

AN EVALUATION OF *SORGHUM BICOLOR* (Linn.) MOENCH LEAF SHEATH EXTRACT IN HIGH FAT DIET-INDUCED OBESE RATS

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ABSTRACT

Obesity, resulting from energy imbalance caused by an increased ratio of caloric intake to energy expenditure is a global health problem. This study investigated the effects of *Sorghum bicolor* leaf extracts (SBE) on blood lipid levels in high fat diet-induced obese rats. *Sorghum bicolor* leaf was extracted with 70% ethanol using Soxhlet extractor. Male Wistar rats (n=36) divided into six groups: (A) normal control, (B) obese control (High Fat Diet–HFD), (C) HFD+150 mg/kg body weight SBE (first treatment), (D) HFD+300 mg/kg body weight SBE (second treatment), (E) HFD+600 mg/kg body weight SBE (third treatment) and (F) HFD+ 1200 mg/kg body weight SBE (fourth treatment) were used. The SBE was administered orally to the animals daily for 28 days. Body weight was taken weekly and Lee index was calculated alongside. Plasma, erythrocytes and lipoproteins' lipid profiles were determined using spectrophotometric methods. Results from the study revealed that generally, SBE significantly reduced ($p<0.05$) the levels of cholesterol, phospholipids, triacylglycerol by varying levels, in the various blood compartments of the obese animals. It was observed that SBE caused a significant ($p<0.05$) decrease in plasma cholesterol level of the fourth treatment group. Also, SBE significantly ($p<0.05$) reduced the elevated atherogenic and coronary risk indices by 50%. This study suggests that SBE might be useful in the management of obesity and its co-morbid conditions.

Keywords: Obesity, *Sorghum bicolor*, management, plasma, lipoproteins

INTRODUCTION

Obesity is a chronic condition in which the adipose tissue mass is increased and leads to increase in body weight due to accumulation of fat. It is a global health problem of the 21st century which results from energy imbalance caused by an increased ratio of caloric intake to energy expenditure (Ramgopal *et al.*, 2010). In developing countries, the prevalence of obesity is rising continuously, and is now occurring even at younger ages (Greenberg and Obin, 2006). The accumulation of fat is essentially the only way body weight becomes excessive, especially as the other energy storage tissues like the muscle and liver do not have the potentials of the adipose tissue to exceed requirement limits (Sikaris, 2004). The adipose tissue is the storage tissue for triacylglycerol and also an endocrine organ which releases chemical messengers called adipokines used for communication with other tissues thereby controlling metabolic functions (Kershaw and Flier, 2004). Obesity is usually defined by Body Mass Index (BMI), which equals person's weight in kilograms (kg) divided by the height in meters square (m^2), with values greater than 29.9 considered obese (Greenberg and Obin, 2006).

According to WHO report (2013), about 2.8 million adults die due to being overweight and /or obese. Also, 44% of diabetic patients, 23% of ischemic heart problem and 7% to 41% cases of endometrial, breast and colon cancers have been attributed to overweight and obesity (WHO, 2013). Many factors interact at diverse levels to influence energy balance; therefore, determining the etiology and developmental changes leading to obesity is cumbersome (Bray and Popkin, 1998). As far back as 1983, Hartz *et al.* (1983), in their studies, observed that obesity is associated with dyslipidemia, atherosclerosis, type 2 diabetes, hypertension, hypercholesterolemia, heart attack and osteoarthritis. Besides these hazards, obesity is also associated with infertility in males, polycystic ovary syndrome and breast cancer in females (Vanltallie, 1992). Though, the interaction of genetics and environmental factors is the most accepted model for obesity, the relative importance of these two factors varies across populations (Bouchard and Perusse, 1993). Amongst the causes of obesity, the most important has been linked to diet (Jyothsna1 and Kumar, 2015). This is because the intake of high fat diet and high carbohydrate foods tend to

increase weight (Bleich *et al.*, 2008). Also in children, decreased physical activity and increased energy intake (as sugar or fat) has been linked to obesity (Butte, 2000; Robinson, 2001). Induction of obesity using high fat diets in animal models has been recognized and diet-induced obesity known as “DIO” animal models which is used in investigating obesity outcomes is used as a resemblance for human obesity, though DIO models differ in macronutrient composition (Thibault *et al.*, 2004). The macronutrient composition of DIO models differ irrespective of the percentage contribution of fat to energy in the diet; fatty acid composition plays a significant role in body weight regulation, although, all fat are not equally obesogenic (Kien *et al.*, 2005).

Saturated fatty acids are stored in the adipose tissue rather than being used as fuel, whereas, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) are mainly used as fuel (Moussavi *et al.*, 2008). There is evidence that saturated fatty acid (SFA) intake is positively correlated with obesity (van Dijk *et al.*, 2009; Phillips *et al.*, 2009). However, monounsaturated fatty acid (MUFA) intake reduces the impact of a high fat diet on obesity (Bos *et al.*, 2010; Lai *et al.*, 2009). It has also been suggested that fatty acids and their composition influence the biological characteristics of adipocytes (van Dijk *et al.*, 2009).

Factors such as periodic feeding, circadian rhythm and pattern of feed intake, size, frequency and duration of meals, intermeal interval, satiety ratio and the rate of feeding have been reported to play a significant role in the development of obesity in rodents and humans (Chapelot *et al.*, 2006; Arble *et al.*, 2009). High fat diets have been reported to induce alterations in eating pattern (Bellisle, 2004; Savastano and Covasa, 2005). This alteration was linked to larger meal sizes due to low intra-meal satiety signals, decrease in sensitivity to food intake-lowering effect of cholecystokinin and less control over appetite due to infrequent meals which lead to higher energy intake (Bellisle, 2004; Savastano and Covasa, 2005). Feeding at different circadian time other than the normal nocturnal feeding time has been associated with high risk of obesity in rodents as well as humans (Ma *et al.*, 2003; Arble *et al.*, 2009). The expression of clock genes like *Clock* and *Bmal 1* in the hypothalamus

and in other cells of the body, affect physiological activities such as eating, lipid and glucose metabolism (Rudic *et al.*, 2004). High fat diets and obesity attenuate the expression of these clock genes in the liver and adipose tissue, hence they affect metabolism and body weight balance (Rudic *et al.*, 2004). In obesity, there is an increase in the release of whole body free fatty acid and glycerol from adipocytes due to deficiency of perilipins which prevent lipases from hydrolyzing triacylglycerols to facilitate free fatty acid release (Zhang *et al.*, 2003). Proinflammatory factors like interleukin 6, TNF- α , tissue factor nitric oxide synthase are produced in the adipose tissue as obesity increases; also macrophages are increased with obesity where they effect the production of cytokines in the adipose tissue (Greenberg and Obin, 2006). These inflammatory factors promote free fatty acid release from adipose tissue into blood stream to act on other tissues such as the liver and muscle and consequently result in systemic insulin resistance, thus adipose tissue inflammation is associated with obesity-related complications (Greenberg and Obin, 2006).

