British Journal of Pharmaceutical Research
4(12): 1416-1428, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Comparative Studies of the Effect of *Sphenocentrum jollyanum* Pierre on High Fat and High Carbohydrate Diet Induced Obesity in Rats

Gabriel O. Anyanwu[1*]

[1]Department of Biochemistry, Faculty of Science and Technology, Bingham University, Nasarawa State, Nigeria.

Author's contribution

This whole work was carried out by author GOA.

Original Research Article

Received 28th March 2014
Accepted 20th May 2014
Published 4th June 2014

ABSTRACT

Aim: The aim of this study was to compare the effect of ethanolic extract of *Sphenocentrum jollyanum* on high fat and high carbohydrate diet induced obesity in male wistar rats.
Methodology: 30 male wistar rats used for this study were divided into 5 groups of 6 rats each. Group 1 was fed the normal pellet diet (NPD), Group 2 and 3 were fed with high fat diet (HFD), Group 4 and 5 were with fed high carbohydrate diet (HCD) and all groups had free access to diets and water *ad libitum* for 18 weeks. Treatment with 500mg/kg b.w ethanolic extract of *S. jollyanum* for Group 3 and 5 started in the 14th week, that is, at the end of obesity induction and lasted for another four weeks. The extract was suspended in normal saline and administered orally to the rats using a gavage tube. Thereafter, the food intake, body weight, total fat mass, adiposity index, total cholesterol, triglycerides, high density lipoprotein cholesterol, very low density lipoprotein cholesterol, low density lipoprotein cholesterol, creatine kinase, lactate dehydrogenase, glucose, insulin and leptin were measured.
Results: The results showed that feeding with HFD and HCD significantly increased ($p<0.05$) body weight, total fat mass, adiposity index, total cholesterol (TC), triglycerides (TG), very density low cholesterol (VDL-C), low density low cholesterol (LDL-C), creatine kinanse (CK) activity, lactate dehydrogenase (LDH), glucose, insulin and leptin levels. The

**Corresponding author: Email: gabrielanyanwu@yahoo.com;*

ethanolic extract of *S. jollyanum* significantly decreased total fat mass, adiposity index, TC, TG, VDL-C, LDL-C, CK activity, LDH, glucose and leptin levels in the HFD group. While among the HCD group, *S. jollyanum* significantly decreased total fat mass, adiposity index and CK activity.
Conclusion: The high fat and high carbohydrate diet induced obesity in the wistar rats and the decrease in the lipid profile, heart biomarkers, glucose and leptin by ethanolic extract of *S. jollyanum* shows that the plant might possess anti-obesity effect.

Keywords: Obesity; diet; Sphenocentrum jollyanum; fat; carbohydrate.

1. INTRODUCTION

Obesity is a leading preventable cause of death worldwide, with increasing prevalence in adults and children, and health organizations view it as one of the most serious public health problems of the 21st century [1]. The change in the average weight of the population is occurring quickly, and within a few generations the bell-curve of human-weight distribution has shifted toward greater weight [2]. The reason for this change in body weight has gone beyond genetics alone, rather substantial changes in diet and sedentary lifestyle have contributed to the obesity epidemic.

High carbohydrate and fat diets are fast becoming the trend in developing countries like Nigeria [3]. As life gets better with increased earnings, many Nigerians take pleasure in eating foods high in fat and carbohydrates from their favorite big eateries. These foods are increasingly consumed in association with sweetened drinks, snacks, and other liquid formulations. It has been reported that the trends in soft drink consumption by children are associated with an alarming increase in the prevalence of obesity and co-morbidities in children and adolescents [4,5,6,7]. The long-term effect of increased prevalence of obesity due to trends in soft drink consumption would be early onset obesity as this generation progresses to adulthood [8].

Obesity is not just a cosmetic consideration; it is a dire health dilemma directly harmful to one's health. Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis [9]. Apart from dieting, exercising and surgery, medications such as Sibutramine and Orlistat have been used in the treatment of obesity. These synthetic drugs have side effects and are not safe for everyone. Recently, there has been a paradigm shift in research from synthetic drugs to medicinal plants which are often cheaper, locally available, and easily consumable (raw or as simple medicinal preparations) with less side effects.

