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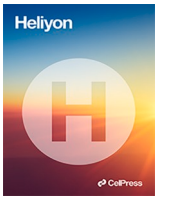

Research article

The significant antidyslipidemic, hypoglycemic, antihyperglycemic, and antiobesity activities of the aqueous extracts of Agave Sisalana juice are partly mediated via modulation of calcium signaling pathways

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ABSTRACT

Plant species in the genus Agave, including *Agave sisalana*, have found extensive application in African and Asian traditional medicine. Inspired by the use of the edible sweet sap known as Aguamiel (obtained from specific mature agave species such as *Agave salmiana*) in Mexico by diabetic patients to improve their diabetic condition, this study investigated the effects of *Agave sisalana* extracts prepared by lyophilization, fermentation, and saponin extraction from sisal juice in a rodent model of metabolic syndrome. The metabolic syndrome was induced by administering a high fat and high fructose diet to freshly weaned Sprague-Dawley rats for eight weeks.

The *A. sisalana* extracts possessed significant hypoglycemic effects [3.883 ± 0.371 mmol/L (normal group) vs. 8.183 ± 0.5845 mmol/L (negative control) vs. 3.767 ± 0.2716 mmol/L (positive control) vs. 4.167 ± 0.4602 mmol/L (FSP) vs. 4.533 ± 0.3169 mmol/L (FerSP) vs. 3.5 ± 0.2309 mmol/L (FS LD) vs. 3.867 ± 0.3353 mmol/L (FS HD) vs. 4.617 ± 0.2725 mmol/L (FerS LD) vs. 4.383 ± 0.3114 mmol/L (FerS HD): $p < 0.0001$]. The extracts also possessed significant antihyperlipidemic effects with significant differences in total serum cholesterol between the groups [1.398 ± 0.1232 mmol/L (normal group) vs. 4.225 ± 0.4135 mmol/L (negative control) vs. 1.582 ± 0.154 mmol/L (positive control) vs. 1.245 ± 0.0911 mmol/L (FSP) vs. 1.393 ± 0.1423 mmol/L (FerSP) vs. 1.387 ± 0.0924 mmol/L (FS LD) vs. 1.761 ± 0.1495 mmol/L (FS HD) vs. 1.698 ± 0.1294 mmol/L (FerS LD) vs. 1.6975 ± 0.0982 mmol/L (FerS HD): $p < 0.0001$]. Further, significant antiobesity effects of the *A.sisalana* extracts were observed with significant differences in weight among the groups [196.3 ± 6.49 g (normal group) vs. 298.9 ± 6.67 g (negative control) vs. 215.3 ± 6.06 g (positive control) vs. 195.4 ± 3.92 g (FSP) vs. 213.1 ± 5.21 g (FerSP) vs. 190.8 ± 6.49 g (FS LD) vs. 198.9 ± 4.31 g (FS HD) vs. 204.7 ± 4.78 g (FerS LD) vs. 208.7 ± 6.21 g (FerS HD): $p < 0.0001$].

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Network pharmacology studies indicated that the chemical components found in sisal juice primarily exert their effects by modulating the voltage-gated calcium channels CACNA1S, CACNA1D, and CACNA1C, in the beta cells of the islets of Langerhans.

1. Introduction

The prevalence of metabolic syndrome, which the World Health Organization (WHO) defines as a pathologic condition characterized by abdominal obesity, insulin resistance, hypertension, and hyperlipidemia, is on the rise globally [1, 2]. Metabolic syndrome is a documented risk factor for the development of various disease conditions, e.g., Type 2 Diabetes Mellitus, ischemic heart disease, stroke, cancer, chronic kidney disease, etc. which have severe effects on morbidity and mortality, underscoring the need to identify novel treatments and lifestyle modifications to prevent and ameliorate this syndrome [3].

The sisal plant *Agave sisalana* Perrine ex Engelm belongs to the genus *Agave*, whose plant species are indigenous to the tropical and subtropical regions of South America, Mexico, the Southern Coast U.S.A., and the Caribbean Islands [4]. This genus, whose many members find extensive application in African and Asian traditional medicine, contains more than 200 species plus 47 intraspecific categories [5, 6]. Although the sisal plant's most famous use is the stiff fiber production after processing the sisal leaves' decortications, it is also used in traditional medicine [6]. Indeed, sisal is a folk remedy for dysentery, leprosy sores, and syphilis. The roots are used to produce an alcoholic drink, and the new shoots are cooked as a vegetable [4].

Since only 2.7–7.3% of the decortications of the sisal leaves produce the stiff fiber that is used for various purposes, the remaining 97.3–92.7%, which consists of solid waste (mucilage) and sisal juice, are usually discarded by sisal farms contributing to the environmental pollution underscoring the need to identify novel value addition approaches in sisal processing [4, 7, 8, 9]. This study investigated the effects of extracts prepared by the lyophilization, fermentation, and saponin-rich extracts prepared from sisal juice in a rodent model of metabolic syndrome. This study was inspired by the use of the edible sweet sap known as Aguamiel (obtained from specific mature agave species such as *Agave salmiana*) in Mexico by diabetic patients to improve their diabetic condition [10].

2. Materials and methods

2.1. Plant material and extraction

The *Agave sisalana* specimen (young succulent leaves) was harvested from Kajiado County, Kenya, in January 2018. Resident taxonomists confirmed its identity at the Department of Botany, School of Biological Sciences, University of Nairobi, and a voucher specimen deposited therein. Voucher number (31122018).

2.1.1. Sisal juice extraction and extract preparation

The sisal leaf juice was extracted immediately after collection by pressing, blending, and filtering the mucilage. The juice was used to obtain four extracts, including Fresh freeze-dried extract (F.S.), fermented freeze-dried extract (FerS), fresh saponin extract (F.S.P.), and fermented saponin extract (FerSP) as follows:

2.1.1.1. Fresh freeze-dried extract. One liter of the fresh sisal juice extracted was immediately frozen and lyophilized to obtain the F.S.E. and diluted according to the dose requirements per group.

2.1.1.2. Fermented freeze-dried extract. One liter of the extracted sisal juice was stored in plastic bottles in an oven at 28 °C ± 2 for four days to allow complete fermentation. On the fourth day, the fermented juice was frozen and lyophilized to obtain a FerSE.

2.1.1.3. Fresh saponin extract. To obtain the fresh saponin crude extract, the protocol described by Leal et al. (2015) was applied as follows; 1 L of the fresh sisal juice was mixed with n-butanol-distilled water (1:1), and the mixture agitated at 250 rpm and 37 °C for 30 min. The mixture was centrifuged for five (5) minutes at 3000 rpm and 4 °C. The organic phase was collected and dried in an oven at 40 °C for 2 h. The extract obtained was stored at 4 °C in amber-colored glass bottles (Leal-Díaz et al., 2015).

2.1.1.4. Fermented saponin extract. One liter of the extracted sisal juice was stored in amber-colored plastic bottles in an oven at 28 °C ± 2 for four days to allow complete fermentation., the saponin extraction protocol described above was followed (Leal-Díaz et al., 2015).

2.2. Experimental animals

The Sprague Dawley rats were housed in standard animal cages containing five rats each in the animal house at the Department of Medical Physiology, University of Nairobi. The following conditions were maintained in the animal house: constant room temperature (23 ± 2 °C) and 30–70% relative humidity with a 12-h light/day cycle. The rats were habituated for a period of 2 weeks. The Biosafety approved the experimental protocol, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi REF No.

FVM BAUEC/2019/202. All experimental procedures complied with the FELASA guidelines on the use and care of laboratory animals.

2.3. Animal model and diet preparation

An animal model of metabolic syndrome was created by the administration of a high fat high sucrose diet. The high fat/high sugar diet was prepared by the addition of 20% w/w saturated fat (Frymate™ manufactured by Bidco industries) to standard rat chow which contained protein 29.82%, fat 13.43% carbohydrates 56.74%, fiber 5.3%, vitamins, and minerals small quantities-ppm (Unga Feeds) to ensure that the pellets delivered approximately 30% of calories as fat. Monosodium glutamate (M.S.G.) was added to the pellets to make up 0.8% of the pellets to increase the pellets' palatability. Fructose was added to drinking water to make up a 20% fructose solution. The resulting high fat/high fructose diet contained 30% fat and 20% fructose in the drinking water. All groups except the standard control group were fed this high fat/high fructose diet ad libitum for eight weeks. The extracts/treatments were administered via oral ga.vage for eight weeks.

2.4. Experimental procedures

Fifty-four (54) freshly-weaned (4 weeks old) Sprague-Dawley rats weighing (70 g–150 g) between were randomly divided into the following nine groups (n = 6 per group): (1) normal control (normal diet and distilled water), (2) negative control (high fat/high fructose diet), (3) positive control (high fat/high sugar diet plus metformin (70 mg/kg), (4) fresh saponin (high fat/high fructose diet plus 50 mg/kg *Agave sisalana* saponin-rich extract), (5) fermented saponin (high fat/high fructose diet plus 50 mg/kg *Agave sisalana* saponin extract), (6) fresh extract low dose (LD) (high fat/high fructose diet plus 50 mg/kg fresh freeze dried *Agave sisalana extract*), (7) fresh extract high dose (HD) (high fat/high fructose diet plus 100 mg/kg F.S. *Agave sisalana*), (8) fermented extract low dose (LD) (high fat/high fructose diet plus 50 mg/kg fermented freeze dried *Agave sisalana* extract), (9) fermented extract high dose (HD) (high fat/high fructose diet plus 100 mg/kg fermented freeze dried extracts *Agave sisalana*).The respective treatments were administered via oral gavage for eight (8) weeks. The doses of treatment used in this experiment were determined from pilot studies as well as from a survey of literature. The following physiological and biochemical parameters were measured in this study.

