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Solanum nigrum show anti-obesity effects on high-fat diet-fed Sprague Dawley *rats* in a randomized study

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ABSTRACT

Solanum nigrum also known as black night shade biosynthesizes various bioactive compounds which have various pharmacological activities including treating cardiovascular diseases, diabetes type 2 among others. To assess the anti-obesity effects of *Solanum nigrum* using high-fat-fed diet rats, Sprague Dawley male rats ($n = 35$) of weights 160–180 g were assigned randomly into seven groups comprising $n = 5$ rats each. Each group was fed for 11 weeks as follows: normal group (normal chow rat feed); high-fat diet control (HFD); HFD and standard drug (Orlistat 30 mg/kg bw); HFD and methanolic extract 150 mg/kgbw; HFD and methanolic extract 300 mg/kgbw; HFD and dichloromethane extract 150 mg/kgbw; HFD and dichloromethane extract 300 mg/kgbw. Body mass index and food intake were monitored per week and an oral glucose tolerance test was measured in weeks 5 and 10. Lipid profiles, liver function tests, adipose tissue, liver weights, and phytochemical analysis of *Solanum nigrum* were later carried out.

Results: High-fat diet control group rats exhibited a significant increase in body mass index (BMI) while rats administered with leaf extracts of *Solanum nigrum* showed a reduction in BMI. Both low dose of dichloromethane (150 mg/kgbw) and high dose of methanol extracts (300 mg/kgbw) showed a better reduction in BMI than the other treatment groups. A significant decrease ($p < 0.05$) on low-density lipoprotein-cholesterol, triglycerides and cholesterol was observed among the rats administered with *Solanum nigrum* extracts compared to those of HFD control. Moreover, the HFD control group significantly increased liver and adipose tissue weights compared to other treatments groups ($p<0.05$). *Solanum nigrum* also decreased glycemic levels and normalized the hepatic enzymes of HFD control. However, food intake among the groups showed no significant difference ($p>0.05$). Qualitative analysis of *Solanum nigrum* leave extracts indicated the presence of various bioactive compounds associated with anti-obesity.

Conclusion: These results validate the use of *Solanum nigrum* in controlling obesity.

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Introduction

Obesity is an increasing concern both in developed and developing countries [1]. It results from an increase in adipose tissue mass due to the accumulation of triglycerides on the adipocytes [2]. A person with BMI above 30 kg/m^2 is considered obese and BMI between 25 kg/m^2 and 29 kg/m^2 is considered overweight [3]. The intake of a high-fat diet augmented with sucrose solution leads to the pathogenesis of obesity and other comorbidities such as cancer, diabetes, cardiovascular diseases, dyslipidemia, atherosclerosis, fatty liver, obstructive sleep apnea, hypertension among others [4]. The global prevalence of obesity among children under 5yrs was 14.4 million in 2018 according to World Health Organization [5]. However, the number increased to 38.2 million and 39 million in the year 2019 and 2020 respectively [6].

Pharmacological drugs reduce obesity through different mechanisms such as alteration of appetite, metabolism or consumption of calories. For example; drugs such as Orlistat, have been found to reduce obesity through inhibition of pancreatic lipase leading to reduction of intestinal fat absorption [7]. However, they appear ineffective in the long-term treatment and management of obesity and are also expensive with reported adverse side effects [8]. Recent approaches for seeking alternative therapies are focused on screening natural therapeutic compounds that have anti-obesity effects with minimal side effects. Study reports indicate that plants with phytochemicals such as saponins, alkaloids, flavonoids among others may be effective in the prevention of obesity through various mechanisms such as; increment of thermogenesis, inhibition of metabolic and digestive lipases, suppression of appetite, and pre-adipocytes proliferation inhibition and differentiation. *Solanum nigrum* is a medicinal vegetable belonging to the *Solanaceae* family used by various countries [9]. It is commonly known as *Managu* in Kenya and has been used globally for different treatments such as management of human bacterial infections, stomach upsets, diabetes, high blood pressure among others [10]. In Kenya, *Solanum nigrum* has folklore claims to have anti-obesity activity yet it is not scientifically validated. Hence, this study focuses on the evaluation of ethnomedicinal anti-obesity importance of *Solanum nigrum* on rats fed with the high-fat diet.

Methods

Collection/preparation of plant leaves

Fresh leaf vegetables of *Solanum nigrum* were collected in October 2019 during a rainy season from Limuru sub-county, Kiambu County, Kenya. The leaves were packed and transported in khaki bags to the Department of Biological Science University of Nairobi, for plant identification and authentication by a taxonomist. *Solanum nigrum* voucher specimen KWN-UON2019/001 was deposited at the department of biology herbarium. Samples were shade dried, grounded into a fine powder using an electric mill and then packed awaiting extraction.

