Saponin-rich extracts reverse obesity and offer protection against obesity-induced inflammation in high-fat diet mice

Background: Obesity is a medical condition that occurs as a result of excess body fat which increases the risk of several metabolic disorders. Non-availability of efficient classical treatment for obesity has led to propose alternative treatment using plant material.

Aim: To investigated the effect of saponin-rich extract of Lindackeria dentata (SLD) on obesity and some specific genes involved in inflammation and insulin resistance in the high-fat diet (HFD) on mice.

Setting: The plant leaves were collected from farmland in Igede Ekiti, South-western Nigeria and authenticated at a herbarium unit of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria.

Methods: Saponin-rich extracts from Lindackeria dentata leaves were extracted using standard procedures, HFD was given to some selected mice for 12 weeks whilst monitoring blood glucose and body weight (bw) of the mice. Obese mice were treated orally with SLD 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw for 3 weeks, sacrificed, organs were collected and some biochemical assays were performed. Expression of some genes and histopathological study of the pancreas were also carried out using a standard scientific protocol.

Results: Saponin-rich extract of Lindackeria dentata treatment significantly reduced (p < 0.05) bw and adipose fat deposit, and caused partial restoration of pancreatic islet expansion (100 mg/kg and 200 mg/kg) coupled with accentuated regulation of leptin, insulin and IL-10 gene (25 mg/kg, 50 mg/kg and 100 mg/kg) when compared with control groups.

Conclusion: The present data clearly showed that SLD could be a good intervention in the treatment of obesity and its attendant metabolic disorders.

Keywords: leptin; Lindackeria dentata; obesity; insulin resistance; anti-inflammatory; saponin.

Introduction

Diet-induced obesity seriously affects the health of the populace because of its association with different metabolic disorders (Darkhal et al. 2015). Recently, it was reported that abdominal adiposity rather than the body mass index is indicator of cardiovascular mortality (Czernichow et al. 2011) and many metabolic disorders (Piya, McTernan & Kumar 2013). Therefore, inability of adipose tissue to store excessive calories as triglycerides results in spillover of lipids into other organs, foremost the liver, thereby resulting in local and systemic inflammation (Fischer et al. 2018). This sets off a cascade of metabolic alterations, resulting in systemic insulin resistance, dyslipidemia and development of metabolic syndrome (Fischer et al. 2018). In an obese state, insulin serves as a key regulator, which enhances the accumulation of triglycerides with the aim of increasing breaking down of glucose and free fatty acid synthesis through inhibition of fat cells lipolysis (Stafeev et al. 2017). Leptin functional role is to achieve an energy balance in the body which is up-regulated by insulin, cortisol and down-regulated by catecholamines. Deficiency of leptin or leptin receptor in human beings results in extreme obesity, thereby implicating leptin mediated signaling in the regulation of food intake, energy expenditure, reproductive, thyroid and immune functions (Wasim 2015). The anti-inflammatory cytokines interleukin-10 (IL-10) identified by Mosmann and colleagues in 1989 is a critical negative regulator of immune responses in which loss of IL-10 leads to inflammatory diseases (Ruts & Ouyang 2016).
Researchers have placed much interest on insulin resistance and its comorbidities in the last decades with the aim of providing alternative modalities to these metabolic disorders rather than the conventional therapy (Nagy & Einwallner 2018). Conventional treatments are available to curb obesity, but these are not effective with prolonged treatment durations (Pothuraju et al. 2016). Therefore, there is a need for alternative therapy such as phytotherapy (Pothuraju et al. 2016). Plants have been used for untold years as natural pharmaceutical aids and scientists are now moving towards natural product-based therapeutic formulations to obtain safer anti-obesity drugs (Sharma & Kanwar 2018). Currently, phytomedicines are prominent alternatives to synthetic drugs (Sharma & Kanwar 2018).

*Lindackeria dentata* (Oliv.) Gilg is a plant of 16 meters tall, which belongs to the family of Flacourtiaeae (Burkill 1985; Jaroszewski, Ekpe & Witt 2004). It is known in Nigeria as ‘Igbo Oru Burkill’ (1985) and Ekiti people in the South-Western region of Nigeria called it ‘*Uagbo*’, which they used locally to treat back pain. Alkaloid has been found in the leaves, stem, bark and roots (Burkill 1985). Saponins as phytocompound are well known bioactive agents that have been investigated for a multitude of biological activities, including antimicrobial, cytotoxic, anti-inflammatory, anti-hyperlipidemic, immune-stimulatory and anti-diabetic properties (Ejelonu, Ekofehinti & Adanlavo 2017; Elekofehinti et al. 2014). But not much has been documented about saponins from *Lindackeria dentata* (Oliv.) and its lipid-lowering effect. Therefore, research on saponin-rich extract of *Lindackeria dentata* (SLD) and its actions on obesity and some specific genes involved in inflammation and insulin resistance in high-fat diet (HFD) mice was investigated.

