Journal of Medicinal Plants for Economic Development
ISSN: (Online) 2616-4809, (Print) 2519-559X

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

Authors:
Oluwamodupe C. Ejelonu[1]
Simeon O. Oluba[2,3]
Bankole O. Awolokun[2]
Olusola O. Elekofehinti[4]
Isaac G. Adanlawo[5]

Affiliations:
[1]Department of Chemical Sciences, Biochemistry Program, Faculty of Science, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria

[2]Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Nigeria

[3]Clinical Biochemistry Units, Department of Medical Laboratory Science, Inland Medical Hospital, Ikare Akoko, Nigeria

[4]Bioinformatics and Molecular Biology Units, Department of Biochemistry, Faculty of Science, Federal University of Technology, Akure, Nigeria

[5]Department of Biochemistry, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria

Corresponding author:
Oluwamodupe Ejelonu
oc.ejelonu@osustech.edu.ng

Dates:
Received: 01 July 2020
Accepted: 17 Oct. 2020
Published: 25 Jan. 2021

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).

How to cite this article: Ejelonu, O.C., Oluba, S.O., Awolokun, B.O., Elekofehinti, O.O., Adanlawo, I.G., 2021, 'Saponin-rich extracts reverse obesity and offer protection against obesity-induced inflammation in high-fat diet mice', *Journal of Medicinal Plants for Economic Development* 5(1), a101. https://doi.org/10.4102/jomped.v5i1.101

Researchers have placed much interest on insulin resistance and it's 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 Flacourtiaceae (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 and some saponins in the leaves, bark and roots (Burkill 1985). Saponins as phyto-compound are well known bioactive agents that have been investigated for a multitude of biological activities, including anti-microbial, cytotoxic, anti-inflammatory, anti-hyperlipidemic, immune-stimulatory and anti-diabetic properties (Ejelonu, Ekofehinti & Adanlawo 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, Ekiti State University, Ado Ekiti, Nigeria with voucher number UHAE/181. The plant leaves were washed, air-dried, pulverised and preserved at room temperature in airtight containers.

Extraction of saponin-rich extract of *Lindackeria dentata* leaves

Saponin-rich compounds were extracted according to the method described by Ejelonu et al. (2017). One thousand grams ground sample was extracted with 5000 mL of methanol for 72 h. The methanolic extract was concentrated using a rotary evaporator and partitioned with n-hexane and water (1:2, v/v). After a thorough shaking, the mixture was allowed to stand overnight and the water layer was concentrated and partitioned between ethyl acetate and n-butanol (1:3, v/v). The ethyl acetate fraction was concentrated to obtain saponin-rich extract.

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

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 lV – Vll 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.

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 hematoxylin 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 used to separate the averages. Differences in the mean were significant when $p < 0.05$. GraphPad Prism version 7.04 was used to plot the graph.

Ethical consideration

Approval to conduct the study was provided by the Ethics Committee of the Olusegun Agagu University of Science and Technology (Project number: oaustechETC032).

TABLE 1: Primer sets for the gene expression.

Factors	Forward primers	Reverse primers
Interleukin-10	5′-ATAACTGCACCCACTTCCCA-3′	5′-GGGCATCACTTCTACCAGGT-3′
Leptin	5′-TGACACCAAAACCCTCATCA-3′	5′-AGCCCAGGAATGAAGTCCA-3′
Insulin	5′-AAGTGGAGGACCCACAAGTG-3′	5′-CTGAAGGTCCCCGGGGCT-3′
β-actin	5′-GCAAGTGCTTCTAGGCGGAC-3′	5′-AAGAAAGGGTGTAAAACGCAGC-3′

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 excitement, 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 OGTT (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.

