], resulting in a high level of fat in storage. The consequence of high fat storage is body weight gain or increase, and then obesity. In the present study, obesity from the continuous increase in body weight as a consequence of the HCD. Similar results had earlier been obtained in body weight changes with the feeding of a high carbohydrate diet[25, 26, 27, 28]and adiposity index[29].

Alstonia boonei caused significant decrease in food intake in HCD obese rats. This suggests that the plant might have appetite suppressing ability. The observed decrease in food intake would be accompanied with lower exposure to calorie intake which would have affected the body weight of the group treated with *A. Boonei.* In a toxicity study, it had been reported that *A.boonei* decreased significantly the body weights of rats treated for 4 and 12 weeks[30].

The significantly higher fat mass and adiposity index of HCD obese control when compared to the normal control rats corroborates the results found in other studies[31]. However, *A. boonei* extract produced a significant decrease in the total fat mass and adiposity index compared to their respective obese controls. This effect of *A. boonei* extract was similar to the result reported earlier by Onyeneke and Anyanwu[23], although it was of a high fat diet. Some plants contain saponins, polyphenols and flavonoids which have pancreatic lipase inhibitory effect[32];it inhibits these lipases from hydrolyzing the ingested fat into absorbable free fatty acids and monoglycerides. The decreased absorption of ingested fat leads to an overall decreased caloric absorption, in turn leading to weight loss[33].

It is well known that excess sugar in the human diet can be converted both into glycerol and fatty acids and, thus, into lipids such as triglycerides[34]. This explains why the triglyceride, total cholesterol, VLDL cholesterol and LDL cholesterol levels in the HCD obese rats were significantly higher than the normal control (Table 3).

The HCD obese rats had significant increase in glucose and insulin concentration. This effect is similar to that found in HFD obese rats as indicated by Zhang *et al*.[35] and Amin and Nagy[25]. The *A. boonei* extract significantly decreased leptin levels. Leptin is one of the hormones that regulate hunger and satiety. Leptin is a powerful appetite suppressant, which help to regulate cravings for sweet foods[36].Leptin levels are excessively high in obese people and this leptin resistance is associated with weight gain. In this study, *A. boonei* extract decreased leptin level which might suggest that the plant possess appetite suppressant ability or ability to boost leptin sensitivity. Its low level will decrease quest for food by the animals and may have been responsible for the low food intake observed by the animals treated with the plant extract.

Leptin is a hormone produced mainly by adipocytes, thus leptin concentration is related to the amount and distribution of body fat[37] such that the heavier the body weight the higher the leptin concentration in human and rodents[38]. Therefore, the decreased leptin level might be due to decreased fat deposits in the treated rats which might be consequent upon some bioactive components in the *A. boonei* extract.

The photomicrograph of HCD obese control showed presence of numerous prominent fat deposits in the livers and this appeared reduced in group 3. There is increasing evidence that non-alcoholic fatty liver disease often represents a component of the metabolic syndrome characterized by obesity, hyperinsulinemia, peripheral insulin resistance, diabetes, hypertriglyceridemia and hypertension[39, 40].

Fat deposits were not present in the heart of HCD obese control, although there were signs of myocardial infarction which was not much different with that of the treated groups. Abnormal blood lipid levels, that is high total cholesterol, high levels of triglycerides, high levels of low-density lipoprotein (LDL, "bad" cholesterol) or low levels of high-density lipoprotein (HDL, "good" cholesterol) cholesterol all increase the risk of heart disease like myocardial infarction and stroke[41]. High total cholesterol levels, which may be caused by many factors, are associated with an increased risk of cardiovascular disease[42, 43].

Conclusion

The high carbohydrate diet formulated for this study induced obesity in the male Wistar rats and gave rise to increased food intake, body weight, fat mass, cholesterol levels, glucose levels, insulin and leptin levels; this may have implications for the progress of obesity related problems. The ethanolic extract of *A. boonei* stem bark which reduced the food intake, body weight, fat mass and leptin levels might possess anti-obesity potential.

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