In order to find a promising plant for this study, a visit was made to different traditional healers in Imo State, Nigeria. The claim by some traditional healers that *Sphenocentrum jollyanum* Pierre is used to treat obesity was the reason this herbal plant was chosen for this study. *S. jollyanum* is a deep rooted plant that grows up to 1.5m high having few branches. It has a wide range of beneficial biological and pharmacological activities. The leaves decoctions of *S. jollyanum* is used as vermifuge and in dressing wounds as first reported by Dalziel [10]. The plant is also used for treating feverish conditions, cough, jaundice, breast swelling related to menstrual cycles, as an aphrodisiac and other inflammatory conditions such as tumours [11]. There is no available information on the effect of *S. jollyanum* on

obesity. Therefore, the aim of this study was to compare the effect of ethanolic extract of *S. jollyanum* on high fat and high carbohydrate diet induced obesity in wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh roots of *S. jollyanum* Pierre were collected from a farm land behind Comprehensive Secondary School in Umuekwune, Imo State, Nigeria. The plant was collected in the month of March, 2012. The plant was identified by the Chief Research Officer (a taxonomist) at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria.

2.2 Experimental Animals

Thirty male wistar rats weighing 80-90g were used for this study. The rats were purchased from Anatomy Department, University of Benin, Nigeria. All animals were housed in steel cages and each cage contained 6 rats. Rats were maintained under controlled temperature (± 23ºC) and a 12:12h light/dark cycle. The rats were acclimatized for two weeks having free access to water and normal pellet diet (NPD) until they were divided into groups. The study was done in accordance with the guidelines of Faculty of Life Sciences at University of Benin for animal use.

2.3 Preparation of Plant Extract

The fresh roots of the plant was washed, sliced into small pieces and air-dried in the laboratory at room temperature. The dried plant roots were grinded into powder. 250g of the powdered plant was soaked in 500ml of 95% ethanol in a container for 72 hour and stirred intermittently with a stirring rod. Thereafter, it was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure. The slurry concentrate was freeze dried to get powder and was stored in a refrigerator (4ºC).

2.4 Phytochemical Screening

Phytochemical screening of the root of *S. jollyanum* Pierre was done using standard methods of analysis [12,13]. The phytochemical screening of the plant was done for saponins, flavonoids, tannins, alkaloids, steroids, terpenoids, cardiac glycosides and reducing sugars.

2.5 Acute Toxicity (LD_{50}) of Plants

The acute toxicity of the ethanolic extract of *S. jollyanum* root was carried out [14,15].

2.6 Composition of Experimental Diet

Three types of diet were formulated for the study as shown in Table 1. First, the normal pellet diet which included 60% of carbohydrate (garri), 5% of fat (butter), 30% of protein (bonga fish), 1.5% of fiber, 2.5% of multi-minerals and 1.0% of multi-vitamins. Second, the HCD which contained 80% of carbohydrate (garri 60% and sucrose 20%), 5% of fat (butter), 10% of protein (bonga fish), 1.5% of fiber, 2.5% of multi-minerals and 1.0% of multi-vitamins.

While the third was HFD which contained of 25% of carbohydrate (garri), 50% of fat (butter), 20% of protein (bonga fish), 1.5% of fiber, 2.5% of multi-minerals and 1.0% of multi-vitamins.

Table 1. Composition of Experimental Diet

Composition	Food/ Supplements	NPD (%)	HCD (%)	HFD (%)
Carbohydrates	Garri	60.0	80.0	25.0
Fat	Butter	5.0	5.0	50.0
Proteins	Bonga Fish	30.0	10.0	20.0
Fiber	Afrodak	1.5	1.5	1.5
Mineral mixture	Multi-minerals	2.5	2.5	2.5
Vitamin mixture	Multi-vitamins	1.0	1.0	1.0
Energy (KCal/g)		4.095	4.095	6.345

2.7 Induction of Obesity in the Rats

A total of 30 rats were randomly assigned into two groups, normal 6 rats and obese 24 rats. Obesity was induced in the 24 rats by feeding 12 rats with high fat diet (HFD) and the other 12 rats with high carbohydrate diet (HCD) for 14 weeks (Tables 1 and 2). Rats fed with HFD and HCD whose body weight was significantly increased compared to the normal control were considered obese [16,17].