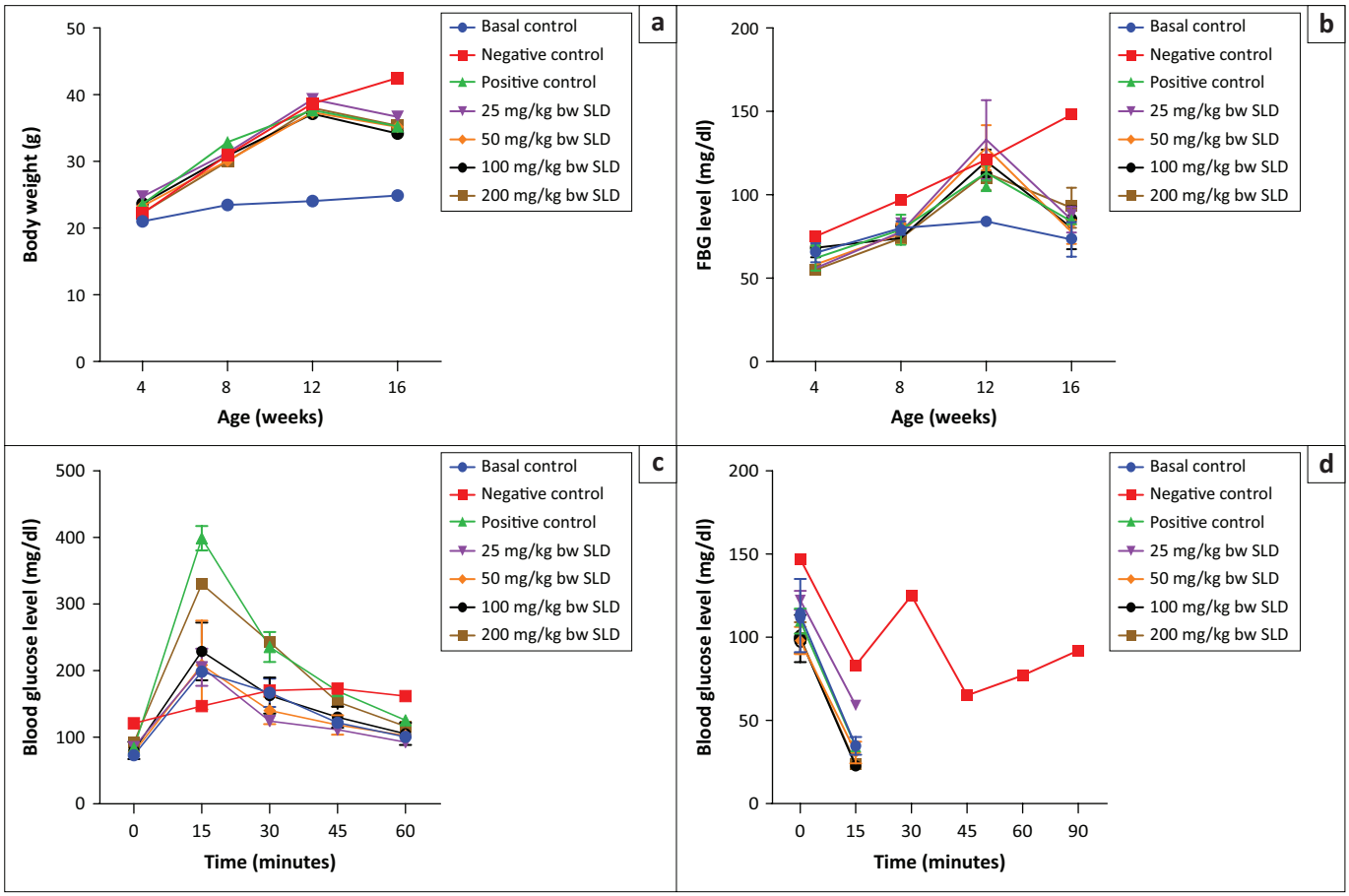
The effect of oral administration of saponin-rich extract of *Lindackeria dentata* on some biochemical parameters, visceral fats and liver weight in high-fat diet mice

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.