Obesity increases VLDL (triacylglycerols) through increased production and decreased clearance of triacylglycerol rich lipoproteins due to lack of stimulation of lipoprotein lipase; and also lowers HDL in men and women of all ages (Sikaris, 2004). Plasma lipid dynamics have been widely studied during various metabolic malfunctions due to their involvement in vascular disorders (Rotimi *et al.*, 2015). The lipid composition of erythrocytes is presently becoming of great interest as the aetiology of many diseases has been linked to erythrocyte lipid abnormalities (Allen and Manning, 1994). Several studies have shown that erythrocyte lipid is composed mostly of unesterified (free) cholesterol, phospholipids and little cholesterol esters which are different from that of the plasma environment (Suda *et al.*, 2002). Despite this difference in the lipid composition of the erythrocyte and its plasma environment, circulating mature erythrocytes from mammals are limited in their lipid metabolism and there is little or no evidence of *de novo* synthesis of lipid by the erythrocyte (Rotimi *et al.*, 2015). Also, *in vivo* and *in vitro* evidences have suggested that one of the major pathways for replacement of

erythrocyte lipids is through exchange with plasma lipids (Rotimi *et al.*, 2015). For instance, a rapid rate of exchange of lipids between HDL and erythrocytes has been reported (Nikolic *et al.*, 2004; 2007; Meurs *et al.*, 2005).

Obesity has been managed and treated by weight-loss programs involving dietary changes, exercise and activity, behavioural changes, weight-loss prescriptions and on rare cases, weight-loss surgery (Shick *et al.*, 1998; Stoppler, 2012). In some researches using laboratory animals like rats, ad libitum low-fat diets caused weight loss and reversed dietary obesity, while in other studies, the approach was inefficient (Wade, 1983; Huang *et al.*, 2004). The use of traditional medicines and/or medicinal plants as a cheap remedy accessible to all and as therapeutic agents for the maintenance of good health in developing countries has been reported (UNESCO, 1998). Reports showed that about 80% of the population of developing countries relies on traditional medicines for their primary health care (Schmincke, 2003). Various parts of these medicinal plants which include leaves, stems, flowers, fruits, barks and roots, are used in the preparation of decoctions (Schmincke, 2003). Amongst these medicinal plants, is *Sorghum bicolor* (Linn.) Moench - a tropical cane-like grass, belonging to the family Poaceae plant (Odugbemi, 2008). According to previous research, *Sorghum bicolor* leaf sheath is a rich source of phytochemicals which are tannins, saponins, simple phenolic acids, flavonoids, alkaloids, anthocyanins, phytosterols, and policosanols. Some of these bioactive components have been reported to have antioxidants (Kim *et al.*, 2010; Sikwese and Duodu 2007). Awika and Rooney (2004) reported that the consumption of *S. bicolor* reduced the risk of cancer in humans compared to other cereals and also promoted cardiovascular health in animals. Tannins in *S. bicolor* are widely reported to decrease caloric availability and hence reduce weight gain in animals, indicating its potential in reducing obesity in humans (Awika and Rooney, 2004). Tsuda (2003) reported the inhibitory effect of anthocyanins extracted from *S. bicolor* on increases in both body weight and adipose tissue weight of rats consuming high-fat diets. Also, extracts of *S. bicolor* leaf sheath have

been reported to possess antilipidemic activity (Chung *et al.*, 2010).

Therefore, this study was conducted to investigate the possibility of managing dyslipidemic complications associated with obesity using *Sorghum bicolor* leaf extract (SBE) in high fat diet-induced obese rats.

MATERIALS AND METHODS

Collection and Authentication of Plant

Bundles of *Sorghum bicolor* leaf sheath were purchased from Rukayat herbarium in Abeokuta, Ogun state. The bundles were authenticated by Dr. O. R. Adeyemi, a Weed Scientist and Dr. A. S. Oyelakin, a taxonomist in the Federal University of Agriculture, Abeokuta where a voucher specimen was deposited (*Voucher No: FUNAABH0021*).

Preparation of Plant Extract

The bundles of *Sorghum bicolor* were rinsed in clean water and thereafter air-dried in the laboratory. The dried plant was pulverized and extracted with 70% ethanol using a Soxhlet extractor, Thermo scientific model, UK. The extract obtained was concentrated using a rotary evaporator and then air-dried to obtain a solid powdery mass which was dissolved in distilled water for oral administration to experimental animals.

Animals

Thirty-six young male Wistar rats (5-6 weeks old) weighing 50 ± 10 g were used. They were housed in cages under ambient conditions and allowed to acclimatize for two weeks before commencement of obesity induction using high fat diet. All procedures used followed the *Principles of Laboratory Animal Care* from NIH Publication No. 85-23 and were approved by the Ethics Committee of the College of Natural Sciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Phytochemical Analysis of Plant Extract

Quantitative determination of phytochemicals in the *Sorghum bicolor* leaf was carried out according to the methods described for saponins (Brunner, 1984), tannins (Swain, 1979), phenols (AOAC,

1984), alkaloids (Henry, 1973) and flavonoids (Adeyemi and Olorunsanya, 2012).