2.8 Experimental Design and Animal Grouping

After induction of obesity, the rats were assigned in 5 groups of 6 rats per group. Group 1 was fed the NPD, Group 2 and 3 were fed HFD, Group 4 and 5 were fed HCD and all groups had free access to water *ad libitum* for 18 weeks (Table 2). Treatment with 500mg/kg b.w of *S. jollyanum* extract started in the 14th week and lasted for another four weeks. The extract was suspended in normal saline and administered orally to the rats using a gavage tube.

Table 2. Experimental design and animal grouping

Group	Nutrition for 14 weeks	Treatment for 4 weeks
1	Normal Pellet Diet (NPD)	Normal Control
2	High Fat Diet (HFD)	HFD Obese Control
3		500 mg/kg b.w *S. jollyanum*
4	High Carbohydrate Diet (HCD)	HCD Obese Control
5		500 mg/kg b.w *S. jollyanum*

2.9 Food Intake and Body Weight Measurement

The food intake was measured daily in grams (g). Food intake was calculated by subtracting the refusal and spillage for the individual solid diets per cage from the measured amount of food provided at the previous day. The average of food intake was represented in gm/day/group. The body weight of the rat was measured weekly in grams (g).

2.11 Blood Sample Preparation and Bioassays

At the end of the experiment, rats were fasted for 12 to 14 hours. After being anesthetized with chloroform, blood was collected by cardiac puncture from the rats at fasting state. The blood which was collected in plain tubes was allowed to stand for 15 minutes to coagulate before centrifugation at 3500rpm for 15 minutes at room temperature for separation of serum. The clear, non-haemolysed supernatant was separated using clean dry Pasteur pipette and stored at -20ºC. Serum was used for subsequent biochemical measurements as follows: total cholesterol [18], triglycerides [19], high density lipoprotein cholesterol [20], very low density lipoprotein cholesterol (triglycerides/5), low density lipoprotein cholesterol [21], creatine kinase activity [22], lactate dehydrogenase [23], glucose [18], insulin (DRG Insulin ELISA kit, USA) and leptin levels (DRG Leptin ELISA kit, USA).

2.12 Total Fat Mass Determination

Five different white adipose depots (Two subcutaneous and three intra-abdominal) and interscapular brown adipose tissue were harvested from each rats after abdominal incision. The fats were weighed and the sum was regarded as the total fat mass.

2.13 Adiposity Index

Adiposity index was estimated by the sum of epididymal, visceral and retroperitoneal fat pads divided by the body weight × 100, and expressed as adiposity percentage [24].

2.14 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 using SPSS 17 software. $P<0.05$ was considered to be significant.

3. RESULTS

The phytochemical constituents in the ethanolic root extract of *S. jollyanum* had alkaloid, saponin, tannin, flavonoid, cardiac glycoside, terpenoid and reducing sugar (Table 3). In the acute toxicity test, at 6400mg/kg b.w. oral dose, the rats showed signs of general weakness and sluggishness which were the major behavioral changes. These behavioral changes disappeared after 1 hour of observation. Since no death was recorded at any of the doses administered, the oral LD_{50} of *S. jollyanum* was determined as >6400mg/kg b. w. in wistar rats.

The food intake of the HCD control group was increased significantly compared to the normal control, but there was no significant difference in the HFD control group compared to the normal control at the 18^{th} week. Apart from the food intake, there was no significant difference in the body weight, fat mass and adiposity index between the HFD and HCD obese controls. The food intake of the HFD and HCD groups treated with *S. jollyanum* was not significantly decreased compared to HFD obese control and HCD obese control. The body weight of the HFD and HCD obese controls significantly increased compared to the normal control, however, the obese rats treated with *S. jollyanum* significantly decreased in

total fat mass and adiposity index, but not in body weight when compared to the HFD and HCD obese controls (Table 4).