**Materials and methods**

**Plant materials**

*Lindackeria dentata* leaves were harvested from a farmland in Igede Ekiti, South-western Nigeria. The fresh plant was identified, authenticated in the herbarium unit of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko. The principles of Laboratory animal care National Institutes of Health (NIH) Publication (1985) were followed throughout the duration of the experiment. Thirty-five healthy male albino mice of 20.5 g – 25.0 g were obtained from the animal house in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko. The principles of Laboratory animal care National Institutes of Health (NIH) Publication (1985) were followed throughout the duration of the experiment.

**Experimental design**

The mice were grouped into seven groups of five mice. High-fat diet of 70% fat and 30% carbohydrate were fed to the mice for 12 weeks to make them obese. Changes in their body weight (bw) and blood glucose level were monitored weekly, with their bw ≥ 35 g. Group I normal mice and group II negative control (NC) (obese untreated mice) were administered with 0.5 mL distilled water, group III positive control (obese mice) was administered 10 mg/kg bw of atorvastatin and group IV – VII test groups were obese mice administered with SLD at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg orally for 21 days, respectively. The mice food was withdrawn over the night and sacrificed by cervical dislocation to obtain their whole blood, visceral fat and liver for biochemical assays, whilst the pancreatic glands were used for histopathological and gene expression studies.

**Acute toxicity study**

Acute toxicity study of SLD was investigated using healthy male albino mice 25 g – 30 g according to the procedure of Organization for Economic Co-operation and Development (OECD 2008). The mice were observed for general behavioural change and mortality for a period of 48 h post-treatment.

**Experimental animals**

**Body weight and fasting blood glucose level**

Bodyweight and fasting blood glucose level of the mice were taken at a regular interval with the use of electronic weighing balance and Accu-Chek® active glucometer, respectively.

**Oral glucose tolerance test**

Oral glucose tolerance test was carried out after 16 weeks of feeding with HFD and oral administration of the saponin-rich extract. All mice were fasted overnight for 14 h before testing. All mice had free access to drinking water and a 20% D-glucose solution was freshly prepared on the day of the experiment. Blood samples were taken from a tail vein at 0 min, 15 min, 30 min, 45 min and 60 min, respectively, after glucose administration and blood glucose levels were measured using Accu-Chek® active glucometer.

**Intraperitoneal insulin tolerance test**

Intraperitoneal insulin tolerance test was carried out a week after OGTT, food was removed at least 2 h before testing. With all mice having free access to drinking water, the animals were administered human regular insulin (0.75 U insulin/kg bw) dissolved in 0.9% sodium chloride (NaCl) solution, coupled with freshly prepared 20% D-glucose solution on the day of the
experiment, in case of any hypoglycemia because of blood sugar falling below 35 mg/dl. Whole blood was collected from a tail vein at 0 min, 15 min, 30 min, 45 min, 60 min and 90 min, respectively, after the injection.

**Collection of blood and tissues**

The animals were sacrificed by cervical dislocation. Whole blood was collected via cardiac puncture, whilst visceral fats and liver were collected and weighed. The whole blood were centrifuged at 3500 revolutions per minute (rpm) for 10 min and the supernatant was separated and stored at −20 °C until required for some biochemical analysis using Randox test kits and protocols.

**Histopathological of the pancreas**

Histopathology of the pancreatic glands was carried out according to the method described by Chen et al. (2014). Pancreatic tissue specimens were fixed in 10% formalin overnight and then paraffin-embedded and sectioned at a thickness of 7 μm. The tissue sections were de-paraffinised and rehydrated sequentially in xylene, xylene/ethanol and gradient ethanol, and then placed in distilled water for 10 min. Pancreatic tissue sections were then stained with haematoxylin and eosin (H&E).