EXPERIMENTAL DESIGN

Induction of Obesity and Treatment

Protocol

The animals were divided into 6 groups consisting of 6 animals each. Group A served as the normal control and was fed with normal diet throughout

the experiment; while the remaining animals were fed *ad libitum* with high fat-diet to induce obesity (Tuzcu *et al.*, 2011). They were fed this diet continuously for twenty-five weeks and were judged obese with the use of Lee index, defined by the body weight of the animal in grams divided by the naso-anal length in centimeters (Bernadis and Patterson, 1968). Rats with the Lee index of

Table 1: Composition of diets in g/Kg.

| Constituents | Normal diet | High fat diet |
|---------------------|-------------|---------------|
| Casein | 200 | 200 |
| Starch | 615 | 145 |
| Sucrose | - | 150 |
| Corn oil | 80 | - |
| Beef tallow | - | 400 |
| Cellulose | 50 | 50 |
| Vitamin-Mineral Mix | 50 | 50 |
| DL-Methionine | 3 | 3 |
| Choline chloride | 2 | 2 |

The vitamin-mineral mix is made up of all-*trans*-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-*rac*- α -tocopherol acetate, 12.5 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B-6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B-12, 0.02 mg; folic acid, 0.55 mg; *d*-biotin, 0.1 mg; manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

Thereafter, the obese rats were distributed according to their Lee index into five groups. Group B served as the obese control group and was fed only high-fat diet (HFD) throughout the experiment while groups C to F were orally administered daily with *Sorghum bicolor* leaf extract (SBE) while also still on HFD for the rest of the experiment. The animals in groups C to F were administered 150 mg/kg (first treatment), 300 mg/kg (second treatment), 600 mg/kg (third treatment) and 1200 mg/kg body weight SBE (fourth treatment) respectively.

Determination of Body Weight

Body weights of animals were determined once a week using a measuring scale and the change in

body weight was noted. Lee index of each rat was also calculated.

Sample Collection

After four weeks of extract administration, the animals were sacrificed after an overnight fast under light ether anaesthesia. Blood was drawn from the animals through cardiac puncture into heparinized tubes and the plasma was separated from the erythrocytes by centrifuging the whole blood at 3500 rpm for 10 minutes. The erythrocytes were washed thrice with normal saline to remove extraneous materials.

Biochemical Analyses

Separation of HDL Fraction of Plasma

The HDL fraction of the plasma was separated after very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were precipitated using heparin-MnCl₂ solution (Gidez *et al.*, 1982). Heparin-manganese chloride solution (1 volume) was added to 10 volume of plasma in a test tube. The resultant mixture was thoroughly mixed and allowed to stand at 25°C for 10 min. It was then centrifuged at 4000 rpm for 10 min. The supernatant was decanted into Eppendorf tubes and stored in the refrigerator until further analysis.

Extraction of Lipids from Plasma, HDL and VLDL+LDL Fractions

Plasma, HDL and VLDL+LDL lipids were extracted using chloroform-methanol mixture (2:1, v/v) (Folch *et al.*, 1956). Aliquot (0.1 ml) of the plasma and HDL fraction were measured into test tubes and 0.9 ml chloroform-methanol mixture was added. The mixture was mixed thoroughly and allowed to stand at 25°C for 30 min. The mixture was then centrifuged at 4000 rpm for 10 min. The chloroform layer was removed into a separate tube with a Pasteur's pipette. The VLDL+LDL precipitate was first returned to the original volume of plasma from which HDL had been precipitated with 0.1M phosphate buffer pH 7.4, before the extraction process was carried out.

Extraction of Lipids from the Erythrocyte

Chloroform-isopropanol mixture (7:11 v/v) was used to extract lipids from the erythrocytes (Rose and Oklander, 1965). To 0.1 ml of the washed erythrocytes, 0.9 ml chloroform-isopropanol mixture (7:11, v/v) was measured into test tube, mixed and left to stand for 1 hour for complete extraction of lipids. The suspension was then centrifuged at 4000 rpm for 10 min and the supernatant containing the lipid was removed using Pasteur's pipette and stored at -4°C till further analysis.

Determination of Cholesterol, Triacylglycerol and Phospholipids Concentrations

The concentrations of cholesterol in the plasma and HDL were determined using commercially available diagnostic kits. To determine cholesterol concentrations in VLDL+LDL and erythrocytes, 0.1 ml of the lipid extracts were evaporated to dryness at 50°C. For erythrocyte cholesterol determination, 20 µl of Triton X- 100 : chloroform mixture (1:1, v/v) was added to the dried extract and this was evaporated again. Then 1.0 ml of the cholesterol kit reagent was added, mixed and incubated for 30 min before reading the absorbance at 505nm against a reagent blank.

Triacylglycerol concentrations were also determined in the plasma and HDL using commercially available diagnostic kits. To determine triacylglycerol concentrations in VLDL+LDL and erythrocytes, 0.1ml of the lipid

extracts were evaporated and 0.1 ml of 97% ethanol was added to re-suspend the dried lipid extract. To this, 1.0 ml of the triacylglycerol kit reagent was added, mixed and incubated for 30 minutes before the absorbance was read against a reagent blank at 505 nm.

Determination of phospholipid concentration was assessed using 0.1N ammonium ferrothiocyanate (Stewart, 1979). Aliquots of the lipid extracts of plasma, HDL, VLDL-LDL and erythrocytes (0.1 ml) were evaporated to dryness at 50°C. After cooling, 2 ml chloroform was added to each of the dried extract, mixed by inversion and 2 ml of ammonium ferrothiocyanate was then added and mixed by inversion for 1 minute. The mixture was left for 10 min for separation to occur. The chloroform layer was later removed into a glass cuvette and the absorbance was read at 488nm against a reagent blank.

Estimation of VLDL - and LDL – Cholesterol Concentrations

The concentrations of VLDL-cholesterol and LDL-cholesterol were calculated by modification of Friedewald's formulae (Sandkamp *et al.*, 1990).

$$\text{VLDL-cholesterol (mg/dl)} = \frac{\text{Triacylglycerol (mg/dl)}}{5}$$

$$\text{LDL-cholesterol (mg/dl)} = \text{Total plasma cholesterol (HDL-cholesterol + VLDL-cholesterol)}$$

Estimation of Atherogenic and Coronary Risk Indices

Atherogenic index was calculated as the ratio of LDL-cholesterol and HDL-cholesterol while Coronary risk index was estimated as the ratio of plasma total cholesterol and HDL-cholesterol as described by Ademuyiwa *et al.* (2008).