Preparation of extract from solanum nigrum *leaf powder*

The preparation of plant extract was done in the Department of Chemistry at the University of Nairobi. The grounded plant material was soaked in 100% Hexane for 24 h then re-soaked in 100% Dichloromethane (DCM) for 48 h filtered or re-soaked for another 48 h in methanol [11]. The solvents were added to the powdered plant material at a ratio of 2:1. Whatman No.1 filter paper was used during filtration and the filtrate was concentrated using a rotary evaporator at 68 °C, 39 °C and 64 °C respectively. The concentrate was weighed and left to dry under a shade for one month until a sticky solid was formed then stored at 4 °C ready for bioassay [12].

Study design

Thirty-five [35] Sprague Dawley male rats weighing 160- 180 g were purchased from Kabete Veterinary Laboratories and transported to the animal house department of Biochemistry, University of Nairobi. The rats were acclimatized for one week in standard cages at normal laboratory conditions (22.5 $\pm$ 2 °C, 12 hrs light and 12 hrs dark cycles) before experimentation. Thirty-five [35] rats were kept in one cage and thereafter randomly assigned into seven groups of five animals each ($n = 5$). Sample size determination was calculated using the G* power software 2007 whereby each group had 5 rats as indicated above [13].

The rat groups were given a high-fat diet (HFD) concurrently with treatments, except for the normal group, as follows: normal group (normal rat chow), positive control (Orlistat 30 mg/kgbw), negative control (high-fat diet only) and treatment groups received either a low dose of 150 mg/kgbw or a high dose of 300 mg/kgbw of methanol extract or 150 mg/kgbw or high dose of 300 mg/kgbw of dichloromethane extract. The treatments were administered daily by oral gavage on the experimental groups except for the normal group, which was given water only. This study was approved by the Institutional Ethical Review Committee of the Institute of Primate Research (ISERC/06/19).

Preparation of feed

HFD was prepared by addition of 30 g of fat (Seagull* frying fat, Kapa oil refineries, Kenya) to 100 g of rat chow pellets (Fat: 10%, Protein: 20% and Carbohydrate: 70%) (Unga Feeds Limited, Nairobi). To improve the palatability of HFD 0.8% of

monosodium glutamate (MSG) (Oshwal Flavours Limited, Nairobi, Kenya) was added. This was done by heating the mixture for 20 min with constant mixing to absorb the fat into the feed [14].

Body mass index (BMI)

The rat lengths (nasal-anal length) and weights were examined every week for 11 weeks using a ruler and an electronic balance respectively. To verify the rats body mass index; Lee index was computed, as follows:

$$Lee\ index = \sqrt[3]{(Body\ weight)/Nasal-anal\ length\ (cm)\}} \times 1000$$

Rats with a lee index $\geq$ 310 mass index were considered obese [15].

Determination of food consumption

Food consumption was measured daily during the experimental period. The animals were given 200 g of rat chow per cage in the morning and the food remaining weighed 24 h before the administration of the extract [16].

Oral glucose tolerance test

This test was determined twice, on weeks five and ten of the experimental period using a glucometer (on-call plus) (Acon Laboratories, Sandiego, USA). The animals were fasted overnight (12 h) before the test and blood samples were obtained by snipping the rat lateral tail vein [17]. To minimize pain lidocaine was applied before the procedure [18]. The baseline glucose level was measured and immediately the rats were orally given a glucose dose of 2 g/kgbw. Blood glucose levels were then measured at 30, 60, 90 and 120 min.

Blood sample collection and serum biochemical analysis

On the 11th week, the rats were euthanized using carbon dioxide to reduce pain during the sacrifice. The animals were dissected to draw blood by cardiac puncture. The blood samples were then transferred into plain micro vacutainer tubes, left to clot for 15 min and centrifuged at 2500 rpm for 10 min. Sera obtained was collected and put in cryovials for biochemical analysis such as low-density lipoprotein (LDL-c), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL-c), Alanine aminotransferase (ALT) Alkaline phosphatase (ALP) and Gamma-glutamyl transferase (GGT). These analyses were determined using an automated biochemical analyzer (Olympus 640) according to the standard operating procedures (Sops) written at the Department of Clinical Laboratory Medicine, Kenyatta National Hospital.

Excision of liver and adipose tissue

After euthanization, liver and the adipose tissue were excised and weighed using an electronic balance (WANT Balance Instrument Co. Ltd, Jiangsu China).