**Gene expression**

Messenger RNA (mRNA) was isolated from the mice pancreas with TRIzol Reagent (ThermoFisher Scientific) and converted to complementary DNA (cDNA) using ProtoScriptFirst Strand cDNA Synthesis Kit (NEB). Polymerase chain reaction (PCR) amplification was carried out using OneTaq®2X Master Mix (NEB) and the intensities of the bands from agarose gel electrophoresis were quantified densitometrically using ImageJ software.

**Statistical analysis**

Data collected was analysed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20 and were expressed as mean ± standard error of mean (SEM). Duncan's multiple ranges was performed using Statistical Package for Social Sciences (SPSS) version 20 and were expressed as mean ± standard error of mean (SEM). Duncan’s multiple ranges was used to separate the averages. Differences in the mean were significant when compared with control groups. Saponin-rich extract of Lindackeria dentata significantly increased the body weight, fasting blood glucose, serum urea, serum alkaline phosphatase (ALP) activity and visceral fats weight (Table 2) at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw in every 15 min of metabolising blood glucose, when compared with control groups. After intra-peritoneal insulin administration (Figure 2d), SLD at various dosages significantly decreased insulin resistance at 15 min, which resulted in hypoglycemia and had to be resuscitated by the administration of a dose of D-glucose solution when compared with control groups.

**Ethical consideration**

Approval to conduct the study was provided by the Ethics Committee of the Olusegun Agagu University of Science and Technology (Project number: oauastechETC032).

<table>
<thead>
<tr>
<th>TABLE 1: Primer sets for the gene expression.</th>
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<tbody>
<tr>
<td><strong>Factors</strong></td>
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<tr>
<td>Interleukin-10</td>
</tr>
<tr>
<td>Leptin</td>
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<tr>
<td>Insulin</td>
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<td>P-actin</td>
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</table>

**Results**

**Comparison of normal mice and high-fat diet mice**

**Acute toxicity study**

Oral administration of SLD was found to be safe up to the dose of 2000 mg/kg bw and produced no signs of toxicity. However, from 5000 mg/kg bw SLD caused sluggish movement of the healthy male albino mice, decreased aggressiveness, altered touch and pain sensibility but did not cause any negative behavioural changes such as excitation, respiratory distress, convulsions or coma. No mortality was observed up to 48 h. Therefore, the median lethal dose (LD$_{50}$) of the SLD was then greater than 2000 mg/kg bw. Therefore doses of 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw were selected for in vivo experiments, conforming to Pandhare et al.’s (2012) dosages recommendation.

**The effect of oral administration of saponin-rich extract of Lindackeria dentata on body weight, fasting blood glucose, oral glucose tolerance test and intra-peritoneal insulin tolerance test in high-fat diet mice**

At the end of first week after oral administration of SLD, there was significant reduction in the body weight and fasting blood glucose of HFD mice at various dosages when compared with control groups (Figure 2a and 2b). During OGGT (Figure 2c), blood glucose metabolised faster at 200 mg/kg bw, which decreased significantly at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw in every 15 min of metabolising blood glucose, when compared with control groups. After intra-peritoneal insulin administration (Figure 2d), SLD at various dosages significantly decreased insulin resistance at 15 min, which resulted in hypoglycemia and had to be resuscitated by the administration of 20% of D-glucose solution when compared with control groups.

**The effect of oral administration of saponin-rich extract of Lindackeria dentata on some biochemical parameters, visceral fats and liver weight in high-fat diet mice**