Statistical Analysis

Results are expressed as mean ± S.E.M. One-way Analysis of Variance (ANOVA) followed by Tukey's test was used to analyze the results with $p < 0.05$ considered significant. Relationships among the parameters were assessed by using Pearson's correlation.

RESULTS

Table 2 shows the levels of phytochemicals in SBE. SBE contained 11.5g/100g phytochemicals. Alkaloids, flavonoids, tannins, saponins and

phenols make up 15%, 26%, 19%, 31% and 8% respectively. The quantity of phytochemicals in decreasing order is Saponins > Flavonoids > Tannins > Alkaloids > Phenols.

Table 2: Levels of phytochemicals in SBE

| Phytochemical | Composition (g/100g) |
|---------------|----------------------|
| Alkaloids | 1.78 |
| Flavonoids | 2.98 |
| Phenols | 0.96 |
| Saponins | 3.62 |
| Tannins | 2.16 |

Table 3: Body weight gain (g) and Lee indices

| Group | Body Weight gain (g) | Initial Lee index | Final Lee index |
|-------|-------------------------|------------------------|------------------------|
| A | 45.40±1.78 ^b | 0.30±0.00 ^a | 0.28±0.00 ^a |
| B | 70.00±0.45 ^d | 0.32±0.00 ^b | 0.31±0.00 ^b |
| C | 35.00±1.58 ^a | 0.31±0.00 ^b | 0.29±0.00 ^a |
| D | 64.00±1.58 ^c | 0.31±0.00 ^b | 0.29±0.00 ^a |
| E | 55.00±2.24 ^c | 0.32±0.00 ^b | 0.29±0.01 ^a |
| F | 46.00±1.76 ^b | 0.31±0.00 ^b | 0.29±0.02 ^a |

Values are mean ± S.E.M (n=6). Values in the column with different superscripts are significantly different from one another at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150mg/kg body weight SBE (First treatment), D = HFD+300mg/kg body weight SBE (Second treatment), E = HFD+600mg/kg body weight SBE (Third treatment), F = HFD+1200mg/kg body weight SBE (Fourth treatment)).

Weight gain and Lee index of the animals are presented in Table 3. There was a significant ($p < 0.05$) increase of 43% in the weight gain of the obese control (group B) over that of the normal control (group A). SBE reduced the weight gain of the treated groups by 50%, 9%, 21% and 34% in groups C, D, E and F respectively compared with group B. SBE lowered the Lee index of the animals to an insignificant ($p > 0.05$) value which was not different from that of group A, the normal control.

Figs. 1-2 depict cholesterol levels in the plasma, erythrocyte and the lipoproteins. Plasma cholesterol level of group B was the highest and it was significantly different ($p < 0.05$) from the values obtained for the treated groups with 8%, 9%, 11% and 35% for groups C, D, E and F respectively. SBE elevated the HDL-cholesterol

level of the treatment groups by 3-fold that of group B. Also, SBE reduced the LDL-cholesterol level of the treated groups by 60% in groups C and D, 54% and 75% in groups E and F when compared with group B. A significant ($p < 0.05$) increase of 46% in the VLDL-cholesterol level of group B in comparison with group A was observed. SBE reduced the VLDL-cholesterol concentration between 1.4% and 29% in the treatment groups. Erythrocyte cholesterol concentration of group B was 3-fold that of group A. SBE reduced the cholesterol concentrations of the obese rats by 23% - 57%. Erythrocyte cholesterol levels of the treatment groups were however still significantly ($p < 0.05$) higher than that of group A by 27% - 130%. The treatment group E administered 1200 mg/kg body weight SBE was the most efficient.

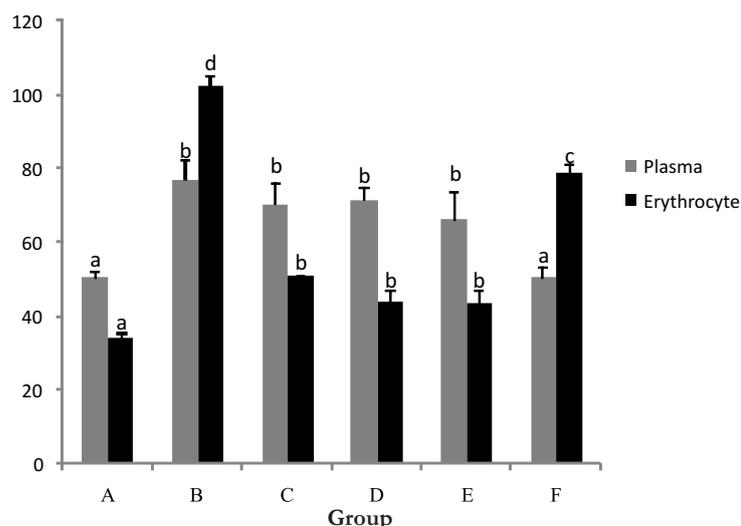


Fig. 1. Cholesterol Concentrations in Plasma and Erythrocyte of the Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150mg/kg body weight SBE (First treatment), D = HFD+300mg/kg body weight SBE (Second treatment), E = HFD+600mg/kg body weight SBE (Third treatment), F = HFD+1200mg/kg body weight SBE (Fourth treatment)).