2.4.1. Weight measurement, retroperitoneal adipose tissue, pericardial adipose tissue, and mesenteric adipose tissue

The animals were weighed weekly on a standard laboratory weighing scale throughout the eight-week study period.

The adipose tissue (Retroperitoneal adipose tissue, Pericardial adipose tissue, and Mesenteric) were carefully extracted and weighed using a standard laboratory weighing scale.

2.4.2. Fasting blood glucose (F.B.G.) test

Blood was sampled from the lateral tail vein after a 6-h fast. The tail was warmed by dipping in warm water for five (5) minutes to cause vasodilation of the vein and ease visualization and blood sample collection. The blood glucose was determined using a glucometer (StatStrip Xpress, Nova Biomedical).

2.4.3. Oral glucose tolerance test (OGTT)

An oral glucose tolerance test (OGTT) was carried out at week four and week 8 of the study using the procedure described by Barrett-Connor [11]. Briefly, the experimental animals were fasted for eight (8) hours, after which a glucose load of 2 g/kg was administered via oral gavage with a glucose solution. Blood samples were collected from the tail vein at 0, 30, 60, 90, and 120 min, and the blood glucose was determined using a glucometer (StatStrip Xpress, Nova Biomedical, Waltham, MA, U.S.A.).

2.4.4. Serum lipid profiles

At the end of the study, following an overnight fast, all the rats were euthanized Diethyl ether, and blood was immediately collected through a cardiac puncture. After that, the blood was then allowed to clot and centrifuged at 1500 revolutions per minute for 10 min to produce Serum, then transferred into labeled vacutainers. The serum samples were refrigerated and used for lipid profile assay. The assay was carried in the clinical chemistry department from university of Nairobi.

2.4.5. Assay of hepatic triglycerides

After euthanization as described above, a midline incision was made on the ventral surface on the body of each rat to expose the abdominal cavity and the liver excised. The liver samples were then deep frozen at -90^{O} C and the hepatic triglycerides determined using the procedure by Bulter and Mailing (Butler et al., 1961). Briefly, two (2) g portions of the respective livers were homogenized in eight (8) mls of phosphate Buffer. One (1) ml of the resulting homogenate was then added to four (4) g of activated charcoal which had been pre-moistened with two (2) mls of chloroform. The resulting paste was then topped up with eighteen (18) mls of chloroform and gently shaken for ten (10) minutes after which it was filtered. The resulting filtrate was then divided into 3 test tubes. One (1) ml of standard oil solution (1%) was pipetted into 3 additional test tubes. All the test tubes were placed in a water bath at 80 °C and excess chloroform evaporated. 0.5 ml alcoholic potassium hydroxide were added to the first & second tube and 0.5 ml of 95% alcohol was added to the third tube containing the filtrate and the test tube containing the standard corn oil solution. The test tubes were maintained in water at 60 °C for twenty (20) minutes after which 0.5 ml of 0.2N sulphuric acid were added to each tube and the resulting mixtures heated in a water bath (100 °C) for twenty (20) minutes. They were then cooled after which 0.1 ml sodium metaperiodate followed by 0.1 ml sodium arsenide were added. Five (5) ml of chromotropic acid were then added to each test tube after

ten (10) minutes. The tubes were placed in a water bath (100 °C) for half an hour. The optical densities at 540 nm were then determined using spectrophotometer. The optical densities obtained were then used to calculate the hepatic triglyceride content using the following formula:

$$\text{Let R} = \frac{\text{optical density (O.D) saponified unknown} - \text{O.D unsaponified unknown}}{\text{O.D saponified corn oil standard} - \text{O.D unsaponified corn oil standard}}$$

and

A = volume of aliquot of chloroform extract in ml (1 ml was used in the present study)

Then triglyceride contents in milligram per gram of tissue

$$\frac{200}{A} \times R \times 0.05 = 10\frac{R}{A}$$

2.4.5.1. LC-MS of Agave sisalana. One (1) gram of each the dry samples were weighed using an analytical balance in a 100 ml glass beaker and reconstituted in 100 ml, of LC-MS grade water (Tedia, Inc U.S.A.) and stored into laboratory prior to analysis. 20 μl of each sample was injected using Hamilton syringe into Agilent triple quad LC-ESI-MS/MS and analyzed.

The LC-MS operating conditions were as follows: a quaternary L.C. pump (Model 1200) coupled to Agilent M.S.D. 6120-Single quadrupole M.S. with an electrospray source (Palo Alto, CA) was used. The system was controlled using ChemStation software (Hewlett-Packard). Reversed-phase liquid chromatography was performed on an Agilent technologies 1200 infinite series, Zorbax SB C18 column, 2.1 × 50 mm, 1.8 μm (Phenomenex, Torrance, CA) using the following gradient program 0 min, 5% B; 0–5 min, 5–50% B; 5–10 min, 50–80% B; 10–15 min, 80–100% B; 15–25 min 100% B; 25–30 min 5% B; 30–35 min 5% B. The flow rate was held constant at 1 mL/min, the injection volume was 1.0 μL, and data were acquired in a full-scan positive-ion mode using a 100–1500 m/z scan range. The dwell time for each ion was 50 ms. Other parameters of the mass spectrometer were as follows: capillary voltage, 3.0 kV; cone voltage, 70 V; extract voltage, 5 V; R.F. voltage, 0.5 V; source temperature, 110 °C; nitrogen gas temperature for desolvation, 380 °C; and nitrogen gas flow for desolvation, 400 L/h. LC-MS analyzed serial dilutions of kaempferol standard (1–100 ng/μL) in full scan mode to generate a linear calibration curve (peak area vs. concentration) with the following equation; [$y = 6008.9x - 5250.3$ ($R^2 = 0.9987$)] which served as a basis for external quantification. Most of the compounds in the M.S. exhibited abundant (M + H) in the positive ion mode.

The single point external standards analyte response is assumed to be linear over concentration. All peaks for the analyte were subjected to the same method of quantitation.

LC-MS in full scan mode to generate linear calibration curve (peak area vs. concentration) with the following equation; [$y = 6008.9x - 5250.3$ ($R^2 = 0.9987$)], which served as a basis for external quantification. 1 g of each sample was reconstituted in LC-MS water from Tedia Inc., U.S.A. and 20 μl injected in LC-MS as above. The identification of compounds and abundance was based on the existing Nist Library with over 10,000 Natural products compounds-based fragmentation of mass ions.

2.4.5.2. Target prediction and network construction. As previously described, LC-MS was utilized to identify the *Agave sisalana* extracts' composite compounds, i.e., freeze-dried extracts of fresh sisal juice, fresh sisal juice saponin-rich extract, freeze-dried extracts of fermented sisal juice, and fermented sisal juice saponin-rich extract. Twenty seven (27) compounds were identified in the freeze-dried extracts of fresh sisal juice, twenty compounds (20) in the fresh sisal juice saponin-rich extract, twelve (12) compounds in the freeze-dried extracts of fermented sisal juice, and five (5) compounds in the fermented sisal juice saponin-rich extracts had adequate structural information in the PubChem™ database that could facilitate target prediction.

The Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM) database was used to carry out target predictions of the *Agave sisalana* constituent compounds, which were constructed into interaction networks and visualized using Cytoscape 3.7.1 (https://cytoscape.org/). The procedure followed, and the functionality of the BATMAN-TCM tool is as described by [13].

2.5. Statistical analysis

All data were expressed as Mean ± S.E.M. and analyzed using One-way ANOVA and Tukey's posthoc tests in cases of significance set at $p < 0.05$. GraphPad Prism 7 suite of statistical software was used to perform the statistical tests.

3. Results

The extract yields were as follows: 50 g F.S.E. from 1 L of sisal juice (20% w/w), 60 g FerSE from 1 L of fermented sisal juice (24% w/w), 14 g F.S.P. extract from 1.7 L of fresh sisal juice (3.3% w/w), and 8 g FerSP extract from 1 L of fermented sisal juice (3.2% w/w).