Saponin-rich extract of Lindackeria dentata treatment significantly reduced (p < 0.05) serum urea, serum alkaline phosphatase (ALP) activity and visceral fats weight (Table 2) in a dose concentration-dependent manner when compared with control groups. However, SLD significantly reduced (p < 0.05) serum creatinine (Table 2) at 200 mg/kg bw when compared with other dosages and control groups. The SLD treatment group at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw significantly decreased (p < 0.05) in high density lipoprotein (HDL) (Table 2) with significant improvement in a dose concentration-dependent manner when compared with control groups. Saponin-rich extract of Lindackeria dentata significantly reduced (p < 0.05) total cholesterol (TC), liver weight, activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Table 2) at 25 mg/kg, 100 mg/kg and 200 mg/kg bw,
TABLE 2: The effect of oral administration of saponin-rich extract of Lindackeria dentata on some biochemical parameters, visceral fats and liver weight in high-fat diet mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum ALT (U/L)</th>
<th>Serum AST (U/L)</th>
<th>Serum ALP (U/L)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Visceral fat weight (g)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal control</td>
<td>450.94 ± 0.57†</td>
<td>0.15 ± 0.01†</td>
<td>12.00 ± 0.58†</td>
<td>31.00 ± 0.58†</td>
<td>146.28 ± 0.58†</td>
<td>96.22 ± 0.01†</td>
<td>47.28 ± 0.58†</td>
<td>44.24 ± 0.58†</td>
<td>0.70 ± 0.00††</td>
<td>1.47 ± 0.03††</td>
</tr>
<tr>
<td>Negative control</td>
<td>454.74 ± 0.58†</td>
<td>0.42 ± 0.01†</td>
<td>25.00 ± 0.58†</td>
<td>52.00 ± 0.58†</td>
<td>538.20 ± 0.06†</td>
<td>281.27 ± 0.58†</td>
<td>89.62 ± 0.58†</td>
<td>136.92 ± 0.58†</td>
<td>1.30 ± 0.06††</td>
<td>2.60 ± 0.64††</td>
</tr>
<tr>
<td>Positive control</td>
<td>616.47 ± 0.58§</td>
<td>1.40 ± 0.00§</td>
<td>17.00 ± 0.58§</td>
<td>31.00 ± 0.58§</td>
<td>270.48 ± 0.58§</td>
<td>186.90 ± 0.52‡</td>
<td>76.21 ± 0.57‡</td>
<td>28.09 ± 0.58‡</td>
<td>0.57 ± 0.12§</td>
<td>1.93 ± 0.18§</td>
</tr>
<tr>
<td>25 mg/kg bw SLD</td>
<td>319.60 ± 0.58§</td>
<td>0.69 ± 0.01§</td>
<td>16.00 ± 0.58§</td>
<td>36.00 ± 0.58§</td>
<td>276.00 ± 0.77†‡</td>
<td>194.90 ± 0.52‡</td>
<td>115.44 ± 0.58‡</td>
<td>28.09 ± 0.58‡</td>
<td>1.20 ± 0.06‡</td>
<td>1.73 ± 0.18‡</td>
</tr>
<tr>
<td>50 mg/kg bw SLD</td>
<td>207.39 ± 0.58‡</td>
<td>0.46 ± 0.01‡</td>
<td>21.00 ± 0.58§</td>
<td>47.00 ± 0.58‡</td>
<td>245.64 ± 5.77†‡</td>
<td>198.00 ± 0.58†</td>
<td>57.16 ± 0.58‡</td>
<td>32.30 ± 0.64†</td>
<td>0.80 ± 0.06‡</td>
<td>2.03 ± 0.03‡</td>
</tr>
<tr>
<td>100 mg/kg bw SLD</td>
<td>329.16 ± 0.58†‡</td>
<td>0.44 ± 0.01‡</td>
<td>12.00 ± 0.58§</td>
<td>10.00 ± 0.58‡</td>
<td>201.48 ± 0.01‡</td>
<td>146.19 ± 0.57†</td>
<td>48.00 ± 0.58‡</td>
<td>67.4 ± 0.64†</td>
<td>0.63 ± 0.09‡</td>
<td>1.77 ± 0.15‡</td>
</tr>
<tr>
<td>200 mg/kg bw SLD</td>
<td>106.54 ± 0.58‡</td>
<td>0.17 ± 0.01‡</td>
<td>5.00 ± 0.58‡</td>
<td>16.00 ± 0.58‡</td>
<td>171.12 ± 0.58‡</td>
<td>109.18 ± 0.58‡</td>
<td>27.52 ± 0.58‡</td>
<td>86.36 ± 0.64†‡</td>
<td>0.30 ± 0.06‡</td>
<td>1.60 ± 0.06‡</td>
</tr>
</tbody>
</table>

†, ††, †‡ Values with the same superscript do not differ significantly from each other whereas values carrying different superscript are significantly different from each other (p < 0.05).
§, ¶, ‡, † Values indicate a statistical difference (p < 0.5).

ALP, alkaline phosphatase; SLD, saponin-rich extract of Lindackeria dentata; U/L, unit per Litre; HDL, high density lipoprotein; TC, total cholesterol; TG, Triglycerides; bw, body weight. 

with marginal increase at 50 mg/kg when compared with control groups. Whilst SLD treatment significantly reduced (p < 0.05) total triglyceride (Table 2) at 50 mg/kg, 100 mg/kg and 200 mg/kg bw when compared with control groups.