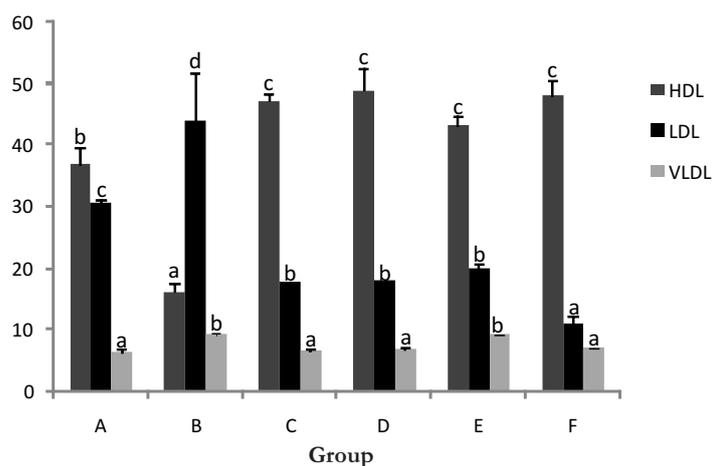


Fig. 2. Cholesterol Concentrations in the Lipoproteins of the Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150mg/kg body weight SBE (First treatment), D = HFD+300mg/kg body weight SBE (Second treatment), E = HFD+600mg/kg body weight SBE (Third treatment), F = HFD+1200mg/kg body weight SBE (Fourth treatment)).

Plasma, erythrocyte and lipoprotein triacylglycerol levels are depicted in Figs. 3-4. There was a significant increase ($p < 0.05$) of 60% in the total triacylglycerol level of group B when compared with group A. Administration of SBE reduced the total triacylglycerol level by 30%, 8%, 22% and 36% for groups C, D, E and F respectively in comparison with group B. A 1.74-fold increase was observed in the HDL-triacylglycerol level of group C with oral administration of 150 mg/kg

body weight SBE. In addition, SBE reduced the VLDL+LDL-triacylglycerol level by 56%, 48%, 47% and 69% in groups C, D, E and F accordingly when compared with group B. The fourth treatment proved to be the best treatment. Erythrocyte triacylglycerol concentration of group B was 1.4-fold higher than group A. SBE reduced the triacylglycerol concentration of the obese rats by 5% to 36%.

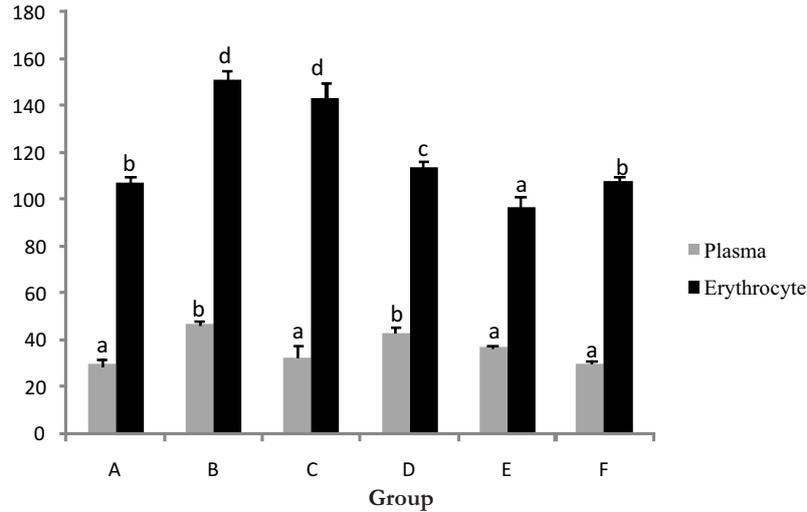


Fig. 3. Triacylglycerol Concentrations in Plasma and Erythrocyte of the Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150 mg/kg body weight SBE (First treatment), D = HFD+300 mg/kg body weight SBE (Second treatment), E = HFD+600 mg/kg body weight SBE (Third treatment), F = HFD+1200 mg/kg body weight SBE (Fourth treatment)).

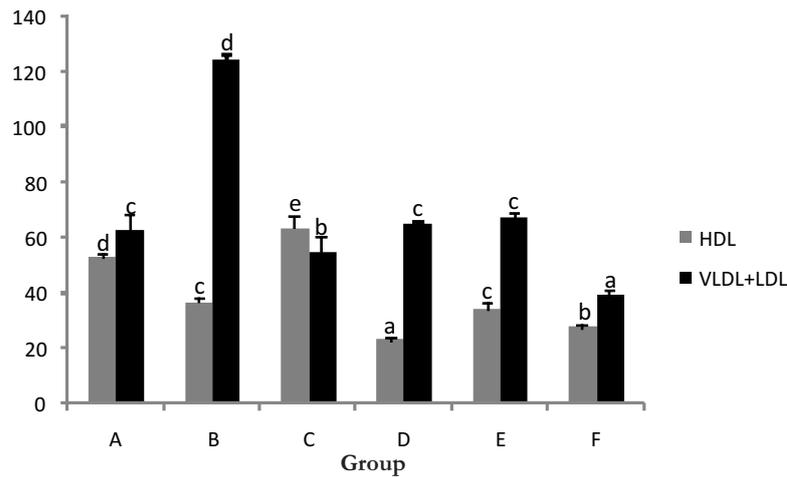


Fig. 4. Triacylglycerol Concentrations in the Lipoproteins of the Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150 mg/kg body weight SBE (First treatment), D = HFD+300 mg/kg body weight SBE (Second treatment), E = HFD+600 mg/kg body weight SBE (Third treatment), F = HFD+1200 mg/kg body weight SBE (Fourth treatment)).

In Figs. 5-6, plasma, erythrocyte and lipoproteins phospholipid level are depicted. Significant increase of 320% and 89% were obtained in the plasma and VLDL+LDL-phospholipids levels of group B when compared with group A. In the HDL-phospholipids, a significant ($p < 0.05$) reduction of 46% was observed in group B compared with group A. SBE decreased the total phospholipid level by 4.84, 2.28, 1.34 and 1.09 fold in groups C, D, E and F accordingly, when compared with group B. The level of HDL-phospholipid was increased by 39%, 85%, 115%

and 81% in groups C, D, E and F respectively in comparison with group B. Moreover, SBE diminished the VLDL+LDL-phospholipids level by 33%, 36%, 40% and 38% in groups C, D, E and F respectively when compared with group B. Erythrocyte phospholipid concentration of group B was 1.6-fold higher than that of group A. SBE reduced the phospholipid concentrations of the obese rats by 11% to 41% when compared with group B.