Groups	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Serum ALT (U/L)	Serum AST (U/L)	Serum ALP (U/L)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	Visceral fats weight (g)	Liver weight (g)
Basal control	450.94 ± 057††	0.15 ± 0.01†	12.00 ± 0.58‡	31.00 ± 0.58§	146.28 ± 0.58†	96.22 ± 0.01†	47.28 ± 0.58‡	44.24 ± 0.58§	0.70 ± 0.00‡§	1.47 ± 0.03†
Negative control	454.74 ± 0.58‡‡	0.42 ± 0.01‡	25.00 ± 0.58††	52.00 ± 0.58‡‡	538.20 ± 0.06‡‡	281.27 ± 0.58‡‡	89.62 ± 0.58††	136.92 ± 0.58‡‡	1.30 ± 0.06¶	2.60 ± 0.64‡
Positive control	616.47 ± 0.58§§	1.40 ± 0.06¶	17.00 ± 0.58§	31.00 ± 0.58§	270.48 ± 0.58††	186.90 ± 0.52††	76.21 ± 0.57¶	28.09 ± 0.58†	0.57 ± 0.12‡	1.93 ± 0.18†‡
25 mg/kg bw SLD	279.60 ± 0.55§	0.69 ± 0.01§	16.00 ± 0.58§	36.00 ± 0.58¶	276.00 ± 5.77††	194.30 ± 0.52††	115.44 ± 0.58‡‡	28.09 ± 0.58†	1.20 ± 0.06¶	1.73 ± 0.18†‡
50 mg/kg bw SLD	207.39 ± 0.58‡	0.46 ± 0.01‡	21.00 ± 0.58¶	47.00 ± 0.58††	245.64 ± 5.77¶	198.00 ± 0.58††	57.16 ± 0.58§	32.30 ± 0.64‡	0.80 ± 0.06§	2.03 ± 0.03†‡
100 mg/kg bw SLD	329.16 ± 0.57¶	0.44 ± 0.01‡	12.00 ± 0.58‡	10.00 ± 0.58†	201.48 ± 0.01§	146.19 ± 0.57§	48.00 ± 0.58‡	67.4 ± 0.64¶	0.63 ± 0.09‡§	1.77 ± 0.15†‡
200 mg/kg bw SLD	106.54 ± 0.58†	0.17 ± 0.01†	5.00 ± 0.58†	16.00 ± 0.58‡	171.12 ± 0.58‡	109.18 ± 0.58‡	27.52 ± 0.58†	86.36 ± 0.64††	0.30 ± 0.06†	1.60 ± 0.06†

†, ‡, 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.



bw, body weight; SLD, saponin-rich extract of *Lindackeria dentate*.

FIGURE 1: The effect of oral administration of saponin-rich extract of *Lindackeria dentata* on (a) body weight, (b) fasting blood glucose, (c) oral glucose tolerance test and (d) intra-peritoneal insulin tolerance test in high-fat diet mice.