The effect of oral administration of saponin-rich extract of Lindackeria dentata on the histopathology of the pancreas in high-fat diet mice

There was no significant inhibition of pancreatic islet cells expansion at 25 mg/kg weight after treatment with SLD (Figure 3c), but there is partial inhibition of pancreatic islet cells expansion at 50 mg/kg bw (Figure 3e). However, at 100 mg/kg and 200 mg/kg bw of SLD (Figure 3f and 3g) there was total inhibition of pancreatic islet cells expansion that caused poor architecture of the pancreatic islet (Figure 3g) when compared to the control groups.

The effect of oral administration of saponin-rich extract of Lindackeria dentata on the expression of insulin, leptin and interleukin-10 gene in the pancreas of high-fat diet mice

Oral administration of SLD significantly increased (p < 0.05) the expression of insulin gene (Figure 4a), leptin gene
(Figure 4b) and IL-10 gene (Figure 4c) at 25 mg/kg, 50 mg/kg and 100 mg/kg bw with significant reduction at 200 mg/kg bw when compared with control groups.

Discussion

Diets high in saturated fatty acids promote the greater accumulation of fats than mono and polyunsaturated fats because saturated fatty acids are inefficiently used for energy production (Hariri, Gougeon & Thibault 2010). Common pathologic conditions arising from diet-induced obesity include weight gain, hyperglycemia, insulin resistance and fatty lipid droplet accumulation in multiple tissues (Heydemann 2016). The high-fat diet used in feeding healthy male albino mice for 12 weeks (Figure 1) induced obesity as a result of increase in weight gain and fat accumulation that led to different pathological conditions. Saponin-rich extract of *Lindackeria dentata* in this study was investigated for its actions on obesity and some specific genes involved in inflammation and insulin resistance in HFD mice. The significant reduction in the bw and blood glucose level as shown in (Figure 2a and 2b), revealed that oral administration of SLD at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw reduced obesity and associated hyperglycemia. The reduction in bw was in accordance with the study of Zhao and co-workers, in which platycodin saponins from *Platycodi radix* induce bw reduction in HFD rats (Zhao et al. 2005). The ability of SLD to reduce the blood glucose level in concentration dose-dependent manner could be as a result of its regenerative action on the pancreatic islet (Keller et al. 2011).

High-fat diet induced obesity leads to insulin resistance accompanied by impaired glucose tolerance and insulin sensitivity (Song et al. 2018). During OGTT (Figure 2c) oral administration of SLD had higher blood glucose level and could metabolise blood glucose faster at 15 min. This insulinotropic action of SLD with improved glucose tolerance...
in HFD mice at 200 mg/kg bw, correlated with the findings of Metwally, Mohamed and ELSharabasy (2012), suggesting that saponins exert direct insulinotropic effect by the release of insulin from the pancreas. Hua et al. (2015) confirmed that furostanolic saponins from *Trigonella foenum-graecum* improved glucose tolerance in HFD mice. It was also revealed that SLD treatment decreased insulin resistance during intra-peritoneal insulin tolerance test (Figure 2d), therefore cause insulin sensitivity of HFD mice at various dosages of SLD: a finding consistent with Yuming et al. (2018), that sea cucumber saponins ameliorate obesity-induced insulin resistance in HFD mice.

Urea and creatinine are markers for a renal function that indicates glomerular filtration rate, concentrating and diluting capacity (tubular function). An increase in the values of these markers indicates a dysfunction of the kidney (Ejelonu et al. 2017). Oral administration of SLD significantly reduced ($p < 0.05$) serum urea (Table 2) and serum creatinine (Table 2) at 200 mg/kg bw. This revealed that oral administration of SLD did not pose any toxic effect to the kidney, but alleviated and restored renal dysfunction caused by HFD, demonstrated Yan et al. (2015), in which panaxadiol saponins improved renal function in lipo-polysaccharide-induced mouse model of acute kidney injury. The liver plays a vital role in biochemical reactions and biological processes.
pathways and in the regulation of body homeostasis (Han, Hui & Wang 2008). The functions of the liver are often compromised during exposure to xenobiotic substances daily (Alli-Smith & Adanlawo 2015). The serum ALT, AST and ALP levels serve as markers for hepatic toxicity (Ejelonu et al. 2017) and are rapidly increased when the liver is damaged because of any reason, including hepatitis or hepatic cirrhosis. Saponin-rich extract of Lindackeria dentata treatment significantly reduced (p < 0.05) serum ALT and serum AST activities at 25, 100 and 200 mg/kg (Table 2), serum ALP at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw (Table 2). These findings revealed that SLD treatment did not pose any toxic threat to the liver of HFD mice but indicated the hepato-protective potentials of SLD. This is consistent with the previous report of Alli-Smith and Adanlawo (2015) that saponin extract from the root of Garcinia kola offer hepato-protection against paracetamol-induced hepatotoxicity in albino rats.