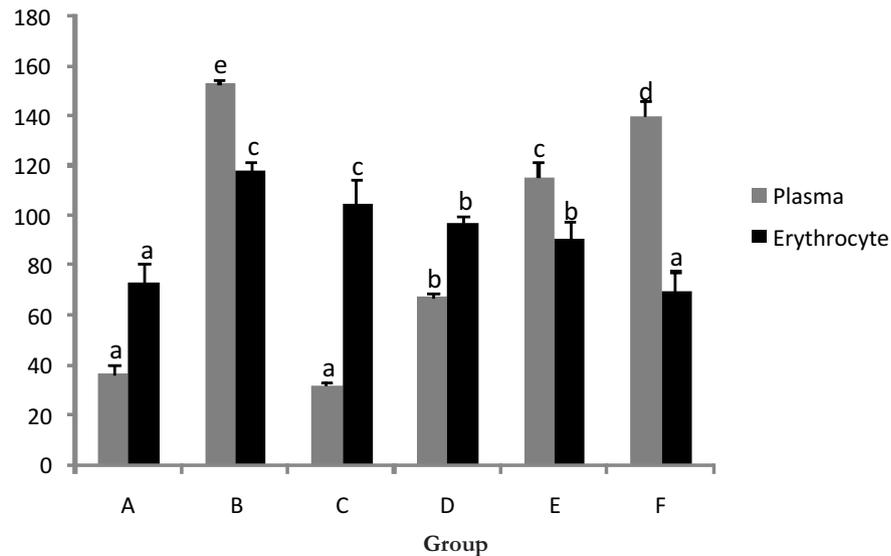


Fig. 5. Phospholipid Concentrations in Plasma and Erythrocyte of the Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150 mg/kg body weight SBE (First treatment), D = HFD+300 mg/kg body weight SBE (Second treatment), E = HFD+600 mg/kg body weight SBE (Third treatment), F = HFD+1200 mg/kg body weight SBE (Fourth treatment)).

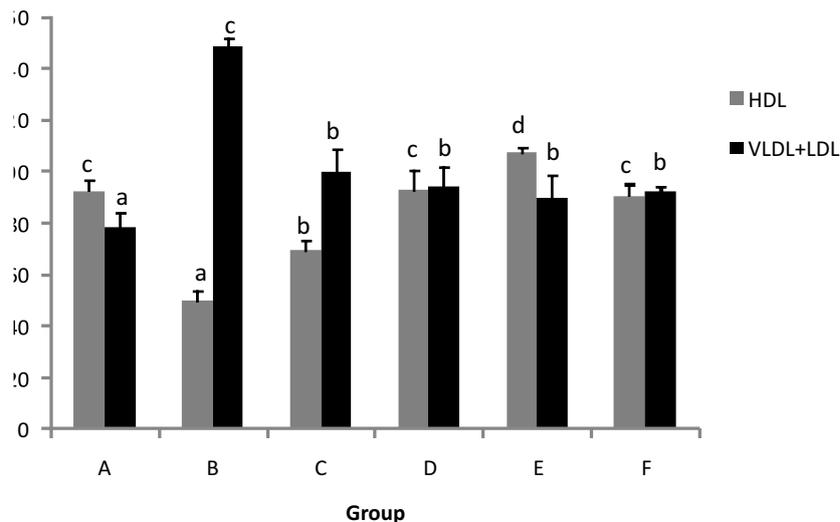


Fig. 6. Phospholipid Concentrations in the Lipoproteins of the Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150 mg/kg body weight SBE (First treatment), D = HFD+300 mg/kg body weight SBE (Second treatment), E = HFD+600 mg/kg body weight SBE (Third treatment), F = HFD+1200 mg/kg body weight SBE (Fourth treatment)).

Fig. 7 present the coronary risk and atherogenic indices of the animals. Group B had a value thrice that of group A. The extract brought about a decrease in the coronary risk index of the treatment groups by 65% in group C, 70% in group D and E, while the percentage reduction in group F was 76% when compared with group B. The values obtained for groups C, D, E and F were significantly ($p < 0.05$) similar to the value obtained for group A. SBE had the same effect on the

atherogenic index of the animals. Group B had an atherogenic index that was 4-fold that of group A. Administration of SBE significantly lowered the index to values lesser than that of group A (45%, 47%, 64% and 90% for groups C, D, E and respectively). The most efficient treatment was however the fourth treatment which reduced the coronary risk and atherogenic indices drastically. Table 4 presents intensity of associations between body weight gain, atherogenic and coronary risk

indices with blood lipid profiles in the animals. Significant positive and negative associations were observed among the parameters. While there was a positive relationship between body weight gain and HDL-phospholipid ($r = 0.403$; $p = 0.027$), there was a negative association between atherogenic and coronary risk indices and HDL-

phospholipid ($r = -0.722$ and $r = -0.726$ at $p < 0.001$ respectively). The reverse was observed for erythrocyte cholesterol level. It positively correlated with atherogenic and coronary risk indices and negatively associated with body weight gain of animals significantly.

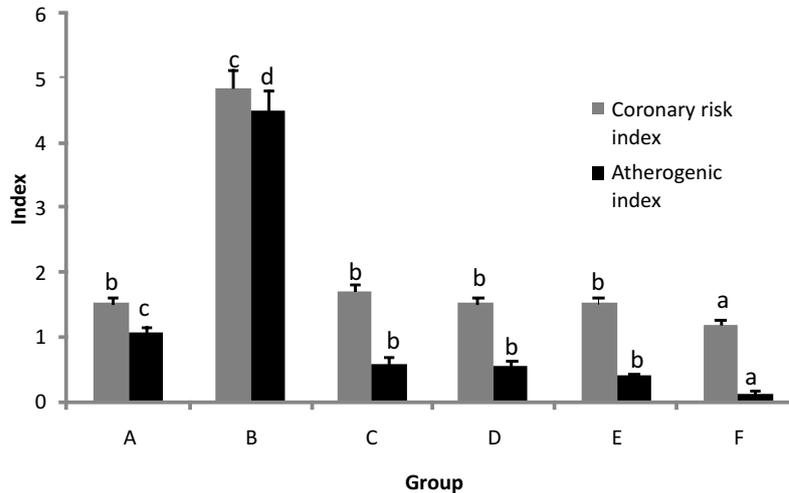


Fig. 7. Coronary Risk and Atherogenic Indices of Animals.