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

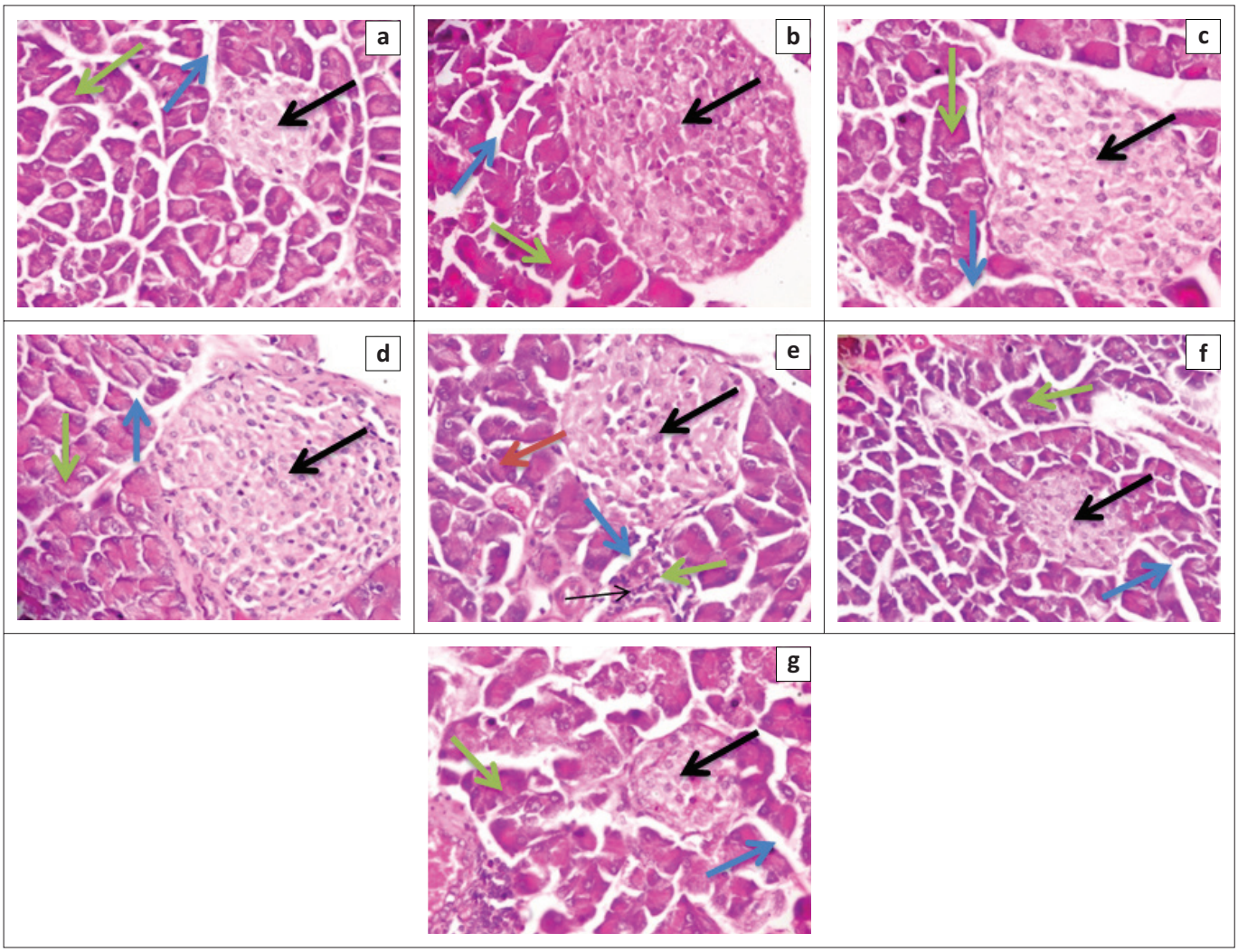


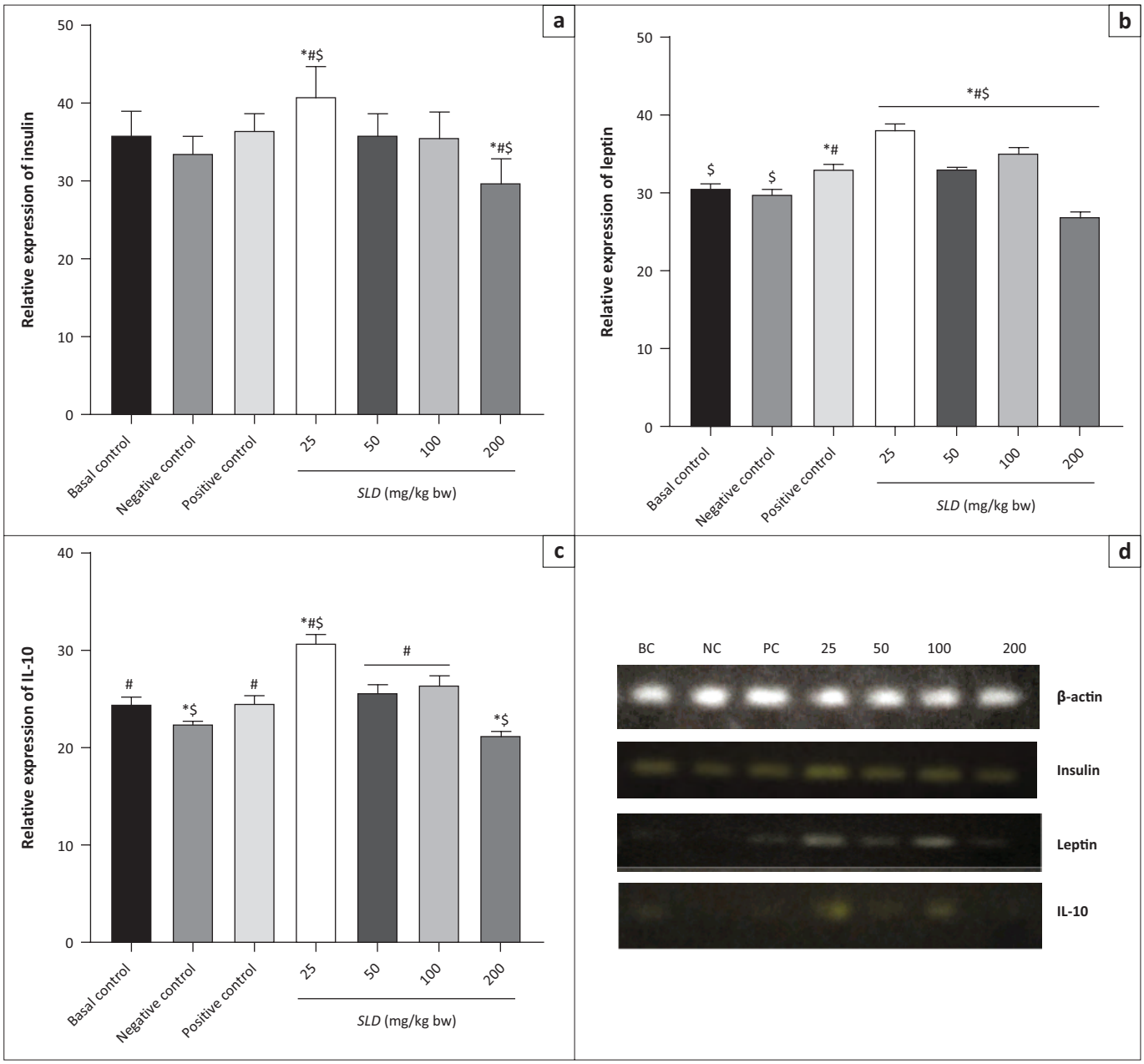
FIGURE 2: The effect of oral administration of saponin-rich extract of *Lindackeria dentata* on the histopathology of the pancreas in high-fat diet mice (hematoxylin and eosin stain × 400). Photomicrograph of the pancreas showed normal serous acinar and zymogenic cells (light green arrow), abundant granular eosinophilic cytoplasm and normal septa exist between the lobules (blue arrow). The pancreatic islet cells appeared to be essentially normal (black arrow) in basal control (a), expansion of pancreatic islet cells (black arrow) as a result of pancreatic cells hyperplasia and hypertrophy in negative control (b), slight expansion of pancreatic islet cells (black arrow) in positive control (c), expansion of pancreatic islet cells (black arrow) as a result of pancreatic cells (d). However, pancreatic islet cells showed poor serous acinar and zymogenic cells with vacuolated nuclei (light red arrow), thickened ductal wall (thin black arrow) with peripheral infiltration of the ducts by inflammatory cells (light green arrow) at 50 mg/kg body weight (e) with partial pancreatic islet expansion (black arrow) with areas of mild necrosis. There was total (f and g) inhibition of pancreatic islet cell expansion (black arrow).