Qualitative phytochemical screening

Phytochemical screening of the crude extract was done to identify the bioactive compounds associated with anti-obesity effects. These bioactive compounds included alkaloids, terpenoids, saponins, anthraquinones, flavonoids, steroids, phenols, tannins diterpenes which were isolated as follows:

Alkaloids

One percent (1%) v/v of hydrochloric acid (HCL) was mixed to 1 g of crude extract, warmed, then filtered and the filtrate treated with a saturated picric acid solution. A yellow-colored precipitate indicated presence of alkaloids [19].

Terpenoids (Salkolski test)

One gram (1 g) of the extract was mixed with 1 ml of ethyl acetate, a few drops of concentrated sulphuric acid and 2 ml of chloroform. Reddish-brown color formation at the interface after shaking indicated the presence of terpenoids [20].

Saponins (Froth test)

One gram of leaf extract was added to 2 ml of distilled water in a test tube and sodium bicarbonate ($NaHCO_3$) solution was added drop wise. The occurrence of frothing after vigorously shaking the mixture indicated the presence of saponins [21].

Anthraquinones

Ten percent (10%) of hydrochloric acid was mixed with 1 g of the extract and boiled in a water bath for a few minutes. This was then filtered, cooled and afterward, 1 ml of chloroform and 10% of ammonia were added drop wise. This was followed by heating of the filtrate and the formation of rose pink coloration showed the presence of anthraquinones [22].

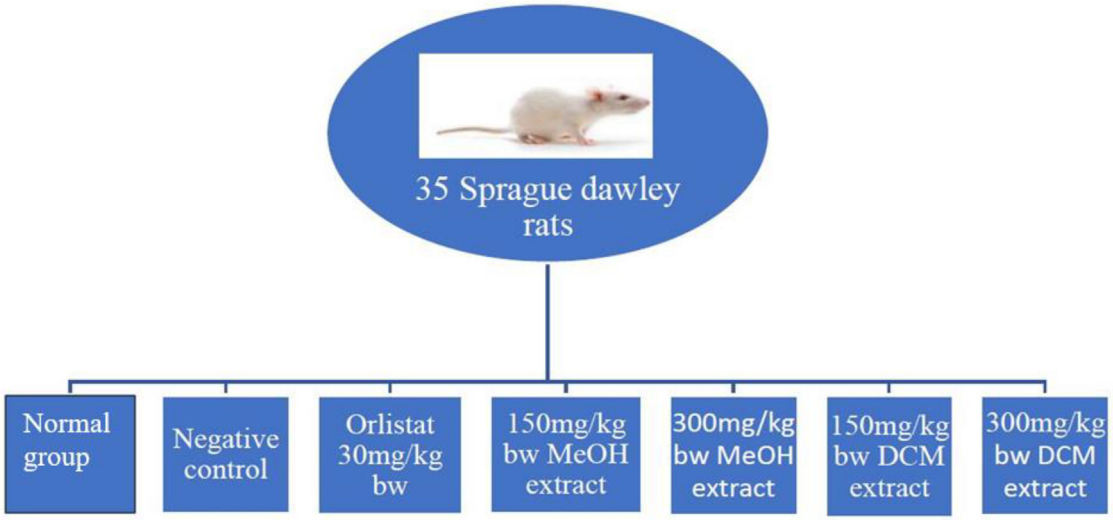

Fig. 1. Various treatment groups. (MeOH- Methanol, DCM- Dichloromethane).

Flavonoids (Sodium hydroxide - NaOH Test)

Two milliliters (2 ml) of diluted NaOH (aq) was mixed with 1 g of extract. The presence of flavonoids was indicated by a golden yellow precipitate [23].

Steroids

Two milliliters (2 ml) of chloroform was used to dissolve 1 g of extract and later 3 ml of concentrated sulphuric acid - H_2SO_4 (aq) was added by the sides to form a layer. The presence of steroids was indicated by a reddish-brown color [24].

Phenols

One gram (1 g) of extract was added to 1 ml of ferric chloride solution and a color change from blue to green indicated presence of phenols [25].

Tannins

A gram of extract was added to 1 ml of distilled water followed by the addition of two drops of 5% iron chloride. Tannin presence was indicated by blue-black color formation [26].

Diterpenes

One gram (1 g) of the extract was mixed in water and later three drops of copper sulphate ($CuSO_4$ (aq) were added. A color change to emerald green from blue showed the presence of diterpene [27].