Bars carrying different letters of the alphabet are significantly different from each other at $p < 0.05$. (A = Normal Control, B = Obese control, C = HFD+150 mg/kg body weight SBE (First treatment), D = HFD+300 mg/kg body weight SBE (Second treatment), E = HFD+600 mg/kg body weight SBE (Third treatment), F = HFD+1200 mg/kg body weight SBE (Fourth treatment)).

There was a negative correlations between HDL-cholesterol and atherogenic and coronary risk indices ($r = -0.894$ and $r = -0.878$ at $p < 0.001$). Aside this, the indices associated positively with the plasma and VLDL+LDL lipid profiles and selectively associated with erythrocyte cholesterol and phospholipid. Among these significant associations, the highest positive significant association was observed between atherogenic index and VLDL+LDL-triacylglycerol while the lowest positive association was with plasma cholesterol ($p < 0.05$).

DISCUSSION

Obesity is a global health problem which is related to several disease conditions including cardiovascular disease and diabetes (Shick *et al.*, 1998; Awika and Rooney, 2004; Dwivedi and Dwivedi, 2007). Due to this, a number of weight loss regimens which include diets, pill and so on has emerged (Awika and Rooney, 2004). However, these regimens do not seem to produce desired

effects and obesity cases continue to be on the increase (Shick *et al.*, 1998; Hill et al., 2003; Wyatt, 2003). Consumption of food with high calorie and a lifestyle that lack physical activity account for most obesity cases (Awika and Rooney, 2004). However, the expected change in either of these factors, as demanded by many weight loss strategies, is not easily sustained for long periods of time, hence the failure of these strategies to produce that desired impact on obesity (Shick *et al.*, 1998; Awika and Rooney, 2004). The use of traditional medicines and/or medicinal plants for the maintenance of good health in developing countries has been reported (UNESCO, 1998) and reports have shown that about 80% of the population of developing countries rely on traditional medicines like *Sorghum bicolor* for their primary health care (Schmincke, 2003).

From this study, it was observed that SBE administration was effective in reducing the body fat mass as depicted by reduced weight gain and

Table 4: Intensity of Associations between Body Weight Gain, Atherogenic and Coronary Risk Indices with Blood Lipid Profiles in the Animals

| Parameter | Correlation coefficient | P-value |
|--------------------------------|-------------------------|---------|
| Body weight gain vs. | | |
| Plasma phospholipids | -0.441 | 0.015 |
| HDL-phospholipids | 0.403 | 0.027 |
| VLDL+LDL phospholipids | -0.524 | 0.003 |
| RBC cholesterol | -0.620 | <0.001 |
| Atherogenic index vs. | | |
| Plasma cholesterol | 0.374 | 0.042 |
| Plasma triacylglycerol | 0.540 | 0.002 |
| Plasma phospholipids | 0.431 | 0.017 |
| HDL-cholesterol | -0.894 | < 0.001 |
| HDL-phospholipids | -0.722 | < 0.001 |
| VLDL+LDL triacylglycerol | 0.923 | < 0.001 |
| VLDL+LDL phospholipids | 0.785 | < 0.001 |
| VLDL-cholesterol | 0.527 | 0.003 |
| LDL-cholesterol | 0.816 | < 0.001 |
| RBC cholesterol | 0.683 | < 0.001 |
| RBC phospholipids | 0.538 | < 0.001 |
| Coronary risk index vs. | | |
| Plasma cholesterol | 0.449 | 0.013 |
| Plasma triglycerides | 0.571 | 0.001 |
| Plasma phospholipids | 0.465 | 0.010 |
| HDL-cholesterol | -0.878 | <0.001 |
| HDL-phospholipids | -0.726 | < 0.001 |
| VLDL+LDL triglycerides | 0.902 | < 0.001 |
| VLDL+LDL phospholipids | 0.817 | < 0.001 |
| VLDL-cholesterol | 0.555 | 0.001 |
| LDL-cholesterol | 0.733 | < 0.001 |
| RBC cholesterol | 0.719 | < 0.001 |
| RBC phospholipids | 0.616 | < 0.001 |

Lee index. This is in agreement with the findings of Lizardo *et al.* (1995); Al-Mamary *et al.* (2001); Muriu *et al.* (2002) and Tsuda (2003) that some functional food components of sorghum reduced body weight gain and adipose tissue weight. Tsuda (2003) provided evidence that anthocyanins present in *S. bicolor* inhibited increases in both

body weight and adipose tissue weight of rats consuming high-fat diets and then suggested that anthocyanins should be used as a functional food component in order to aid the prevention of obesity. On the other hand, Lizardo *et al.* (1995); Al-Mamary *et al.* (2001) and Muriu *et al.* (2002) recorded reduced weight gain in animals fed

tannin-rich sorghum. Tannin sorghums carried out this mechanism by directly binding to digestive enzymes such as sucrase, amylases, trypsin, chymotrypsin and lipases, thus inhibiting their activity (Lizardo *et al.*, 1995; Carmona *et al.*, 1996; Nguz *et al.*, 1998; Al-Mamary *et al.*, 2001).

Another finding of this study is that treatment with SBE lowered the elevated cholesterol levels in plasma, LDL and erythrocyte while significantly increasing the level in HDL. These results corroborate the findings of other researchers (Carr *et al.*, 2005; Cho *et al.*, 2008; Hoi *et al.*, 2009; Chung *et al.*, 2010; Kim and Park, 2012) that SBEs have cholesterol-lowering properties. This finding indicates that SBE possesses antilipidemic property and might be useful in the treatment of cardiovascular diseases since elevated levels of plasma and LDL-cholesterol have been associated with increased risk of the diseases, whereas increase in HDL cholesterol is known to reduce the risk of cardiovascular diseases (Kwiterovich, 2000). Cho *et al.* (2008) and Chung *et al.* (2010) reported that Sorghum hexane extracts inhibited rat liver microsomal 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase in a dose-dependent manner and also stated that fecal bile acid excretion, as well as levels of HDL cholesterol was increased, when whole sorghum, was fed to rats at 30% of diet. Our result however disagreed with that of Rooney *et al.* (1992) where blood serum total cholesterol was increased in rats fed sorghum bran. This may be due to the interference of some components of the extract with the metabolism of these lipids.