(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



*, indicates statistical difference ($p < 0.05$) to basal control.

**, indicates statistical difference ($p < 0.05$) to negative control.

***, indicates statistical difference ($p < 0.05$) to positive control.

AST, aspartate aminotransferase; BC, basal control; NC negative control; IL, interleukin-10; SLD, saponin-rich extract of *Lindackeria dentata*.

FIGURE 3: The effect of oral administration of saponin-rich extract of *Lindackeria dentata* on the expression of insulin, leptin and interleukin-10 (IL-10) gene in the pancreas of high-fat diet mice. Quantitative (a–c) and qualitative (d) representation mRNA expression of insulin, leptin and IL-10 in the pancreas of high-fat diet mice. Basal control (BC), Negative control (NC), Positive control (PC) and various dosages of SLD treatment in mg/kg body weight were compared.

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

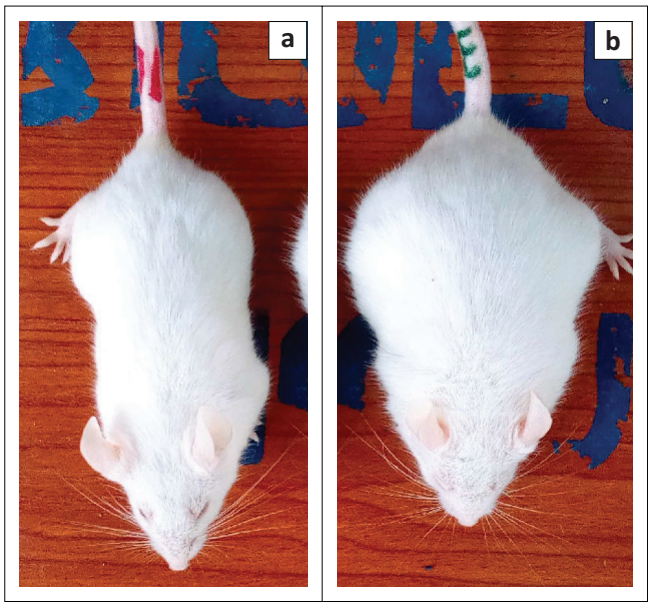


FIGURE 4: Comparison of normal mice (a) and high-fat diet mice (b) after 12 weeks of feeding.