3.1. Effects on body weight

There were significant differences in the body weight between the experimental groups on Day 35: [122 ± 1.50 g (normal group)

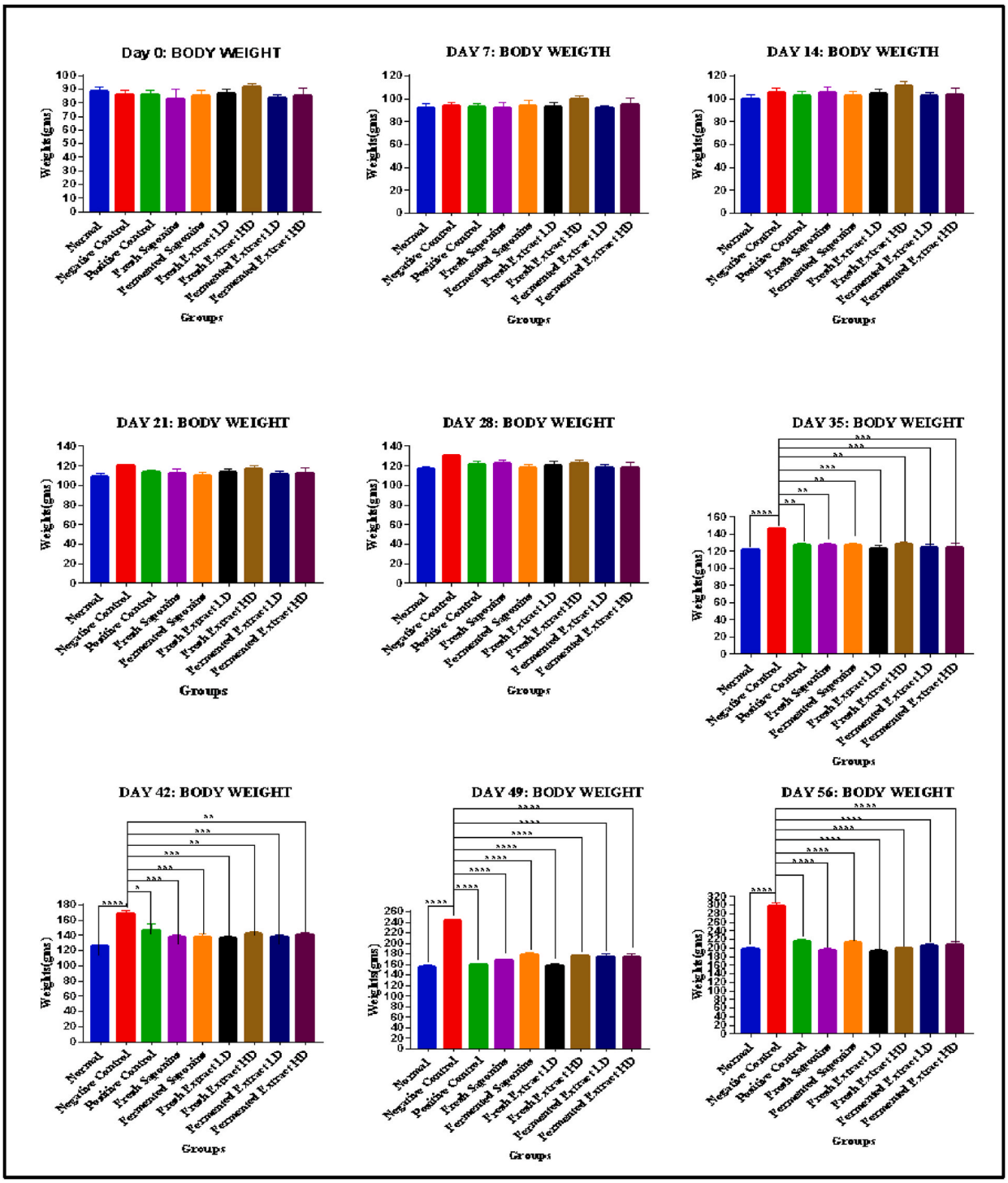

Figure 1. Graphs showing weekly mean body weight (g) over the 8-week experimental period expressed as Mean ± SEM (*- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$, ****- $p < 0.0001$).

vs. 146.3 ± 1.53 g (negative control) vs. 126.7 ± 2.98 g (positive control) vs. 126.7 ± 3.13 g (FSP) vs. 126.4 ± 2.66 g (FerSP) vs. 123.5 ± 4.08 g (FS LD) vs. 128.3 ± 2.99 g (FS HD) vs. 124.4 ± 3.37 g (FerS LD) vs. 124.4 ± 4.62 g (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p = 0.0019$), the negative control and FSP ($p = 0.0019$), the negative control and FerSP ($p = 0.0015$), the negative control and FS LD ($p = 0.0002$), the negative control and FS HD ($p = 0.0056$), the negative control and FerS LD ($p = 0.0004$), negative control and FerS HD ($p = 0.0004$).

There were significant differences in the body weight between the experimental groups on Day 42: [126 ± 1.39 g (normal group) vs. 168.1 ± 4.18 g (negative control) vs. 145.9 ± 9.60 g (positive control) vs. 138.1 ± 2.65 g (FSP) vs. 137.7 ± 3.90 g (FerSP) vs. 136.3 ± 2.76 g (FS LD) vs. 141.7 ± 3.48 g (FS HD) vs. 137.1 ± 2.88 g (FerS LD) vs. 140.8 ± 3.02 g (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p = 0.0211$), the negative control and FSP ($p = 0.0005$), the negative control and FerSP ($p = 0.0004$), the negative control and FS LD ($p = 0.0002$), the negative control and FS HD ($p = 0.0029$), the negative control and FerS LD ($p = 0.0003$), negative control and FerS HD ($p = 0.0018$).

There were significant differences in the body weight between the experimental groups on Day 56: [196.3 ± 6.49 g (normal group) vs. 298.9 ± 6.67 g (negative control) vs. 215.3 ± 6.06 g (positive control) vs. 195.4 ± 3.92 g (FSP) vs. 213.1 ± 5.21 g (FerSP) vs. 190.8 ± 6.49 g (FS LD) vs. 198.9 ± 4.31 g (FS HD) vs. 204.7 ± 4.78 g (FerS LD) vs. 208.7 ± 6.21 g (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p < 0.0001$), the negative control and FerS LD ($p < 0.0001$), negative control and FerS HD ($p < 0.0001$). The graphical presentation of the experimental data is shown in Figure 1.

3.2. Effects on fasting blood glucose

There were no significant differences in the fasting blood glucose between the groups at the beginning of the experiment [3.583 ± 0.1447 mmol/L (normal group) vs. 3.517 ± 0.174 mmol/L (negative control) vs. 3.483 ± 0.1493 mmol/L (positive control) vs. 3.467 ± 0.07149 mmol/L (FSP) vs. 3.417 ± 0.1851 mmol/L (FerSP) vs. 3.45 ± 0.1176 mmol/L (FS LD) vs. 3.55 ± 0.1945 mmol/L (FS HD) vs. 3.45 ± 0.09916 mmol/L (FerS LD) vs. 3.5 ± 0.1653 mmol/L (FerS HD): $p = 0.9979$].

There were no significant differences in the fasting blood glucose between the experimental groups on Day 7: [3.267 ± 0.1022 mmol/L (normal group) vs. 3.7 ± 0.177 mmol/L (negative control) vs. 3.333 ± 0.1256 mmol/L (positive control) vs. 3.467 ± 0.1256 mmol/L (FSP) vs. 3.267 ± 0.9888 mmol/L (FerSP) vs. 3.417 ± 0.0601 mmol/L (FS LD) vs. 3.25 ± 0.09916 mmol/L (FS HD) vs. 3.3 ± 0.1414 mmol/L (FerS LD) vs. 3.34 ± 0.1122 mmol/L (FerS HD): $p = 0.2087$].

On Day 14, there were significant differences in fasting blood glucose between the experimental groups [3.533 ± 0.0667 mmol/L (normal group) vs. 4.217 ± 0.1721 mmol/L (negative control) vs. 3.733 ± 0.1229 mmol/L (positive control) vs. 3.75 ± 0.1522 mmol/L (FSP) vs. 3.667 ± 0.1838 mmol/L (FerSP) vs. 3.583 ± 0.1447 mmol/L (FS LD) vs. 3.817 ± 0.2104 mmol/L (FS HD) vs. 3.733 ± 0.143 mmol/L (FerS LD) vs. 3.717 ± 0.07923 mmol/L (FerS HD): $p = 0.1162$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0500$).

There were significant differences in fasting blood glucose between the four experimental groups On Day 21: [3.65 ± 0.0763 mmol/L (normal group) vs. 5.017 ± 0.0601 mmol/L (negative control) vs. 3.6 ± 0.0730 mmol/L (positive control) vs. 3.917 ± 0.3124 mmol/L (FSP) vs. 4.13 ± 0.2921 mmol/L (FerSP) vs. 3.7 ± 0.2875 mmol/L (FS LD) vs. 4.3 ± 0.2921 mmol/L (FS HD) vs. 4.567 ± 0.2319 mmol/L (FerS LD) vs. 4.383 ± 0.3609 mmol/L (FerS HD): $p = 0.0021$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0085$), the negative control and positive control ($p = 0.0056$), the negative control and FS LD ($p = 0.0128$).

On Day 28, there were significant differences in fasting blood glucose between the experimental groups [3.533 ± 0.1145 mmol/L (normal group) vs. 6.083 ± 0.2676 mmol/L (negative control) vs. 3.6 ± 0.294 mmol/L (positive control) vs. 3.883 ± 0.2892 mmol/L (FSP) vs. 3.783 ± 0.2868 mmol/L (FerSP) vs. 3.567 ± 0.2875 mmol/L (FS LD) vs. 4.267 ± 0.2044 mmol/L (FS HD) vs. 4.683 ± 0.3962 mmol/L (FerS LD) vs. 3.55 ± 0.1025 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p = 0.0005$), the negative control and FerS LD ($p = 0.0152$), negative control and FerS HD ($p < 0.0001$).