Table 3. Phytochemical constituents of the ethanolic extract of *S. jollyanum*

S/No.	Phytochemical Constituents	*S. jollyanum* (root)
1.	Alkaloid	+
2.	Saponin	+
3.	Tannin	+
4.	Steroid	-
5.	Flavonoid	+
6.	Cardiac glycosides	+
7.	Terpenoids	+
8.	Reducing sugar	+

(+) = Presence, (-) = Absent

Table 4. Food intake, body weight, fat mas and adiposity index of rats treated with the 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^{d}	3.29±0.18^{d}
HFD Obese Control	57.33±1.26^{b}	395.00±6.35^{a}	31.30±0.59^{a}	5.17±0.16^{a}
HFD + *S. jollyanum*	58.17±1.82^{b}	379.67±9.77^{ab}	27.05±0.78^{b}	4.29±0.11^{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 + *S. jollyanum*	65.94±2.07^{c}	384.67±11.86^{ab}	24.36±0.58^{c}	3.54±0.24^{c}

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

As shown in Table 5, the HFD and HCD obese rats had significantly increased serum triglycerides (TG), total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C), with a decrease in high density lipoprotein cholesterol (HDL-C) which was not significant. The HFD obese control had significantly higher TC and LDL-C levels than the HCD obese control. The HFD group treated with *S. jollyanum* showed significant decrease in TG, TC, VLDL-C and LDL-C but a significant increase in HDL-C when compared to the HFD obese rats. Whereas in the HCD group treated with *S. jollyanum*, there was no significant difference in the TG, TC, HDL-C, VLDL-C and LDL-C compared to the HCD obese rats.

The CK-activity and LDH level were significantly elevated in the HFD and HCD obese groups compared to the normal control. The HFD obese control had significantly higher CK activity than the HCD obese control; The reverse was the case after treatment with *S. jollyanum* extract. The HCD obese control showed significant increase in LDH when compared to the HFD obese control. Treatment with *S. jollyanum* extract significantly reduced the CK activity and LDH in the HFD obese group when compared to the HFD obese control; whereas only CK activity was significantly reduced by treatment with *S. jollyanum* extract in the HCD obese group when compared to the HCD obese group (Figs. 1 and 2).

Table 5. Lipid profile of rats treated with the plant extracts

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^{d}	43.78±1.45^{d}	29.97±1.70^{ab}	9.12±0.11^{d}	4.68±0.50^{c}
HFD obese control	95.72±7.59^{a}	73.34±2.76^{a}	23.38±1.13^{e}	19.14±1.52^{a}	30.82±3.06^{a}
HFD + *S. jollyanum*	66.24±2.43^{bc}	57.69±1.76^{b}	35.67±2.08^{a}	13.25±0.49^{bc}	8.77±2.05^{bc}
HCD obese control	83.57±5.00^{ab}	54.50±2.09^{bc}	23.98±1.51^{c}	16.71±1.00^{ab}	13.82±1.39^{b}
HCD + *S. jollyanum*	75.97±9.15^{abc}	51.02±4.77^{bcd}	26.68±3.41^{bc}	15.19±1.83^{abc}	9.15±1.7^{bc}