Statistical analysis

Experimental data was tabulated on Microsoft Excel, analyzed using Minitab version 17, and results were presented as Mean ± SEM. One-way analysis of variance (ANOVA) and a post hoc Tukey test was used to determine whether there are significant differences between the treatment groups. Values of $p \leq 0.05$ were considered significant and those of the same superscript were not statistically significant (Fig. 1).

Results

Effects of solanum nigrum *on body mass index, liver and adipose tissue weights*

Effects of solanum nigrum *on body mass index (BMI)*

The leaf extracts of *Solanum nigrum* reduced the body mass index (BMI) of high-fat-fed rats (HFD) (Table 1). From week 0 to week 2 the rats did not have significant changes in body mass index among the treatment groups. The rats treated with a methanol extract dose of 150 mg/kg/bw had no significant decrease in BMI compared to the normal group (placebo) at weeks 3–9 but exhibited a significant decrease at weeks 11($p<0.05$; Table 1). However, the rats administered with a methanol extract dose of 300 mg/kgbw significantly decreased in BMI compared to the normal group (placebo) at weeks 5–11 ($p<0.05$; Table 1). Similarly, the BMI of the rats treated with DCM extracts dose of 150 mg/kgbw and methanol extract dose of 300 mg/kgbw significantly decreased compared to positive control at weeks 5–11 ($p< 0.05$; Table 1). In addition,

Table 1
Effects of Methanol and Dichloromethane leaf Extracts of *Solanum nigrum* on Body Mass Index on HFD-fed rats.

Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 11
Normal group	298.89 ± 0.52^{a}	300.37±0.38^{c}	301.67±0.40^{c}	304.25±0.43^{b}	304.59±0.34^{b}	304.68 ± 0.19^{b}
Positive control (orlistat 30 mg/kgbw)	298.54±0.80^{a}	301.28±0.42^{c}	301.33±0.57^{c}	301.49±0.42^{c}	300.03±0.26^{d}	298.20±0.74^{d}
Negative control	298.81±0.60^{a}	304.18±0.61^{ab}	308.57±0.42^{a}	311.62±0.43^{a}	313.24±0.46^{a}	313.08±0.17^{a}
150 mg/kgbw. MeoH	298.41±0.71^{a}	302.30±0.52^{abc}	303.41±0.32^{bc}	304.01±0.20^{b}	303.54±0.46^{bc}	301.5 ± 0.26^{c}
300 mg/kgbw. MeoH	298.71±0.59^{a}	301.92±0.56^{bc}	298.70±0.71^{d}	297.36±0.42^{d}	296.33±0.20^{e}	295.16±0.31^{e}
300 mg/kgbw. DCM	299.14±0.57^{a}	304.52±0.79^{a}	305.37±0.47^{b}	304.68±0.47^{b}	302.04±0.23^{c}	298.85±0.20^{d}
150 mg/kgbw. DCM	299.63±0.59^{a}	302.18±0.3^{abc}	301.87±0.22^{c}	298.74±0.14^{d}	298.66±0.34^{d}	297.84±0.43^{d}

Values expressed as Mean ± SEM for five animals per group. Statistical comparison was made within a column (BMI) and values with the same superscript letter are not significantly different by one-way ANOVA followed by Tukey's post hoc test ($p>0.05$).