On Day 35, there were significant differences in fasting blood glucose between the experimental groups [3.567 ± 0.3138 mmol/L (normal group) vs. 6.483 ± 0.199 mmol/L (negative control) vs. 3.95 ± 0.2513 mmol/L (positive control) vs. 4.367 ± 0.2552 mmol/L (FSP) vs. 3.667 ± 0.3593 mmol/L (FerSP) vs. 3.317 ± 0.2286 mmol/L (FS LD) vs. 4.467 ± 0.5084 mmol/L (FS HD) vs. 4.367 ± 0.05777 mmol/L (FerS LD) vs. 4.533 ± 0.5481 mmol/L (FerS HD): $p = 0.0021$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p = 0.0009$), the negative control and FSP ($p = 0.0094$), the negative control and FerSP ($p = 0.0094$), the negative control and FS LD ($p < 0.0001$)), the negative control and FS HD ($p = 0.0157$), the negative control and FerS LD ($p = 0.0094$), negative control and FerS HD ($p = 0.0220$).

On Day 42, there were significant differences in fasting blood glucose between the experimental groups [3.467 ± 0.2348 mmol/L (normal group) vs. 7.35 ± 0.199 mmol/L (negative control) vs. 3.783 ± 0.4301 mmol/L (positive control) vs. 4.417 ± 0.3198 mmol/L

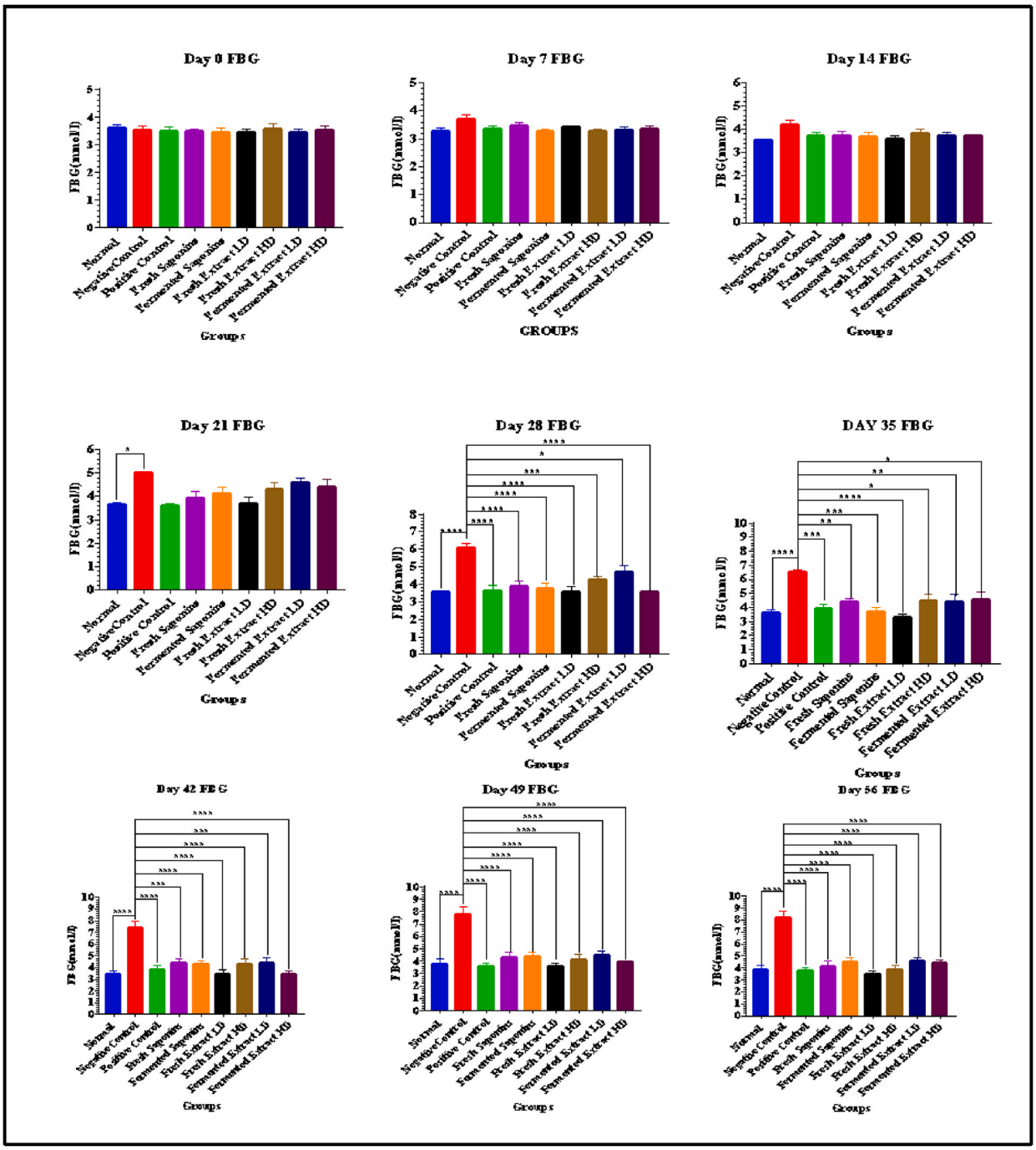

Figure 2. Graphs depicting fasting blood glucose levels (mmol/L) at weekly intervals during the experimental period. Expressed as mean ± SEM (*- $p < 0.05$, **- $p < 0.01$, ***- $p < 0.001$, ****- $p < 0.0001$).

(FSP) vs. 4.317 ± 0.281 mmol/L (FerSP) vs. 3.45 ± 0.3212 mmol/L (FS LD) vs. 4.25 ± 0.5214 mmol/L (FS HD) vs. 4.417 ± 0.3978 mmol/L (FerS LD) vs3.433 ± 0.2704 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p = 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p < 0.0001$), the negative control and FerS LD ($p = 0.0001$), negative control and FerS HD ($p < 0.0001$).

On Day 49, there were significant differences in fasting blood glucose between the experimental groups [3.783 ± 0.4167 mmol/L (normal group) vs. 7.733 ± 0.6657 mmol/L (negative control) vs. 3.617 ± 0.2344 mmol/L (positive control) vs. 4.3 ± 0.4351 mmol/L (FSP) vs. 4.4 ± 0.3759 mmol/L (FerSP) vs. 3.583 ± 0.3081 mmol/L (FS LD) vs. 4.1 ± 0.4858 mmol/L (FS HD) vs. 4.483 ± 0.3351 mmol/L (FerS LD) vs3.933 ± 0.0954 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p < 0.0001$), the negative control and FerS LD ($p < 0.0001$), negative control and FerS HD ($p < 0.0001$).

On Day 56, there were significant differences in fasting blood glucose between the experimental groups [3.883 ± 0.371 mmol/L (normal group) vs. 8.183 ± 0.5845 mmol/L (negative control) vs. 3.767 ± 0.2716 mmol/L (positive control) vs. 4.167 ± 0.4602 mmol/L (FSP) vs. 4.533 ± 0.3169 mmol/L (FerSP) vs. 3.5 ± 0.2309 mmol/L (FS LD) vs. 3.867 ± 0.3353 mmol/L (FS HD) vs. 4.617 ± 0.2725 mmol/L (FerS LD) vs. 4.383 ± 0.3114 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p < 0.0001$), the negative control and FerS LD ($p < 0.0001$), negative control and FerS HD ($p < 0.0001$). The graphical representations of these results are shown in Figure 2.

3.3. Effects on glucose tolerance

There were significant differences in the area under the curve (AUC) values between the experimental groups on Day 28 [561.3 ± 16.31 mmol/L min (normal group) vs. 636.3 ± 11.56 mmol/L min (negative control) vs. 547.8 ± 8.793 mmol/L min (positive control) vs. 545.7 ± 18.14 mmol/L min (FSP) vs. 538.8 ± 12.37 mmol/L min (FerSP) vs. 556 ± 16.66 mmol/L min (FS LD) vs. 546.5 ± 8.065