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

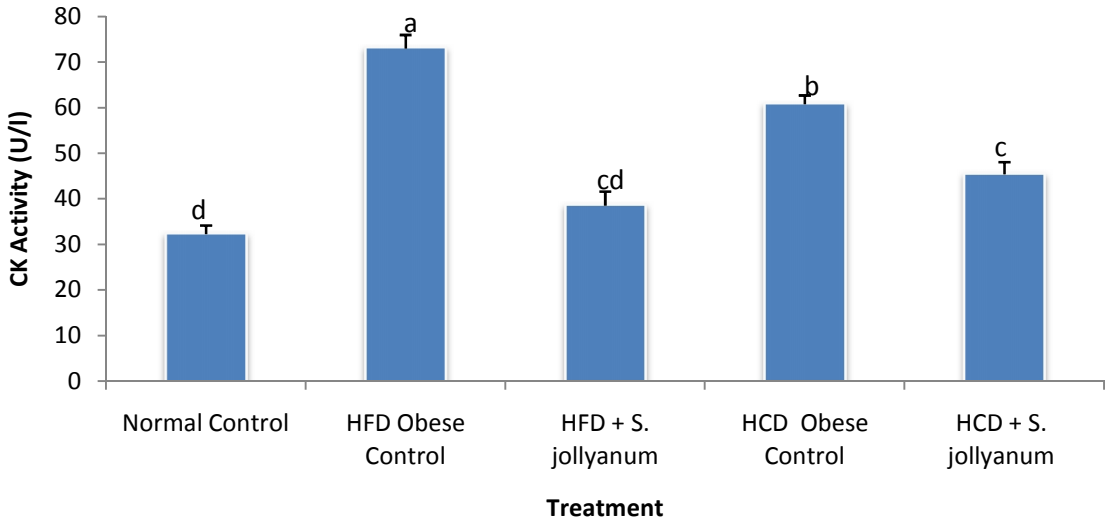

Fig. 1. CK activity of rats treated with *S. jollyanum* extract

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

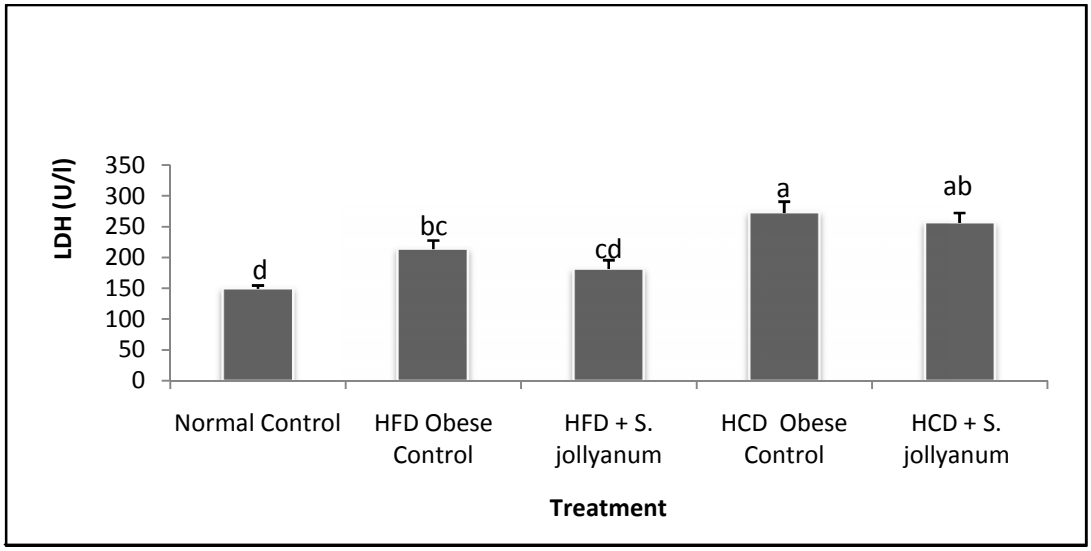

Fig. 2. LDH of rats treated with *S. jollyanum* extract

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 leptin levels were significantly higher in HFD obese control while glucose and insulin levels were higher in HCD obese group when compared to the normal control. The HFD and HCD obese rats had no significant difference in their glucose, insulin and leptin levels. The *S. jollyanum* extract significantly decreased glucose and leptin levels in the HFD treated group and when compared to HFD obese control. No significant difference was observed for the HCD treated group (Table 6).

Table 6. Blood glucose, insulin and leptin of rats treated with the plant extracts

Group	Glucose (mg/dl)	Insulin (µlU/ml)	Leptin (ng/ml)
Normal Control	65.00 ± 2.89^{d}	2.33 ± 0.03^{c}	2.63 ± 0.15^{bcd}
HFD Obese Control	91.67 ± 1.67^{a}	2.37 ± 0.09^{bc}	3.10 ± 0.12^{a}
HFD + *S. jollyanum*	68.33 ± 6.01^{cd}	2.37 ± 0.03^{bc}	2.50 ± 0.06^{cd}
HCD Obese Control	89.67 ± 3.18^{ab}	2.50 ± 0.06^{ab}	2.86 ± 0.09^{ab}
HCD + *S. jollyanum*	101.00 ± 2.08^{a}	2.40 ± 0.06^{abc}	2.77 ± 0.15^{abc}

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

4. DISCUSSION

The phytochemical constituents of the ethanolic root extract of *S. jollyanum* showed the presence of alkaloid, saponin, tannin, flavonoid, cardiac glycoside, terpenoid and reducing sugars. This is in tandem with the earlier study, where the active compounds found in the ethanolic root extract of *S. jollyanum* included; alkaloid, saponin, terpenoid compound, anthraquinones, flavonoids, tannins, cardiac glycosides and reducing sugars [25]. A bitter tasting terpenoid compound had earlier been isolated from the root [11]. Other reports showed the plant root to be rich in tannins and saponins [26].