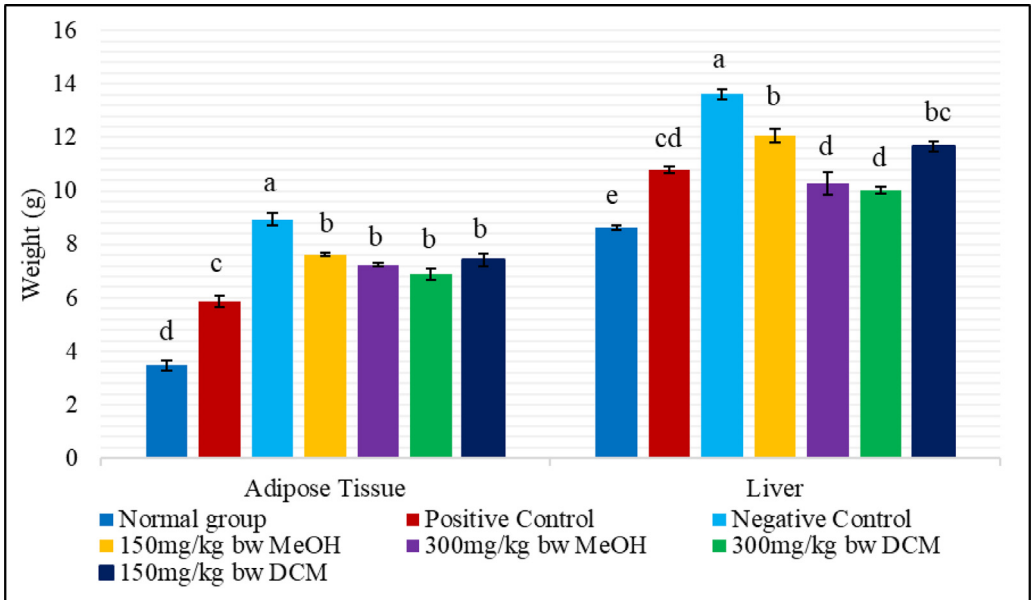

Fig. 2. Effects of MeOH and DCM extracts on adipose tissue and liver.

rats treated with methanol and DCM extract dose of 150 mg/kgbw and 300 mg/kgbw had a significant decrease in BMI compared to HFD rats (negative control) at weeks 5–11 ($p<0.05$; Table 1).

Effects of MEOH and DCM extracts on adipose tissue and liver

Concerning adipose tissue and liver weights, a significant increase in liver and adipose tissue was observed among the HFD control group compared to the other extract groups ($p<0.05$; Fig. 2).

Effects of solanum nigrum *on glucose levels, lipid profiles and hepatic enzymes in HFD-fed rats*

Effects of solanum nigrum *on glucose levels*

Methanol and DCM leaf extracts of *Solanum nigrum* showed no significant difference in glucose levels of high-fat fed rats (HFD)(negative control) at week 5 compared to other treatment groups ($p>0.05$; Fig. 3). However, there was a significant change in glucose levels at week 10 on high-fat diet fed rats (negative control) when compared to other treatment groups ($p<0.05$; Fig. 4).

Effects of solanum nigrum *on lipid profiles and hepatic enzymes*

There was a significant increase in high-fat diet fed rats (negative control) showed a significant increase in total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-c) and a significant decrease in high density lipoprotein (HDL-c) compared to other treatment groups ($p<0.05$; Fig. 5).

A significant increase in gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) was observed on high-fat diet group (negative control) compared to other treatment groups ($p<0.05$; Table 2).

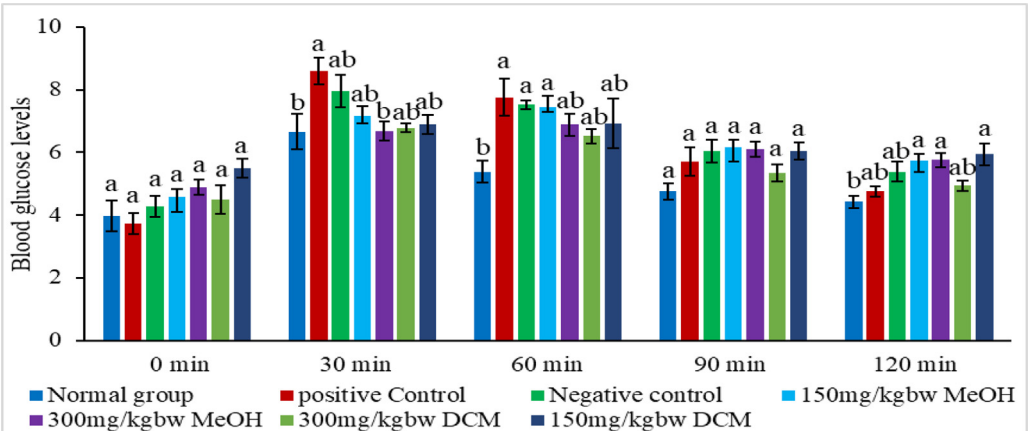

Fig. 3. Effects of MeOH and DCM extracts on blood glucose levels for week 5.