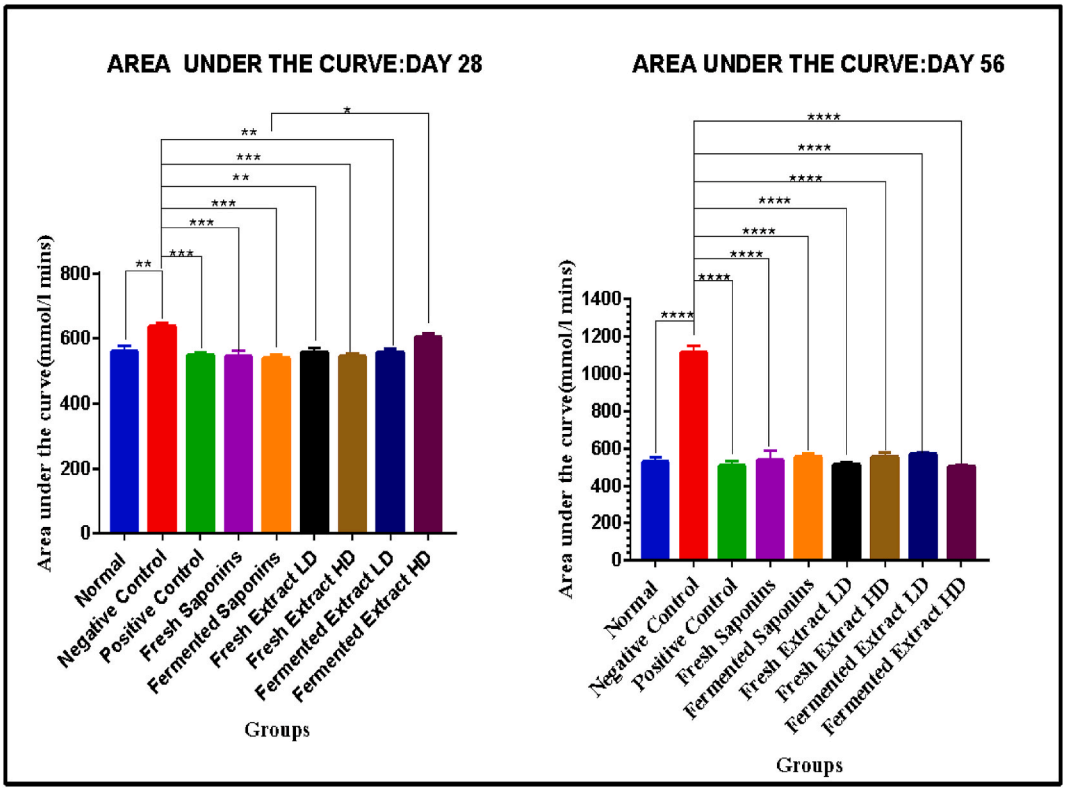

Figure 3. Mean area under the curve (mmol/l) during the oral glucose tolerance tests. Results is expressed as mean ± SEM. ***- $p < 0.001$, ****- $p < 0.0001$.

mmol/L min (FS HD) vs. 557.5 ± 11.34 mmol/L min (FerS LD) vs. 6.03 ± 12.49 mmol/L min (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0067$), the negative control and positive control ($p = 0.0007$), the negative control and FSP ($p = 0.0005$), the negative control and FerSP ($p = 0.0002$), the negative control and FS LD ($p = 0.0029$), the negative control and FS HD ($p = 0.0017$), the negative control and FerS LD ($p = 0.0037$), FerSP and FerS HD ($p = 0.0296$).

There were significant differences in the area under the curve (AUC) values between the experimental groups on Day 56 [525.8 ± 28.18 mmol/L min (normal group) vs. 1112 ± 37.31 mmol/L min (negative control) vs. 507.3 ± 22.85 mmol/L min (positive control) vs. 535 ± 51.91 mmol/L min (FSP) vs. 555 ± 16.35 mmol/L min (FerSP) vs. 511.3 ± 14.15 mmol/L min (FS LD) vs. 553 ± 23.82 mmol/L min (FS HD) vs. 566.3 ± 9.429 mmol/L min (FerS LD) vs. 498.5 ± 11.1 mmol/L min (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p < 0.0001$), the negative control and FerS LD ($p < 0.0001$), negative control and FerS HD ($p < 0.0001$). The graphical representation of these results are shown in Figures 3 and 4.

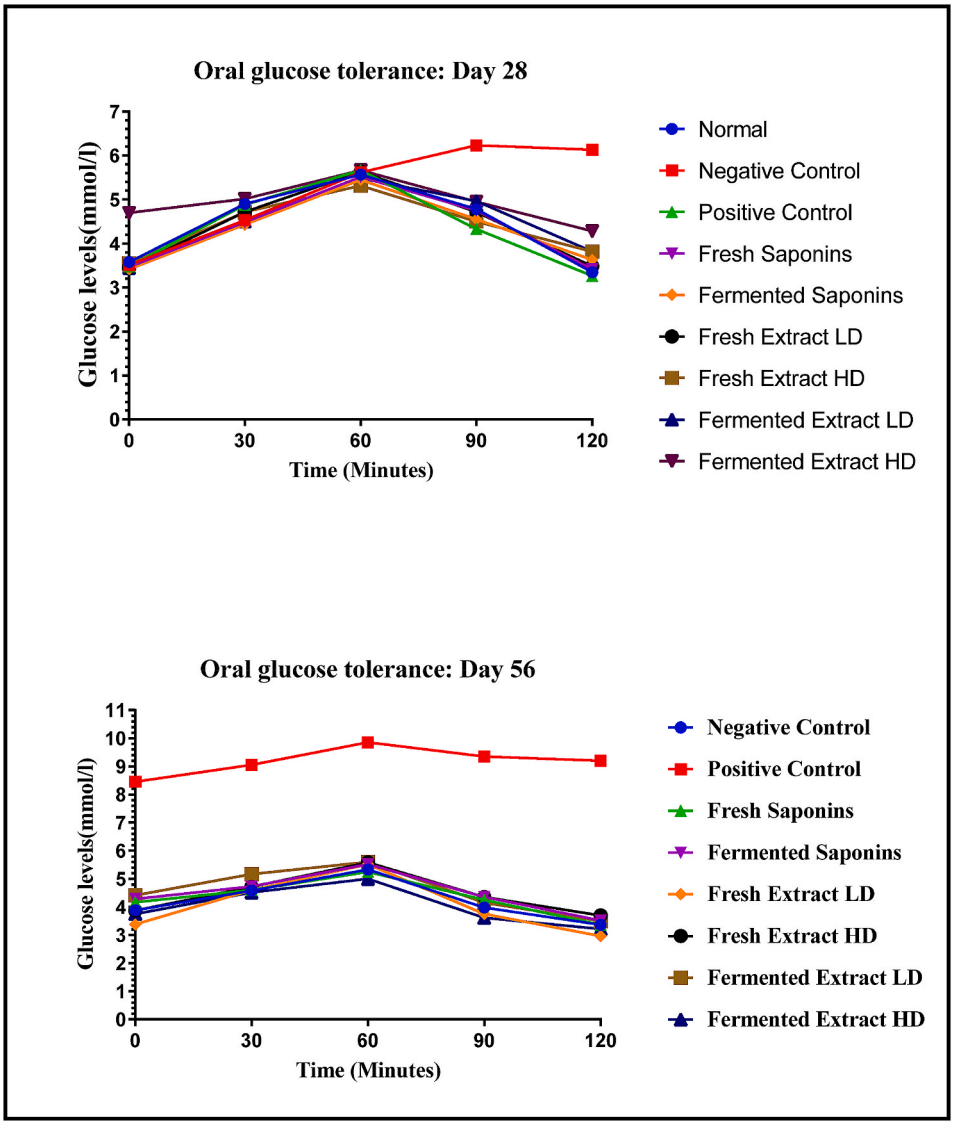

Figure 4. Mean blood glucose response (mmol/L) to an oral glucose bolus 2 g/kg over 2 h.

3.4. Effects on lipid profile

3.4.1. Serum total cholesterol

There were significant differences in total serum cholesterol between the groups [1.398 ± 0.1232 mmol/L (normal group) vs. 4.225 ± 0.4135 mmol/L (negative control) vs. 1.582 ± 0.154 mmol/L (positive control) vs. 1.245 ± 0.0911 mmol/L (FSP) vs. 1.393 ± 0.1423 mmol/L (FerSP) vs. 1.387 ± 0.0924 mmol/L (FS LD) vs. 1.761 ± 0.1495 mmol/L (FS HD) vs. 1.698 ± 0.1294 mmol/L (FerS LD) vs. 1.6975 ± 0.0982 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0065$), the negative control and positive control ($p = 0.0187$), the negative control and FSP ($p = 0.0091$), the negative control and FerSP ($p = 0.0178$), the negative control and FS LD ($p = 0.0054$), the negative control and FS HD ($p = 0.238$), the negative control and FerS LD ($p = 0.0280$), negative control and FerS HD ($p = 0.0202$).

3.4.2. Serum triglyceride

There were significant differences in total serum cholesterol between the groups [0.5967 ± 0.07437 mmol/L (normal group) vs. 2.792 ± 0.1595 mmol/L (negative control) vs. 0.5233 ± 0.1051 mmol/L (positive control) vs. 0.6733 ± 0.07397 mmol/L (FSP) vs. 0.6667 ± 0.06946 mmol/L (FerSP) vs. 0.505 ± 0.07637 mmol/L (FS LD) vs. 0.6717 ± 0.09282 mmol/L (FS HD) vs. 0.6317 ± 0.067 mmol/L (FerS LD) vs. 0.6033 ± 0.03981 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0012$), the negative control and positive control ($p = 0.0014$), the negative control and FSP ($p = 0.0004$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p = 0.0011$), the negative control and FS HD ($p = 0.0019$), the negative control and FerS LD ($p = 0.0023$), negative control and FerS HD ($p = 0.0004$).

3.4.3. LDL -cholesterol

There were significant differences in LDL-cholesterol between the groups [0.6 ± 0.03011 mmol/L (normal group) vs. 2.94 ± 0.1166 mmol/L (negative control) vs. 0.6083 ± 0.057 mmol/L (positive control) vs. 0.69 ± 0.0554 mmol/L (FSP) vs. 0.7017 ± 0.057 mmol/L (FerSP) vs. 0.5883 ± 0.04636 mmol/L (FS LD) vs. 0.7167 ± 0.02789 mmol/L (FS HD) vs. 0.605 ± 0.07343 mmol/L (FerS LD) vs. 0.785 ± 0.04931 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0001$), the negative control and positive control ($p = 0.0004$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p = 0.0002$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p = 0.0002$), the negative control and FerS LD ($p = 0.0005$), negative control and FerS HD ($p < 0.0001$).