There is no established threshold to differentiate obesity from overweight in animal models, such as those established by the WHO for humans [27]. Therefore, in the present study, obesity was considered to be a significant increase in body weight [16,28,17]; and adiposity index [29] of the HFD and HCD groups compared to the normal control. At the end of the obesity induction period, the body weight and adiposity index of the rats fed with HFD and HCD were significantly increased compared to the rats that fed on the normal diet (Table 4).

The body weight of the HFD and HCD obese controls increased when compared to the normal control; this is in agreement with the results of other studies [17,30]. The HFD provided more calories than the normal pellet diet, resulting in a high level of fat storage even though the HFD obese control consumed lesser quantity of food they had the most increase in body weight. The HCD had refined sugar (sucrose) as 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 [31], resulting in a high level of fat storage. Although, *S. jollyanum* decreased the bodyweight in HFD and HCD, the change was not significant.

There was a significant increase in serum TG, TC, VLDL-C and LDL-C levels, but a decrease in HDL-C which was not significant in the HFD and HCD obese rats when compared to normal control. The reason is obvious, as a high fat diet should result in dyslipidemic changes as illustrated by increasing triglycerides, VLDL cholesterol, total cholesterol and low density lipoprotein LDL and a decrease in serum level of high density lipoprotein HDL cholesterol [32,16,33]. Meta-analyses have found a significant relationship between saturated fat, common in HFD and serum cholesterol levels [34]. Also, 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 [35]. This explain why the triglyceride level and VLDL cholesterol in the HCD obese rats is significantly higher than the normal control.

In the present study, among the HFD groups, *S. jollyanum* significantly decreased the TG, TC, and VLDL-C compared to the HFD obese rats. Whereas in the HCD groups, *S. jollyanum* extract had no significant difference in the TG, TC and VLDL compared to the HCD obese rats. On the other hand, HDL- cholesterol known as the “good cholesterol” increased appreciably in *S. jollyanum* treated HFD obese rats. The positive effect of ethanolic root of *S. jollyanum* on HDL level of HFD obese rats could be similar to that found in the seed oil of the same plant as elucidated in a different study [36], and the plant could therefore be said to have beneficial effect on the plasma lipid profile of the animals.

The HFD and HCD obese control rats showed a significant increase in CK activity and LDH level when compared to the normal control rats, which is in agreement to that reported in earlier studies [10,16]. The increased blood levels of total cholesterol, LDL, VLDL as well as lowered levels of HDL in HFD rat have been identified in the development of hypercholestremia, which is one of the risk factors for cardiovascular diseases [37]. The CK activity and LDH significantly reduced in the HFD obese rats treated with *S. jollyanum* when compared to the HFD obese control, but only CK activity was significantly reduced in the HCD obese control. Thus, *S. jollyanum* might be helpful in preventing heart diseases.

Although the HFD obese rats had significant increase in their glucose and leptin levels, there was no significant increase in insulin level. The HCD obese rats had significant increase in glucose and insulin levels. This effect is similar to that found in HFD obese rats as reported in earlier studies [38,16]. Leptin is a powerful appetite suppressant and as humans, we even

have leptin receptors on our taste buds, which help to regulate cravings for sweet foods [39]. Leptin is a hormone produced mainly in adipose tissues which is involved in controlling body weight by increasing both satiety and energy expenditure [40,41]. In this present study, the HFD obese rats showed significant increase in leptin level. This result corroborates other studies that showed high leptin levels in models of rodent DIO obesity [42,40,43]. Since leptin concentration is related to the amount and distribution of body fat, decreased leptin level by *S. jollyanum* extract might suggest anti-obesity effects (Table 6). Insulin which is the most anabolic hormone known and essential to the maintenance of glucose homeostasis, growth and differentiation showed no significant changes after treatment.

The *S. jollyanum* extract lowered the blood glucose in the HFD obese rats. The glucose lowering activity might be through inhibition of glucose uptake, facilitation of glucose entrance into skeletal muscle cells, and/or increased insulin sensitivity which might be due to presence of saponins and flavoniods; which is be the case in a study on anti-obesity effects of *Momordica charantia* [44]. In a different study, finding clearly indicated that the aqueous extract of *S. jollyanum* root was effective in reducing the blood glucose concentration of alloxan diabetic and hyperglycaemic rabbits [25].