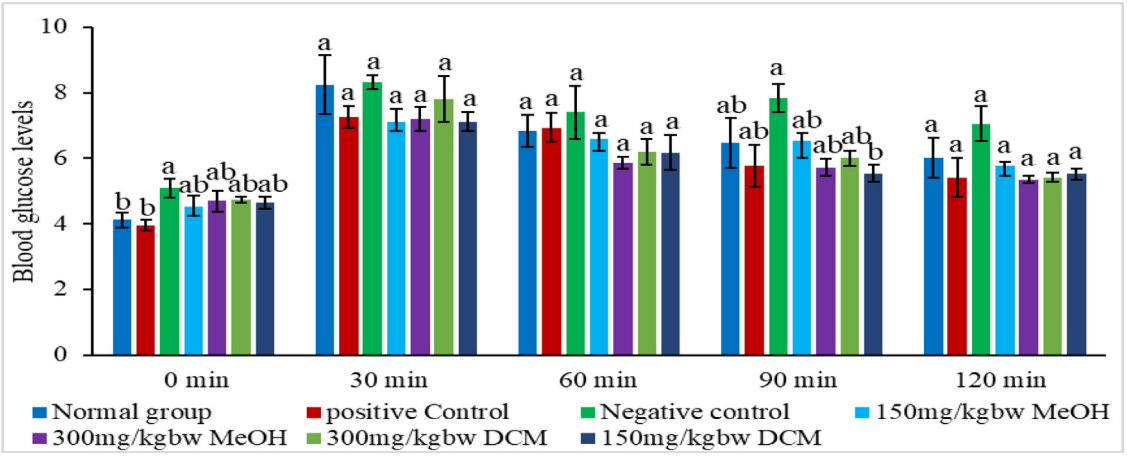

Fig. 4. Effects of MeOH and DCM extracts on blood glucose levels for week 10.

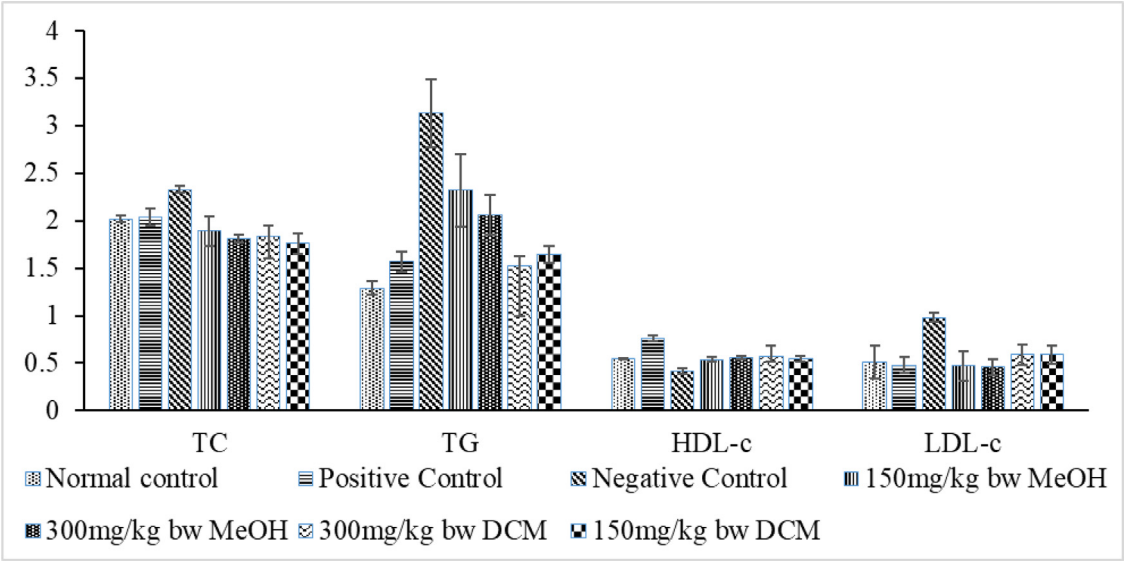

Fig. 5. Effects of MeOH and DCM leaf extracts on lipid profile.

Table 2
Effects of Methanol and Dichloromethane leaf extracts on hepatic enzymes in HFD-fed rats.

Treatment	ALT	ALP	GGT
Normal control	25.07±2.23[b]	152±18.6[b]	5.75±1.03[a]
Positive control (orlistat 30 mg/kgbw)	31.13±3.77[b]	240±7.56[b]	4.25±0.25[a]
Negative control	93.9 ± 3.8[a]	692±191[a]	64.8 ± 43.2[b]
150 mg/kgbw. MeoH	34.83±6.18[b]	224±11.7[b]	5.5 ± 1.50[a]
300 mg/kgbw. MeoH	13.27±1.40[b]	195±7.53[b]	10.75±4.27[a]
300 mg/kgbw.DCM	20.52±1.92[b]	180±11.2[b]	4 ± 0.01[a]
150 mg/kgbw.DCM	21.50±3.62[b]	237.3 ± 11.6[b]	8.50±2.33[a]

The table shows the values of ALT, ALP and GGT in U/L on the different experimental groups.