Obese people tend to be hyperlipidemic with relatively high triglyceride and low HDL-cholesterol, which serve as contributing factors to the prevalence and severity of coronary heart diseases (Elekofehinti et al. 2012). Oral administration of SLD significantly reduced (p < 0.05) serum cholesterol (Figure 2), serum triglyceride (Figure 2) at 50 mg/kg, 100 mg/kg and 200 mg/kg bw, and serum HDL (Figure 2) at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg bw which indicate SLD treatment can prevent some cholesterol linked metabolic disorder which correlates to the findings that several saponins possess hypo-cholesterolemic effects (Marrelli et al. 2016). Likewise, saponin can alter the absorption of cholesterol and bile acid by forming micelles with bile acid, thereby causing lowering of cholesterol through its bile acid sequestrants, or binding capacity of cholesterol in the digestive tract (Ejelonu 2018).

Oral administration of SLD significantly reduced (p < 0.05) visceral fat (Figure 2) at 50, 100 and 200 mg/kg bw and liver weight (Figure 2) at 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg body weight. Thus, the reduction of visceral fat and liver weight by SLD can decrease the production of pro-inflammatory cytokines that cause metabolic dysfunction in HFD mice. This is in accordance with Hamao and coworkers which observed that methanolic extract of flower buds of Camellia sinensis L. inhibits bw gain in HFD mice, which suppress liver weight and weight of visceral fat (Hamao et al. 2011).

In the treatment of HFD mice with inhibited SLD, damage because of poor pancreatic islet cell architecture (Figure 3) is partially restored at 100 mg/kg and 200 mg/kg bw. Therefore oral administration of SLD can restore metabolic function activities that take place in the pancreatic islets of HFD mice. This is consistent with Deng and coworker with the administration of protopanaxadiol (PPD) and protopanaxatriol (PPT)-type saponins which showed partial restoration of the impaired pancreatic cells of HFD mice because of pancreatic cell hyperplasia and islet cell hypertrophy (Deng et al. 2017). The effect of oral administration of SLD on the pancreas of HFD mice could be as a result of its anti-hyperglycemic effect: improving peripheral insulin resistance and stimulating insulin secretion (Zheng et al. 2012), rather than protecting pancreas islet β-cells.

Inflammation is considered to be a pathological mediator in an obese state, and it is associated with metabolic disorders (Henriksbo et al. 2014; Kalupahana, Moustaid-Moussa & Claycombe 2012). It has been established in recent research that there is a relationship between leptin, insulin resistance, obesity and cardiovascular disease (Elekofehinti et al. 2017). Oral administration of SLD in HFD mice up-regulates the expression of insulin gene (Figure 4a), leptin gene (Figure 4b) and IL-10 gene (Figure 4c) in the pancreas at 25 mg/kg, 50 mg/kg and 100 mg/kg bw. Therefore, SLD treatment resulted in increased production of insulin coupled with satiety hormone by increasing the production of anti-inflammatory cytokines in the pancreas of HFD mice. This revealed that SLD treatment could ameliorate insulin resistance, leptin resistance and pro-inflammatory cytokines caused by HFD. This is consistence with Yang and coworker: that Panaxnoto ginseng saponins demonstrated anti-hyperglycemic and anti-obese activities as a result of improved insulin and leptin sensitivity (Yang et al. 2010). Likewise, Yu and coworker also suggested that anti-inflammatory effect of tea saponins was associated with improved glycaemic status in the treated animals, evidenced as a result of improved glucose tolerance (Yu et al. 2013).

**Conclusion**

The results obtained from this study indicate that SLD treatment did not pose any toxic threat to the normal lean tissues of the mice but revealed its anti-obesity, anti-inflammatory and anti-hyperglycemic potential, and the possible mechanism is through increased insulin secretion, increased leptin expression and reduction of inflammation by up-regulating IL-10 gene expression, making it a candidate in combating obesity and its associated disorders.
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Competing interests
The authors have declared no competing interests exist.

Authors’ contributions
All authors contributed equally to this work.

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Data availability statement
Data sharing is not applicable to this article as no new data were created or analysed in this study.

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References