3.4.4. HDL-cholesterol

There were significant differences in HDL-cholesterol between the groups [1.797 ± 0.0597 mmol/L (normal group) vs. 0.4217 ± 0.08459 mmol/L (negative control) vs. 1.543 ± 0.1249 mmol/L (positive control) vs 1.732 ± 0.04665 mmol/L (FSP) vs. 1.758 ± 0.07872 mmol/L (FerSP) vs. 1.593 ± 0.1025 mmol/L (FS LD) vs. 1.807 ± 0.1293 mmol/L (FS HD) vs. 1.32 ± 0.1165 mmol/L (FerS LD) vs. 1.805 ± 0.1727 mmol/L (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0007$), the negative control and positive control ($p = 0.0034$), the negative control and FSP ($p < 0.0006$), the negative control and FerSP ($p = 0.0015$), the negative control and FS LD ($p < 0.0045$), the negative control and FS HD ($p = 0.0022$), the negative control and FerS LD ($p = 0.0517$), negative control and FerS HD ($p = 0.0094$).

The graphical representation of these results is shown in Figure 5.

3.5. Effects on hepatic triglycerides

There were significant differences in hepatic triglyceride between the groups [2.2 ± 0.2251 mg/g (normal group) vs. 5.617 ± 0.4147 mg/g (negative control) vs. 2.233 ± 0.2929 mg/g (positive control) vs. 2.4 ± 0.3011 mg/g (FSP) vs. 2.533 ± 0.2692 mg/g (FerSP) vs. 2.3 ± 0.1915 mg/g (FS LD) vs. 2.633 ± 0.2692 mg/g (FS HD) vs. 2.783 ± 0.2701 mg/g (FerS LD) vs. 2.733 ± 0.2472 mg/g (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0067$), the negative control and positive control ($p = 0.0001$), the negative control and FSP ($p = 0.0015$), the negative control and FerSP ($p = 0.0182$), the negative control and FS LD ($p = 0.0033$), the negative control and FS HD ($p = 0.0265$), the negative control and FerS LD ($p = 0.0387$), negative control and FerS HD ($p = 0.0049$).

3.6. Effects on liver weight

There were significant differences in liver weight between the groups [3.025 ± 0.4039 g (normal group) vs. 8.557 ± 0.4808 g (negative control) vs. 3.665 ± 0.4386 g (positive control) vs. 3.797 ± 0.3256 g (FSP) vs. 4.232 ± 0.4648 g (FerSP) vs. 3.417 ± 0.254 g (FS LD) vs. 3.917 ± 0.3936 g (FS HD) vs. 3.823 ± 0.5542 g (FerS LD) vs. 3.862 ± 0.4324 g (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p < 0.0001$), the negative control and FSP ($p < 0.0001$), the negative control and FerSP ($p < 0.0001$), the negative control and FS LD ($p < 0.0001$), the negative control and FS HD ($p < 0.0001$), the negative control

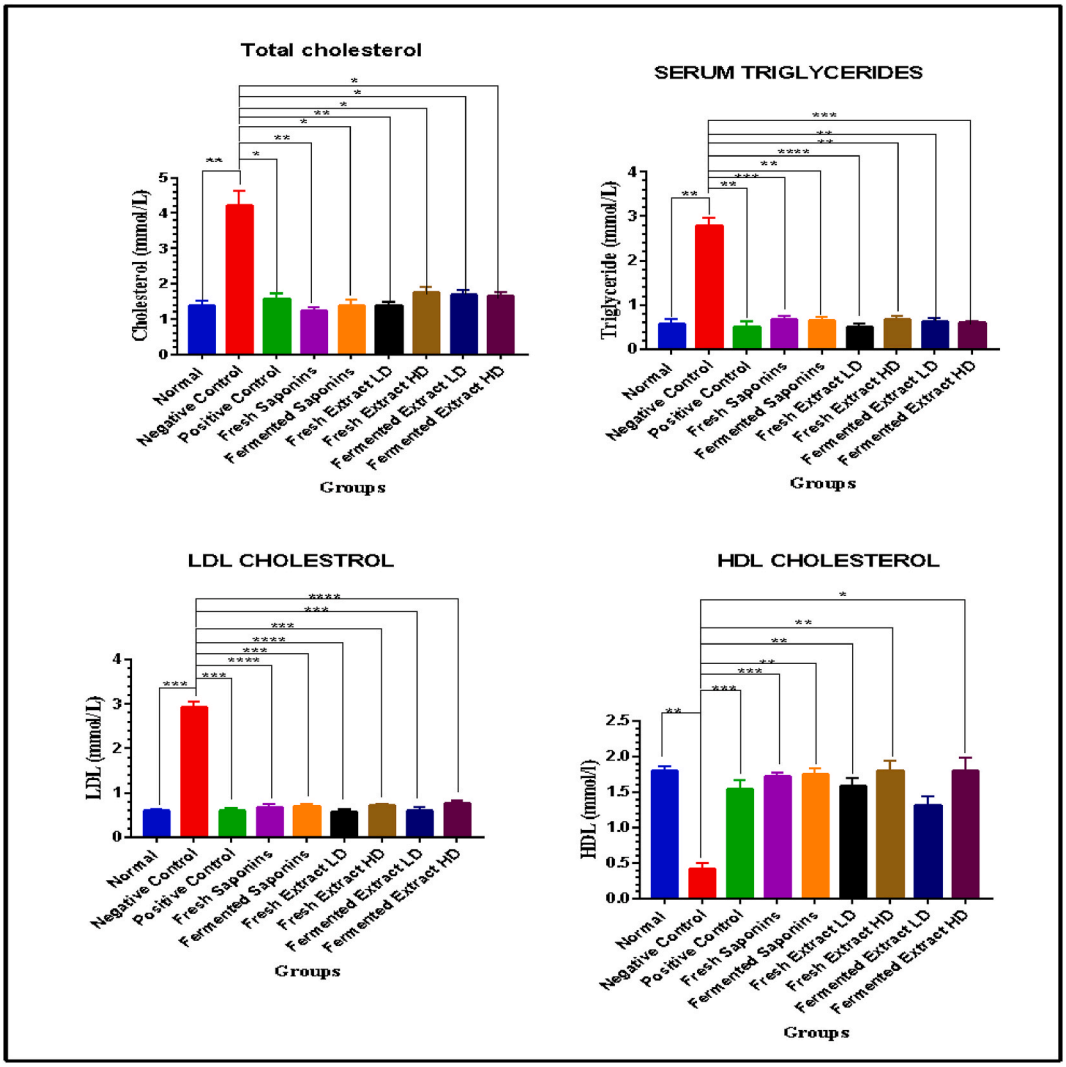

Figure 5. Lipid profile (mmol/L) of the experimental groups. Results are expressed as mean ± S.E.M. (****- $p < 0.0001$).

and FerS LD ($p < 0.0001$), negative control and FerS HD ($p < 0.0001$).

3.7. *Effects on liver weight: body weight ratio*

There were significant differences in mean liver weight: body weight ratio between the groups [3.958 ± 0.2324% (normal group) vs. 6.3 ± 0.4218% (negative control) vs. 4.322 ± 0.2117% (positive control) vs. 4.785 ± 0.0343% (FSP) vs. 4.407 ± 0.2839% (FerSP) vs. 4.267 ± 0.1811% (FS LD) vs. 4.452 ± 0.3077% (FS HD) vs. 4.752 ± 0.3051% (FerS LD) vs. 4.73 ± 0.0.3176 % (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p < 0.0001$), the negative control and positive control ($p = 0.0006$), the negative control and FSP ($p = 0.0181$), the negative control and FerSP ($p = 0.0012$), the negative control and FS LD ($p = 0.0004$), the negative control and FS HD ($p = 0.0017$), the negative control and FerS LD ($p = 0.0145$), negative control and FerS HD ($p = 0.0125$).

The graphical representations of these results are shown in Figure 6.