5. CONCLUSION

The high fat and carbohydrate diet induced obesity in the wistar rats gave rise to the abnormal values of most of the biochemical parameters that may have implications for the progress of obesity related problems. The ethanolic plant extract of *S. jollyanum* might possess anti-obesity potential, although its anti-obesity effect varied among diets as its effect might be relatively lower in HCD induced obesity than in HFD induced obesity. Further work with higher doses of *S. jollyanum* needs to be done to ascertain the anti-obesity effect in HCD induced obesity in rats.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENT

I am grateful to Professor E. C. Onyeneke for supervising this study at the University of Benin, Benin City, Edo State, Nigeria. Thanks to Dr. N. J. Orhue and Mr. O. F. Amegor for providing the equipments used in carrying out this study at University of Benin, Benin City, Edo State, Nigeria.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Barness LA, Opitz JM, Gilbert-Barness E. Obesity: Genetic, molecular, and environmental aspects. Am J Med Genet A 143A. 2007;(24):3016–34.
2. Power ML, Schulkin J. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. British J Nutr. 2008;99(5):931-940.
3. Anyanwu GO, Onyeneke EC, Usunobun U, Adegbegi AJ. Impact of *Anthocleista vogelii* root bark ethanolic extract on weight reduction in high carbohydrate diet induced obesity in male wistar rats. Afr J Biochem Res. 2013;7(11):225-232.
4. Malik VS, Matthias SB, Hu FB. Intake of sugar-sweetened beverages and weight gain: A systematic review. Am J Clin Nutr. 2006;84:274–88.
5. Ludwig DS, Peterson KE Gortmaker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. Lancet. 2001;357:505–08.
6. Ebbeling CB, Pawlak DS, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. Lancet. 2002;360:473–82.
7. Kaur H, Hyder ML, Poston WS. Childhood overweight: an expanding problem. Treat Endocrinol. 2003;2:375–88.
8. Dietz WH. Childhood weight affects adult morbidity and mortality. J Nutr. 1998;128:411–14.
9. Mayes JS, Watson GH. Direct effects of sex steroid hormones on adipose tissues and obesity. Obes Rev. 2004;5(4):197-216.
10. Dalziel JM. In: The useful plants of West Africa. Crown agents for overseas governments and administration, London, 1955.
11. Iwu MM. Handbook of African medicinal plants. CRCPress Inc; 1993.
12. Treatise GE, Evans WC. Pharmacognosy.11th edn. Brailliar Tiridel Can. Macmillan publishers. Ltd, Ibadan; 1989.
13. Sofowora. A Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan; 1993.
14. Shah MA, Garg SK, Garg KM. Subacute toxicity studies on Pendimethalin in rats. Int J Pharmacol. 1997;29:322-324.
15. Burger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC, Soares dos Santos, AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasilinsis*) (*Asteraceae)* in mice. J Pharmacol Sci. 2005;8:370-373.
16. Amin K A, Nagy MA. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. Diabetol Metab Syndr. 2009;1:17-24.
17. Mistry KG, Deshpande SS, Shah GB, Gohil PV. Effect of Sarpogrelate in high fat diet induced obesity in rats. Asian J Pharmacol Biol Res. 2011;1(4):441-446.
18. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 1969;6:24-27.
19. Tietz NW. Clinical Guide to Laboratory Tests, Second Edition W. B. Saunders Company, Philadephia, USA; 1990.
20. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in HDL separated by three different method. Clin Chem. 1977;23:882–884.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of preparative ultracentrifuge. Clin Chem. 1972;18:499-502.
22. Rec. GSCC (DGKC). Recommendation of German Society of Clinical Chemistry. J Clin Chem Clin Biochem. 1977;15:255.