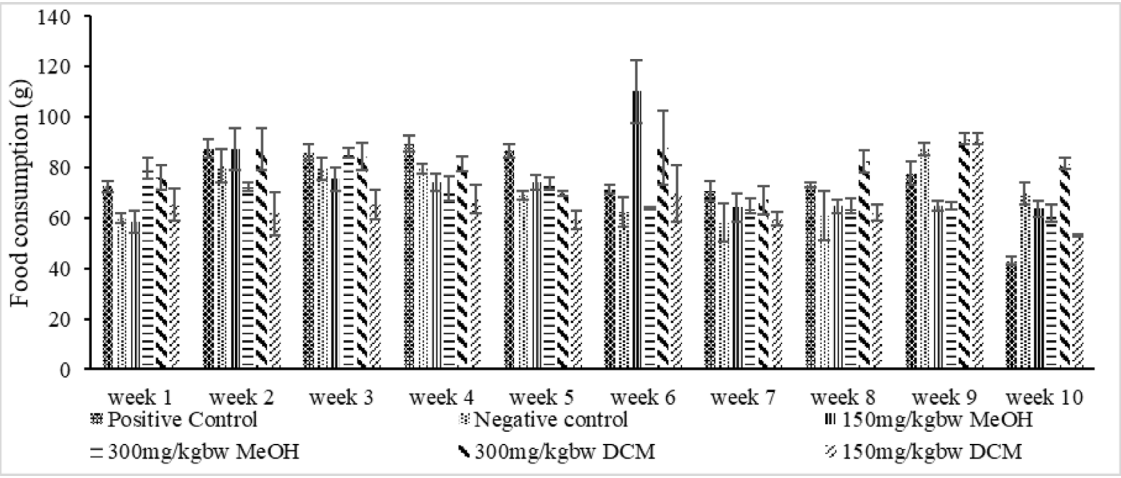

Fig. 6. Effects of MeOH and DCM leaf extracts on food consumption rate in HFD fed rats per week.

Table 3
Qualitative phytochemical screening of crude leaf extract of *Solanum nigrum*.

Phytochemicals	DCM extract	MeOH extract
Alkaloids	+	+
Terpenoids	+	+
Saponins	+	+
Anthraquinones	+	+
Flavonoids	+	+
Steroids	+	+
Phenols	+	+
tannins	+	+
Diterpenes	+	+

Effects of solanum nigrum *on food consumption pattern in HFD fed rats*

The results showed that the average food intake per day in a week was almost similar in all the groups except for rats administered methanolic extract dose of 150 mg/kgbw, which showed a high food intake on week 6 compared to the other groups (Fig. 6).

Qualitative phytochemical composition of methanol and dichloromethane extracts of solanum nigrum

Qualitative phytochemical analysis indicated the presence of terpenoids, alkaloids, saponins, anthraquinones, diterpenes, steroids, flavonoids, phenols and tannins on both methanolic and Dichloromethane extract (Table 3).

Discussion

This study was a randomized control study design that assessed the anti-obesity effects of *Solanum nigrum* on high-fat diet-fed rats. *Solanum nigrum* has been previously reported to have anti-tumor and anti-hepatoprotective effects, anti-inflammatory, antipyretic and anti-diabetic effects but has limited data on its effect on anti-obesity. In this study, we eval-

uated the effect of *Solanum nigrum* as an anti-obesity agent by measuring the BMI, phytochemical compounds, liver and adipose tissue weights, lipid profiles, hepatic enzymes, glucose levels, and food consumption [28].

This study found that the negative control group's BMI significantly increased when compared to the other treatment groups. Most likely, heavy consumption of a high-fat diet led to the rise in BMI. This demonstrates that eating a high-fat diet results in extra calories that increase body weight and, ultimately, BMI. This data was in line with a study produced previously on high consumption of high-fat diets on rats [29]. In addition, DCM and Methanol extracts of *Solanum nigrum* decreased BMI, liver, adipose tissue weights, lipid profiles, hepatic enzymes and glucose levels of high-fat fed rats. Further, phytochemical analysis of *Solanum nigrum* revealed the presence of bioactive compounds included alkaloids, terpenoids, saponins, anthraquinones, flavonoids, steroids, phenols, tannins and diterpenes. The presence of these phytochemicals may have contributed to the reduction of the BMI in *Solanum nigrum* treated high fat fed rats. Indeed, similar previous report indicated plants with phytochemical compounds such as saponins, flavonoids, steroids, and phenols have anti-obesity activity. By reducing oxidative stress in the body, flavonoids, which have an antioxidant function, suppresses obesity [30]. Flavonoids also inhibit the pancreatic lipase enzyme by inhibiting dietary fat absorption and hence increase the excretion of fats in faeces. In addition, the bioactive compounds present in *Solanum nigrum* extract may have suppressed differentiation of the preadipocytes and inhibited adipogenesis through modulation of lipid metabolism pathways [31].