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.

Acknowledgements

Competing interests

The authors have declared no competing interests exist.

Authors' contributions

All authors contributed equally to this work.

Funding information

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Data availability statement

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

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliation agency of the authors.

References

1985, Guide *for the care and use of laboratory animals*, rev. edn., NIH Publication No. 85–93, U.S. Department of Health and Human Services, United States.

Alli-Smith, Y.R. & Adanlawo, I.G., 2015, 'Protective effect of saponin extract from the root of *Garcinia kola* (Bitter kola) against paracetamol-induced hepatotoxicity in albino rats', *International Journal of Biological Biomolecular Agricultural Food and Biotechnological Engineering* 9(2), 130–134.

Burkill, H.M., 1985, *The useful plants of west tropical Africa*, 2nd edn., Royal Botanic Gardens, Kew, viewed 28 November 2019, from https://plants.jstor.org/stable/10.5555/al.ap.upwta.2_307.

Chen, Z-Y., Liu, S-N., Li, C-N., Sun, S-J., Liu, Q., Lei, L. et al., 2014, 'Atorvastatin helps preserve pancreatic β cell function in obese C57BL/6 J mice and the effect is related to increased pancreas proliferation and amelioration of endoplasmic-reticulum stress', *Lipids in Health and Disease* 13, 98. https://doi.org/10.1186/1476-511X-13-98

Czernichow, S., Kengne, A.P., Stamatakis, E., Hamer, M. & Batty, G.D., 2011, 'Body mass index, waist circumference and waist-hip ratio: Which is the better discriminator of cardiovascular disease mortality risk? Evidence from an individual-participant meta-analysis of 82864 participants from nine cohort studies', *Obesity Reviews* 12(9), 680–687. https://doi.org/10.1111/j.1467-789X.2011.00879.x

Darkhal, P., Gao, M., Ma, Y. & Liu, D., 2015, 'Blocking high fat diet-induced obesity, insulin resistance and fatty liver by overexpression of *Il-13* gene in mice', *International Journal of Obesity* 39(8), 1292–1299. https://doi.org/10.1038/ijo.2015.52

Deng, J., Liu, Y., Duan, Z., Zhu, C., Hui, J., Mi, Y. et al., 2017, 'Protopanaxadiol and protopanaxatriol-type saponins ameliorate glucose and lipid metabolism in type 2 diabetes mellitus in high-fat diet/streptozocin-induced mice', *Frontiers in Pharmacology* 8, 506. https://doi.org/10.3389/fphar.2017.00506

Ejelonu, O.C., 2018, 'Bile acids and saponins in incretin modulation: Many similarities, few differences', *Mintage Journal of Pharmaceutical and Medical Sciences* 7(1), 30–37, viewed 28 November 2019, from https://mjpms.in/index.php/mjpms/article/view/301.

Ejelonu, O.C., Ekofehinti, O.O. & Adanlawo, I.G., 2017, '*Tithonia diversifolia* saponins-blood lipid interaction and its influence on immune system of normal wistar rats', *Biomedicine and Pharmacotheraphy* 87, 589–595. https://doi.org/10.1016/j.biopha.2017.01.017

Elekofehinti, O.O., Adanlawo, I.G., Saliu, J.A. & Sodehinde, S.A., 2012, 'Saponins from *Solanum anguivi* fruits exhibit hypolipidemic potential in *Rattusnovergicus*', *Der Pharmacia Lettre* 4(3), 811–814.