The presence of saponins and flavonoids in plant extract has been reported to be responsible for the antilipidemic property of the leaf sheath (Cheeke, 1971). Saponins are antinutritional factors which bind to cholesterol to form an insoluble complex and they have been reported to also bind endogenous cholesterol excreted through bile to prevent reabsorption of cholesterol which ultimately leads to reduction in serum cholesterol (Rosen and MacDougald, 2006; Lai *et al.*, 2009). From our results on levels of phytochemicals in SBE (Table 2), saponins had the highest proportion of 31% of the total phytochemicals. This suggests that SBE was able to decrease the levels of lipid in the blood compartments through

the mechanism of inhibiting the activity of the enzyme, HMG CoA reductase in cholesterol synthesis (Cho *et al.*, 2008; Chung *et al.*, 2010).

SBE was also shown to reverse the increased levels of triacylglycerol in plasma, erythrocyte, HDL and VLDL+LDL. Our result on plasma triacylglycerol supports the report by Kim and Park (2012), that sorghum extract significantly decreased concentration of triglycerides in streptozotocin-induced diabetic rats. However, no report was made concerning the other blood compartments. This finding indicates that SBE could be thermogenic and therefore probably be able to aid in the combustion of calories. It could also possibly affect the differentiation of adipocytes negatively thereby decreasing adipogenicity and fat cell formation by decreasing the expression of fatty acid synthesis genes as well as activating the β -oxidation genes (Athesh *et al.*, 2012).

The phospholipid levels in the plasma, HDL, VLDL+LDL and erythrocytes were elevated by high fat feeding, which was reduced by the oral administration of the extract. Obesity is linked to chronic inflammation, and altered cell-mediated immune responses are affected by multiple mechanisms, due to a positive feedback loop between local inflammation in adipose tissue and dysregulated immune cell activation on one side and production of pro-inflammatory mediators, such as leptin on the other (Benson *et al.*, 2013). The modulation of inflammatory status by bioactive constituents, particularly those present in common and traditional medicinal foods, is becoming an important factor in global preventive health management. Lee and Pan (2003) found that the sorghum residues significantly improved erythrocyte membrane integrity of fish blood cells during winter, thus maintaining normal blood fluidity and preventing RBC hemolysis induced by H₂O₂. They also suggested that the prevention of RBC hemolysis is due to the antioxidant activity of the tannins and other polyphenols present in the sorghum residue. The lipid composition of erythrocytes is now of great interest, as the aetiology of many diseases has been linked to erythrocyte lipid abnormalities (Allen and Manning, 1994). Several studies have shown that erythrocyte lipid is

composed mostly of unesterified (free) cholesterol, phospholipids and little cholesterol esters which are different from that of the plasma environment (Suda *et al.*, 2002). The erythrocyte membranes are basically made up the phospholipids which play a major role in the integrity of the membrane. Our results indicate a decreased fluidity in the erythrocytes of the obese rats considering the cholesterol to phospholipid ratio (result not shown) while the SBE-treated groups had more normal blood fluidity and the effects of pro-inflammatory mediators observed in obesity were prevented. This could be due to the antioxidant property of the SBE as it contained flavonoids, phenols and tannins which have been reported to have antioxidant properties (Awika and Rooney, 2004).

According to this study, SBE reduced the risk of the development of coronary and atherogenic diseases as depicted by decreased coronary risk and atherogenic indices in the treatment groups. LDL levels are not consistently increased in obesity; it is smaller and denser and more atherogenic. It can be suggested that SBE acts by reducing the synthesis of LDL and elevating that of HDL, thus reducing the risk of development of cardiovascular diseases. The consumption of whole grains (like sorghum) is associated with a decrease in the incidence of cardiovascular events (Jyothsna1 and Kumar, 2015). These two lipoproteins are involved in the transportation and metabolism of lipids especially cholesterol. While LDL transports cholesterol to the peripheral tissues thereby increasing risk of cardiovascular disease, HDL scavenges cholesterol from the peripheral tissues back to the liver for excretion through conversion to bile and negating the risk of developing cardiovascular diseases (Kwiterovich, 2000). The reduction of LDL-cholesterol is attributed to the presence of saponins in the extract (Cheeke, 1971), whereas the increase in HDL-cholesterol is as a result of the presence of flavonoids and phenols in the plant extract (Cheeke, 1971; Baba *et al.*, 2007).

Obesity increases VLDL (triacylglycerols) through increased production and decreased clearance of triacylglycerols-rich lipoproteins due to lack of stimulation of lipoprotein lipase. So SBE could probably decrease triacylglycerol by

increasing the stimulation of lipoprotein lipase (Sandkamp *et al.*, 1990; Sikaris, 2004). Cholesterol ester transport protein (CETP) exchanges triacylglycerols from VLDL to LDL in exchange for cholesterol esters. These result in triacylglycerol-rich LDL particles that are rapidly hydrolyzed by hepatic lipase leaving smaller denser LDL particles which can be oxidised or glycated possibly leading to less identification by the LDL receptor and decreased clearance in obesity. CETP also exchanges triacylglycerols from VLDL to HDL in exchange for cholesterol esters. This similarly results in triacylglycerol-rich HDL particles that are rapidly hydrolyzed by hepatic lipase allowing HDL to be cleared from the circulation (Sikaris, 2004). However, with the use of SBE, the atherogenic LDL particles could have been both prevented from being oxidized or glycated, and therefore easily cleared from the system probably by increased recognition by the receptors, hence the decrease in LDL lipid concentrations observed in our results.

It could therefore be concluded based on the present findings that SBE is effective in ameliorating dyslipidemia associated with diet-induced obesity and could probably be useful in managing obesity especially at the highest dose of 1200 mg/kg body weight which proved to be most effective.

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