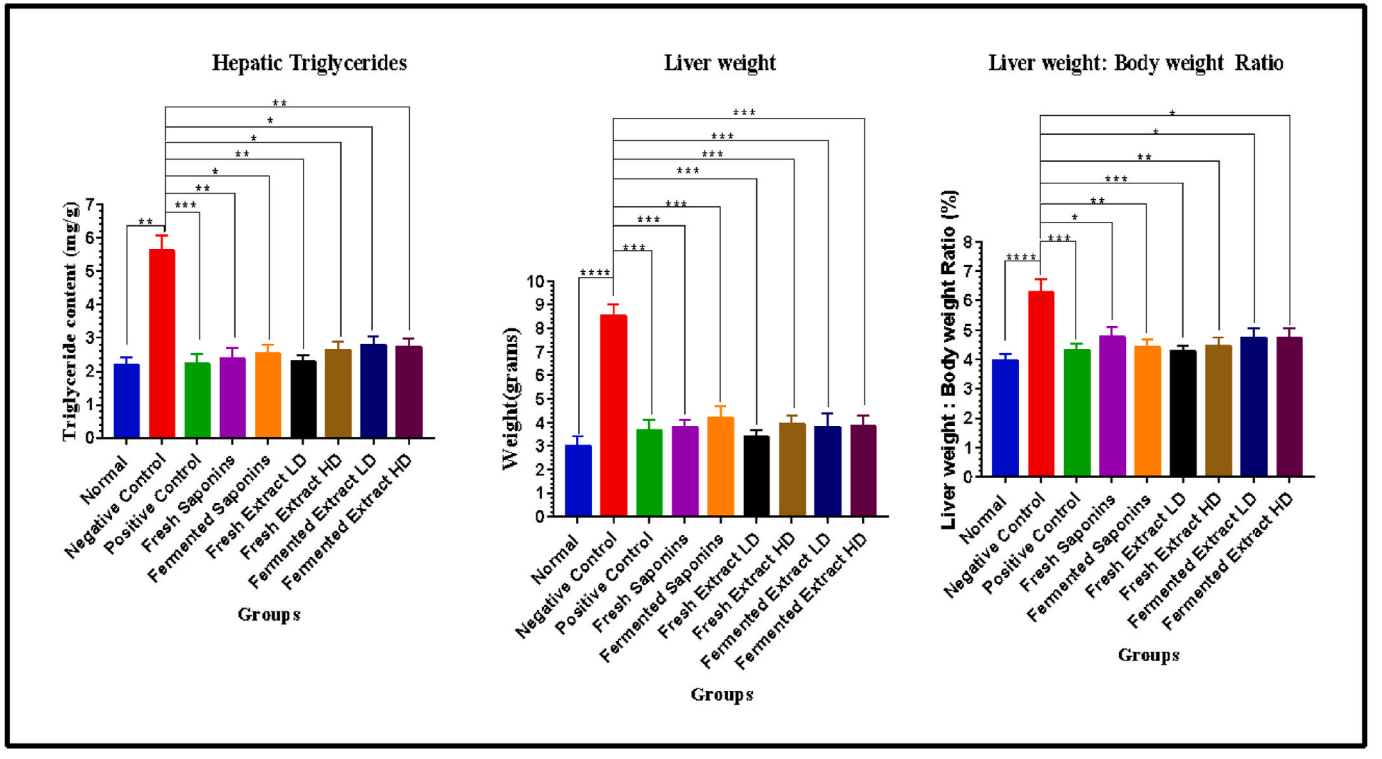

Figure 6. Mean hepatic triglycerides content (mg/g), liver weights (gms), and liver weight: body weight ratio (%). Results are expressed as mean ± S.E.M. (****- $p < 0.0001$).

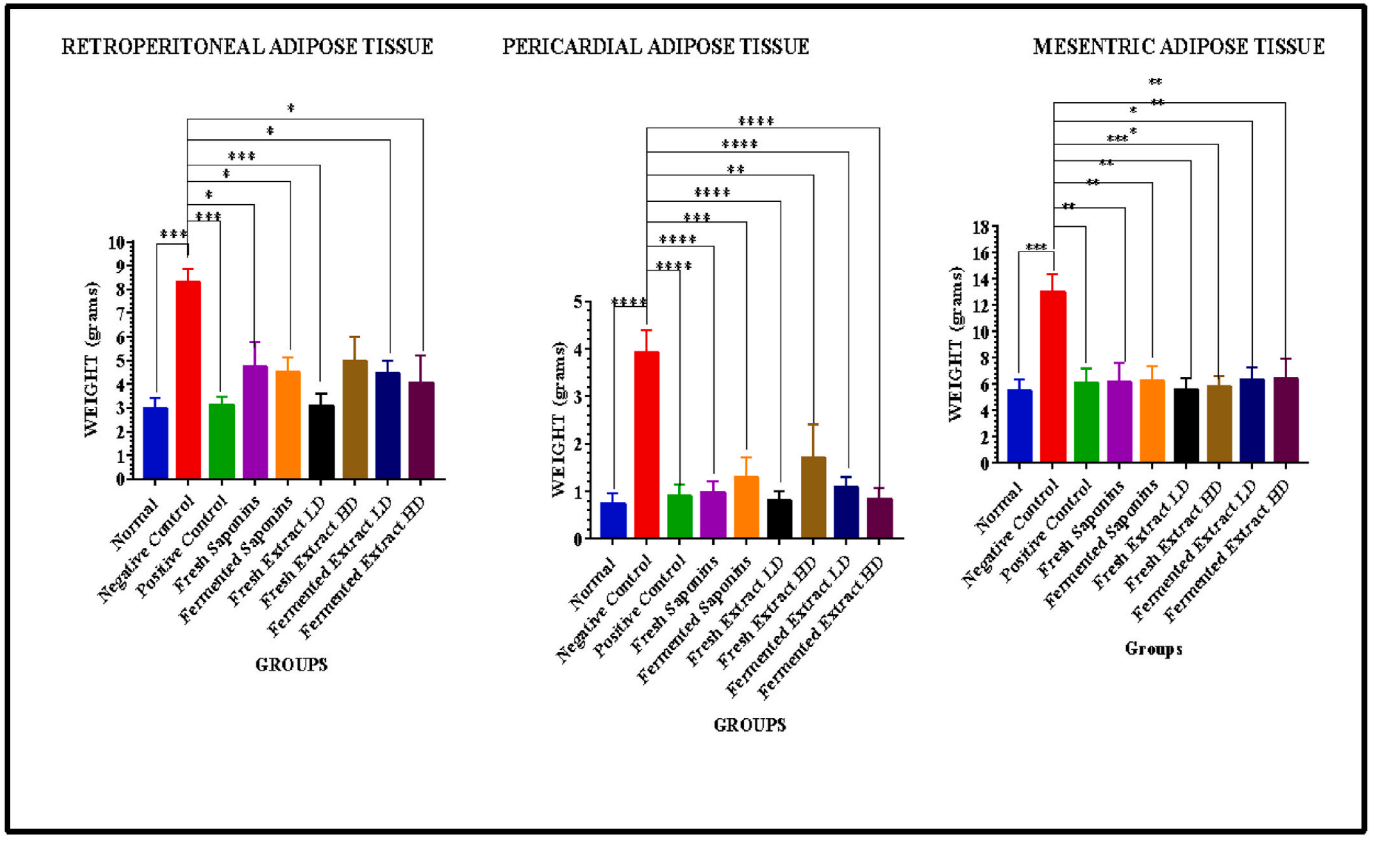

Figure 7. Graph showing mean adipose tissue weights (g). Results expressed as mean ± S.E.M. (****- $p < 0.0001$).

3.8. Effects on adipose tissue

3.8.1. Retroperitoneal adipose tissue

There were significant differences in retroperitoneal adipose tissue weight between the groups [3.008 ± 0.3918 g (normal group) vs. 8.322 ± 0.5602 g (negative control) vs. 3.135 ± 0.3336 g (positive control) vs. 4.738 ± 1.012 g (FSP) vs. 4.508 ± 0.6142 g (FerSP) vs. 3.09 ± 0.5047 g (FS LD) vs. 4.982 ± 1.027 g (FS HD) vs. 4.462 ± 0.5084 g (FerS LD) vs. 4.065 ± 1.171 g (FerS HD): $p = 0.0002$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0002$), the negative control and positive control ($p = 0.0003$), the negative control and FSP ($p = 0.0324$), the negative control and FerSP ($p = 0.0180$), the negative control and FS LD ($p = 0.0003$), the negative control and FerS LD ($p = 0.0159$), negative control and FerS HD ($p = 0.0053$).

3.8.2. Pericardial adipose tissue

There were significant differences in pericardial adipose tissue weight between the groups [0.75 ± 0.2112 g (normal group) vs. 3.935 ± 0.4615 g (negative control) vs. 0.91 ± 0.2237 g (positive control) vs. 0.99 ± 0.2222 g (FSP) vs. 1.297 ± 0.4287 g (FerSP) vs. 0.8233 ± 0.1851 g (FS LD) vs. 1.717 ± 0.7015 g (FS HD) vs. 1.103 ± 0.1892 g (FerS LD) vs. 0.8317 ± 0.2496 g (FerS HD): $p < 0.0001$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0002$), the negative control and positive control ($p = 0.0003$), the negative control and FSP ($p = 0.0324$), the negative control and FerSP ($p = 0.0180$), the negative control and FS LD ($p = 0.0003$), the negative control and FerS LD ($p = 0.0159$), negative control and FerS HD ($p = 0.0053$).

3.8.3. Mesenteric adipose tissue

There were significant differences in mesenteric adipose tissue weight between the groups [5.492 ± 0.8733 g (normal group) vs. 12.99 ± 1.396 g (negative control) vs. 6.112 ± 1.036 g (positive control) vs. 6.223 ± 1.367 g (FSP) vs. 6.277 ± 1.095 g (FerSP) vs. 5.595 ± 0.8369 g (FS LD) vs. 5.823 ± 0.7582 g (FS HD) vs. 6.332 ± 0.9078 g (FerS LD) vs. 6.472 ± 1.424 g (FerS HD): $p = 0.0005$]. Post-hoc statistical analysis using Tukey's multiple comparisons test revealed significant differences between the normal group and negative control ($p = 0.0006$), the negative control and positive control ($p = 0.0020$), the negative control and FSP ($p = 0.0025$), the negative control and FerSP ($p = 0.0027$), the negative control and FS LD ($p = 0.0007$), the negative control and FS HD ($p = 0.0011$), the negative control and FerS LD ($p = 0.0030$), negative control and FerS HD ($p = 0.0040$).

The graphical representation of these results is shown in Figure 7.