23. Klin Z. Chem. Klin Biochem. In: Klin, Z. Kinetic determination of lactate dehydrogenase as recommended by the German Clinical Chemistry Society (DGKC) Biochem. 1970;8:658.
24. Taylor BA, Phillips SJ. Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics. 1996;34(3):389-398.
25. Mbaka G, Adeyemi O, Osinubi A, Noronha C, Okanlawon A. The effect of aqueous root extract of *Sphenocentrum jollyanum* on blood glucose level of rabbits. J Med Plants Res. 2009;3(11):870-874.
26. Moody JO, Roberts VA, Adeniji JA. Antiviral effect of selected medicinal Plants 1: effect of *Diospyros bateri Diospyros monbutensis* and *Sphenocentrum jollyanum* on PolioViruses. Nigeria J Nat Prod Med. 2002;6:4- 6.
27. WHO. World Health Organization: Physical status: the use and interpretation of antromometry. In Report of a WHO Expert Committee. Geneva: World Health Organization; 1995. (WHO Technical Report Series, No. 854).
28. Madsen AN, Hansen G, Paulsen SJ, Lykkegaard K, Tang-Christensen M, Hansen HS, Levin BE, Larsen PJ, Knudsen LB, Fosgerau K, Vrang N. Long-term characterization of the diet-induced obese and diet-resistant rat model: a polygenetic rat model mimicking the human obesity syndrome. J Endocrinol. 2010;206:287–296.
29. Fernandez CDB, Bellentani FF, Fernandes GSA, Perobelli JE, Favareto APA, Nascimento AF, et al. Diet-induced obesity in rats leads to a decrease in sperm motility. Reprod Biol Endocrinol. 2011;9:32.
30. Rivera-Ramírez F, Escalona-Cardoso GN, Garduño-Siciliano L, Galaviz-Hernández, C, Paniagua-Castro N. Antiobesity and Hypoglycaemic Effects of Aqueous Extract of Ibervillea sonorae in Mice Fed a High-Fat Diet with Fructose. J Biomed Biotechnol. 2011;1-6.
31. Hofmann SM, Tschöp MH. Dietary sugars: A fat difference. J Clin Invest. 2009;119(5):1089–1092.
32. Woo MN, Bok SH, Lee MK, Kim HJ, Jeon SM, Do GM, et al. Anti-obesity hypolipidemic effects of a proprietary herb fiber combination (S&S PWH) in rats fed high-fat diets. J Med Food. 2008;11:169–78.
33. Onyeneke EC, Anyanwu GO. Effect of *Alstonia Boonei* De Wild on High Fat Diet Induced Obesity in Male Wistar Rats. Eur J Sci Res. 2013;112(3):292-299.
34. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ (Clinical research ed.). 1997;314(7074):112–7.
35. Murray RK, Granner DK, Mayes PA, Rodwell WV. Harper's Illustrated Biochemistry,Twenty-Sixth Edition, McGraw-Hill Companies, USA; 2003.
36. Mbaka GO, Owolabi MA. Evaluation of Haematinic Activity and Subchronic Toxicity of *Sphenocentrum jollyanum* (*Menispermaceae*) Seed Oil. European J Med Plants. 2011;1(4):140-152.
37. Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, Diamant M. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. Diabetologia. 2005;48:1229–37.
38. Zhang M, LV XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet in multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2008;704045.
39. Ninomiya Y, Shigemura N, Yasumatsu K, Ohta R, Sugimoto K, Nakashima K, Lindemann B. Leptin and sweet taste. Vitam Horm. 2002;64:221-48.

40. Tentolouris N, Pavlatos S, Kokkinos A, Perrea D, Pagoni S, Katsilambros N. Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. Metabolism. 2008;57(3):313-320.
41. Vigueras-Villaseñor RM, Rojas-Castañeda JC, Chávez-Saldaña M, Gutiérrez-Pérez O, García-Cruz ME, Cuevas-Alpuche O, et al. Alterations in the spermatic function generated by obesity in rats. Acta Histochem. 2011;113(2):214-20.
42. Ghanayem BI, Bai R, Kissling GE, Travlos G, Hoffler U. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. Biol Reprod. 2010;82(1):94-104.
43. Fam BC, Morris MJ, Hansen MJ, Kebede M, Andrikopoulos S, Proietto J, Thorburn AW. Modulation of central leptin sensitivity and energy balance in rat model of diet-induced obesity. Diabetes Obes Metab. 2007;9(6):840-852.
44. Oishi Y, Sakamoto T, Udagawa H, Taniguchi H, Kobayashi-Hattori K, Ozawa Y, Takita T. Inhibition of increases in blood glucose and serum neutral fat by momordica charantia saponin fraction. Biosci Biotechnol Biochem. 2007;71(3):735–740.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=548&id=14&aid=4803