Elekofehinti, O.O., Ejelonu, O.C., Kamdem, J.P., Akinlosotu, O.B. & Adanlawo, I.G., 2017, 'Saponins as adipokines modulator: A possible therapeutic intervention for type 2 diabetes', *World Jouranl of Diabetes* 8(7), 337–345. https://doi.org/10.4239/wjd.v8.i7.337

Elekofehinti, O.O., Omotuyi, I.O., Kamdem, J.P., Ejelonu, O.C., Alves, G.V., Adanlawo, I.G. et al., 2014, 'Saponin as regulator of biofuel: Implication for ethnobotanical management of diabetes', *Journal of Physiology and Biochemistry* 70(2), 555–567. https://doi.org/10.1007/s13105-014-0325-4

Fischer, I.P., Irmler, M., Meyer, C.W., Sachs, S.J., Neff, F., Hrabede, A.M. et al., 2018, 'A history of obesity leaves an inflammatory fingerprint in liver and adipose tissue', *International Journal of Obesity* 42(3), 507–517. https://doi.org/10.1038/ijo.2017.224

Hamao, M., Matsuda, H., Nakamura, S., Nakashima, S., Semura, S., Maekubo, S. et al., 2011, 'Anti-obesity effects of the methanolic extract and chaka saponins from the flower buds of *Camellia sinensis* in mice', *Bioorganic and Medicinal Chemistry* 19(20), 6033–6041. https://doi.org/10.1016/j.bmc.2011.08.042

Han, C., Hui, Q. & Wang, W., 2008, 'Hypoglycaemic activity of saponin fraction extracted from *Momordica charantia* in PEG/salt aqueous two-phase systems', *Natural Product Research* 22(13), 1112–1119. https://doi.org/10.1080/14786410802079675

Hariri, N., Gougeon, R. & Thibault, L., 2010, 'A highly saturated fat rich diet is more obesogenic than diets with lower saturated fat content', *Nutrition Research* 30(9), 632–643. https://doi.org/10.1016/j.nutres.2010.09.003

Henriksbo, B.D., Lau, T.C., Cavallari, J.F., Denou, E., Chi, W., Lally, J.S. et al., 2014 'Fluvastatin causes NLRP3 inflammasome-mediated adipose insulin resistance', *Diabetes* 63(11), 3742–3747. https://doi.org/10.2337/db13-1398

Heydemann, A., 2016, 'An overview of murine high fat diet as a model for type 2 diabetes mellitus', *Journal of Diabetes Research* 2016, 14. https://doi.org/10.1155/2016/2902351

Hua, Y., Ren, S.Y., Guo, R., Rogers, O., Nair, R.P., Bagchi, D. et al., 2015, 'Furostanolic saponins from *Trigonella foenum-graecum* alleviate diet-induced glucose intolerance and hepatic fat accumulation', *Molecular Nutrition and Food Research* 59(10), 2094–2100. https://doi.org/10.1002/mnfr.201500197

Jaroszewski, J.W., Ekpe, P. & Witt, M., 2004, 'Cyclopentanoid cyanohydrin glucosides and amides of *Lindackeria dentata*', *Planta Medica* 70(10), 1001–1003. https://doi.org/10.1055/s-2004-832628

Kalupahana, N.S., Moustaid-Moussa, N. & Claycombe, K.J., 2012, 'Immunity as a link between obesity and insulin resistance', *Molecular Aspects of Medicine* 33(1), 26–34. https://doi.org/10.1016/j.mam.2011.10.011

Keller, A.C., Ma, J., Kavalier, A., He, K., Brillantes, A.M.B. & Kennelly, E.J., 2011, 'Saponins from the traditional medicinal plant *Momordica charantia* stimulate insulin secretion *in vitro*', *Phytomedicine* 19(1), 32–37. https://doi.org/10.1016/j.phymed.2011.06.019

Marrelli, M., Conforti, F., Araniti, F. & Statti, G.A., 2016, 'Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity', *Molecules* 21(10), 1404. https://doi.org/10.3390/molecules21101404

Metwally, N.S., Mohamed, A.M. & ELSharabasy, F.S., 2012, 'Chemical constituents of the Egyptian plant *Anabasis articulata* (Forssk) Moq andits antidiabetic effects on rats with streptozotocin-induced diabetic hepatopathy', *Journal of Applied Pharmaceutical Science* 2(4), 54–65. https://doi.org/10.7324/JAPS.2012.2403

Nagy, C. & Einwallner, E., 2018, 'Study of *in vivo* glucose metabolism in high-fat diet-fed mice using oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)', *Journal of Visualized Experiments* 131, 56672. https://doi.org/10.3791/56672

Organization for Economic Co-operation and Development, 2008, *Guidelines for the testing of chemicals: Acute oral toxicity-up-and-down-procedure (UDP)*, Test No. 425, pp. 1–27, Organisation for Economic Co-operation and Development, Paris.