There was a reduction in adipose tissue and liver weights among the treated groups compared to the high-fat-fed rats. Peroxisome proliferator– activated receptor gamma (PPARγ) upregulates the expression of fatty acid oxidation enzymes such as acetyl CoA oxidase (ACO), palmitoyl transferase-1 (CPT-1) and certain proteins such as uncoupling proteins one UCP-1 and uncoupling proteins two UCP- 2. These enzymes are associated with energy balance and thermogenesis in adipose tissue [32]. We, therefore, postulate that reduction in adipose tissue resulted due to increment of peroxisome proliferator – activated receptor gamma (PPARγ) and acetyl Coenzyme A oxidase (ACO) in our treatment groups, hence suggesting *Solanum nigrum* may have anti-obesity effects by increasing fatty acid oxidation and energy expenditure [33,34]. This study also associates reduction in weight of the adipose tissue to the inhibitory effects of the extract, which could have occurred through the inhibition of differentiation of 3T3-L1 preadipocytes cells to adipocytes by downregulation expression of PPARγ [35]

The serum levels of total cholesterol, low-density lipoprotein-cholesterol and triglycerides were higher on the high-fat-fed diet group compared to other treatment groups. Although the rats were hyperlipidemic rats administered with the *Solanum nigrum* extracts were hypolipidemic. Previous reports that show the presence of phytochemical compounds account for the observed bioactivity for example; the presence of phytosterols reduced triacylglycerides and cholesterol by down-regulating the expression of lipogenic genes such as fatty acid synthase (FAS), Sterol regulatory-element binding proteins–1(SREBP1) and proteins involved in cholesterol biosynthesis such as Sterol regulatory-element binding proteins -2 (SREBP) and 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR). A previous study indicated that saponins inhibit cholesterol micellization by interfering with intestinal solubility hence increasing high-density lipoproteins while phytosterols lower low-density lipoprotein [35,36]. Therefore, we postulate that *Solanum nigrum* had the same therapeutic effect since the report was similar to our study.

High-fat diet damages the liver leading to the production of excess Gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and alanine aminotransferase (ALT). In addition, liver enzymes such as ALP, GGT and ALT are used to estimate the liver functions. Previous research indicated a link between abdominal obesity and GGT and ALT. The GGT marker is considered to assess the functionality of kidney and liver whereas ALT is a biomarker for hepatotoxicity evaluation [37,38]. This study showed an increase in ALP, GGT and ALT among the high-fat diet group compared to other treatment groups. This finding was similar to a previous study on mice. According to previous studies, liver damage occurs due to abnormal glycosylation, which is associated with the change in hepatocytes and hepatosteatosis which leads to lipid storage in the cytoplasm thus increasing serum levels of ALT, ALP and GGT. However, the treated groups had lower ALT, ALP and GGT levels comparable to that of the high-fat diet group indicating that *Solanum nigrum* is safe for consumption [39].

We also report an increase in glucose level on high-fat fed rats at week 10 compared to other treatment groups. This may have been due to the impairment of pancreatic beta cells and increase in insulin resistance as reported previously in obese patients due to increased levels of proinflammatory markers, non -esterified fatty acids and other substances leading to insulin resistance. We, therefore, postulate that *Solanum nigrum* was able to attenuate insulin resistance.

Finally, the food intake (quantity consumed) among the groups was comparable meaning that body weight was not necessarily dependent on the amount of food consumed but on the composition of the diet [40].

Though we did phytochemical analysis for *Solanum nigrum*, we did not evaluate the individual compounds for their anti-obesity effect but only evaluated the crude extracts. This was the limitation of this study and future studies should focus on isolated compound analysis on their effect on obesity.

Conclusion

Solanum nigrum extracts possessed anti-obesity, anti-hyperglycemic, and anti-dyslipidemic activities. It reduced body mass index, lipid profiles, hepatic enzymes, and glucose levels. These results validate the use of *Solanum nigrum* in controlling of obesity.

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Credit author statement

Kathryn Nderitu and Atunga Nyachieo contributed to the conception and design of the study. Material preparation, experimental work and data collection was done by Kathryn Nderitu. Data analysis was done by Kathryn Nderitu and Ezekiel Mecha. The first draft of the manuscript was written by Kathryn Nderitu, and Atunga Nyachieo proof read and commented on the previous version of the manuscript. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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