Pandhare, B.R., Sangameswaran, B., Mobile, P.B. & Khanage, S.G., 2012, 'Anti-hyperglycaemic and lipid lowering potential of Adenantherapavonina Linn. in streptozotocin induced diabetic rats', *Oriental Pharmacy and Experimental Medicine* 12(3), 197–203. https://doi.org/10.1007/s13596-012-0074-2

Piya, M.K., McTernan, P.G. & Kumar, S., 2013, 'Adipokine inflammation and insulin resistance: The role of glucose, lipids and endotoxin', *Journal of Endocrinology* 216(1), T1–T15. https://doi.org/10.1530/JOE-12-0498

Pothuraju, R., Sharma, R.K., Rather, S.A. & Singh, S., 2016, 'Comparative evaluation of anti-obesity effect of *Aloe vera* and *Gymnema sylvestre* supplementation in high-fat diet fed C57BL/6J mice', *Journal of Intercultural Ethnopharmacology* 5(4), 403–407. https://doi.org/10.5455/jice.20160623122710

Ruts, S. & Ouyang, W., 2016, 'Regulation of interlukin-10 expression', *Advances in Experimental Medicine and Biology* 941, 89–96. https://doi.org/10.1007/978-94-024-0921-5_5

Sharma, T. & Kanwar, S.S., 2018, 'Phytomolecules for obesity and body weight management', *Journal of Biochemistry and Cell Biology* 1(1), 101.

Song, M.Y., Lim, S-K., Wang, J-H. & Kim, H., 2018, 'The root of atractylodes macrocephala Koidzumi prevents obesity and glucose intolerance and increases energy metabolism in mice', *International Journal of Molecular Sciences* 19(1), 278. https://doi.org/10.3390/ijms19010278

Stafeev, I.S., Vorotnikov, A.V., Ratner, E.I., Menshikov, M.Y. & Parfyonova, Y.V., 2017, 'Latent inflammation and insulin resistance in adipose tissue', *International Journal of Endocrinology* 2017, 5076732, 12 pages. https://doi.org/10.1155/2017/5076732

Wasim, M., 2015, 'Role of leptin in obesity', *Journal of Obesity and Weight Loss Therapy* 5, 258. https://doi.org/10.4172/2165-7904.1000258

Yan, C., Yanwei, D., Yang, L., Xiaoqin W., Pin, G., Guang, Y. et al., 2015, 'Panaxadiol saponins and dexamethasone improve renal function in lipopolysaccharide-induced mouse model of acute kidney injury', *PLoS One* 10(7), e0134653. https://doi.org/10.1371/journal.pone.0134653

Yang, C.Y., Wang, J., Zhao, Y., Shen, L., Jiang, X., Xie, Z.G. et al., 2010, 'Anti-diabetic effects of Panax Notoginseng saponins and its major anti-hyperglycemic components', *Journal of Ethnopharmacology* 130(2), 231–236. https://doi.org/10.1016/j.jep.2010.04.039

Yu, Y., Wu, Y., Szabo, A., Wu, Z., Wang, H., Li, D. et al., 2013, 'Teasaponin reduces inflammation and central leptin resistance in diet-induced obese male mice', *Endocrinology* 154(9), 3130–3140. https://doi.org/10.1210/en.2013-1218

Yuming, W., Tiantian, Z., Cheng, C., Xiuqing, H., Ping, D., Zhaojie, L. et al., 2018, 'Sea cucumber saponins liposomes ameliorate obesity-induced inflammation and insulin resistance in high fat diet fed mice', *Food and Function* 9(2), 861–870. https://doi.org/10.1039/C7FO01599B

Zhao, H., Sim, J., Shim, S., Ha, Y., Kang, S. & Kim, Y., 2005, 'Anti-obese and hypolipidemic effects of platycodin saponins in diet-induced obese rats: Evidences for lipase inhibition and calorie intake restriction', *International Journal of Obesity* 29(8), 983–990. https://doi.org/10.1038/sj.ijo.0802948

Zheng, T., Shu, G., Yang, Z., Mo, S., Zhao, Y. & Mei, Z., 2012, 'Antidiabetic effect of total saponins from *Entadaphaseoloides* (L.) Merr. in type 2 diabetic rats', *Journal of Ethnopharmacology* 139(3), 814–821. https://doi.org/10.1016/j.jep.2011.12.025