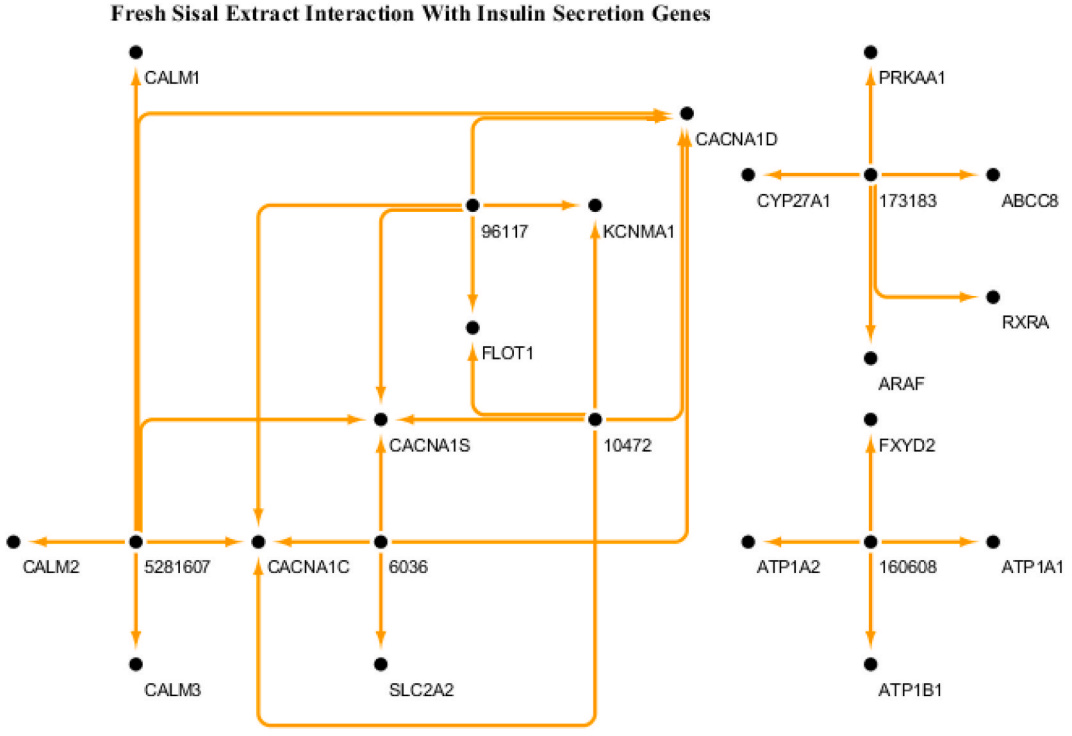

Figure 8. Interaction of compounds from the freeze-dried extract from fresh sisal juice with insulin secretion genes.

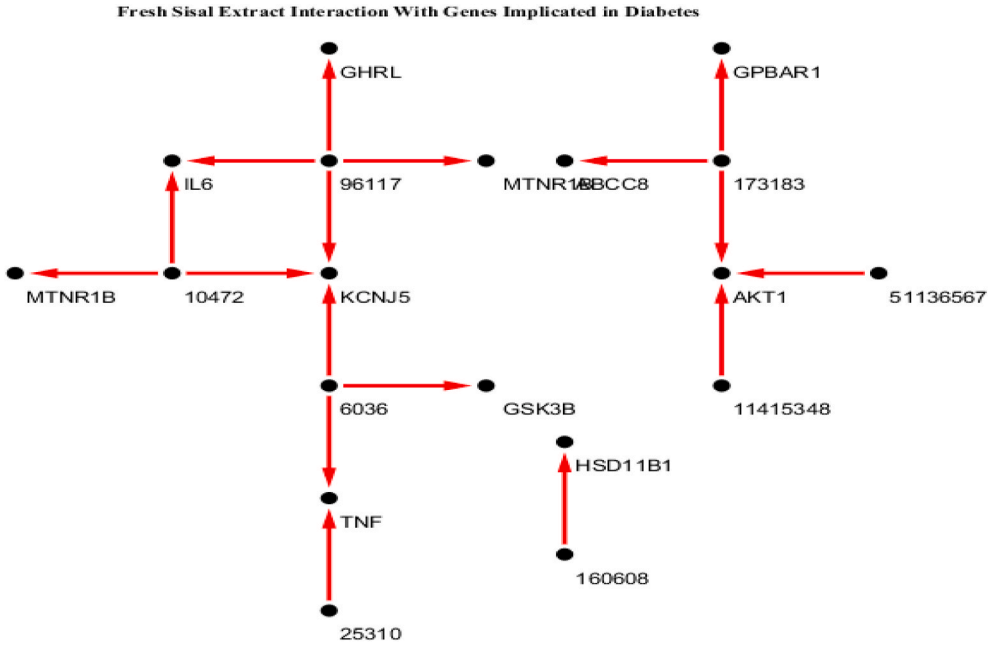

Figure 9. Interactions of chemical components obtained from the freeze-dried extracts of Fresh sisal juice with genes implicated in type 2 diabetes.

3.8.3.1. LC-MS results

3.8.3.1.1. Target prediction. Bioinformatic target prediction analysis of the freeze-dried extract of whole fresh sisal juice and fresh sisal saponin-rich extracts indicated that the composite compounds' main targets were genes in the calcium signaling pathway, with the most common targets being *CACNA1S, CACNA1D*, and *CACNA1C*, as shown in Figure. These genes encode for voltage-gated calcium (Ca_v) channels, which are ubiquitous calcium conducting pores present in cell membranes.

In the network diagrams (Figures 8, 9, 10, 11, 12, 13, 14), the arrow represents the Sisal compound-gene interaction with the arrowhead pointing towards the gene.

The diabetes interaction network analysis revealed the *AKT1* gene that encodes AKT1 as a critical target for the chemical components found in the freeze-dried extracts from fresh sisal juice, as shown in Figure 9.

More notably, the chemical components from the saponin-rich extracts from fresh sisal juice interact with the genes *ADRB2, ADRB3,* which code for beta 2 and 3 adrenergic receptors, as shown in Figure 10.

]Only 12 compounds present in freeze-dried extracts obtained from fermented sisal extract possessed structural information that facilitated their target prediction. However, only three had prediction scores above the set BATMAN-TCM cutoff point, and only one of these three compounds interacted with insulin signaling genes, as shown in Figure 11. However, the Cav channel encoding genes CACNA1C, CACNA1D, and CACNA1S in this interaction network are noteworthy.

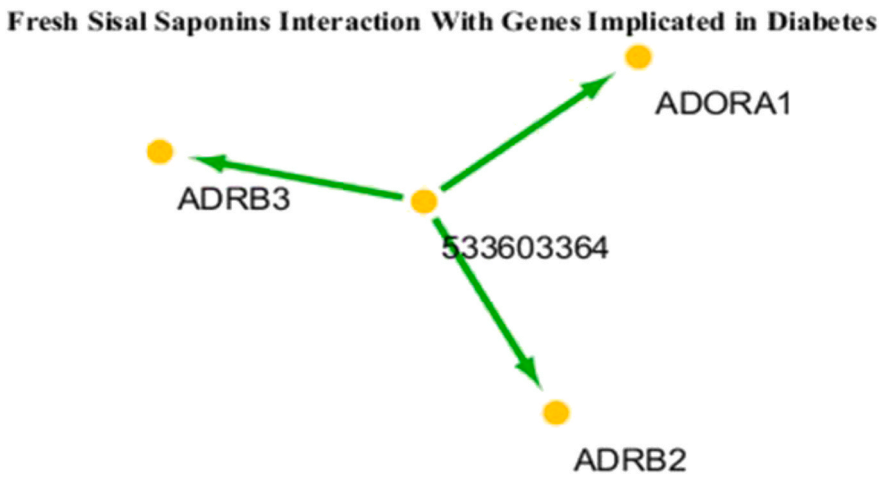

Figure 10. Interaction of the chemical components found in the saponin-rich extract obtained from fresh sisal juice with diabetes-related genes.

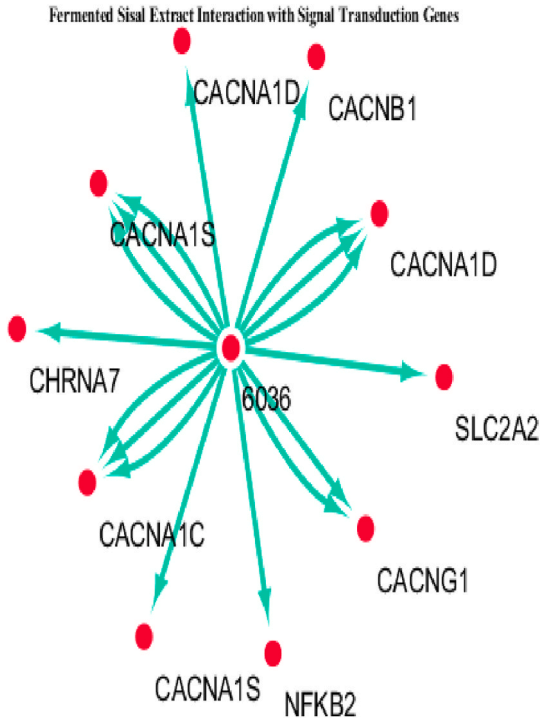

Figure 11. Interaction of the chemical components obtained from the freeze-dried extracts obtained from fermented sisal juice with various signaling pathway genes implicated in diabetes.

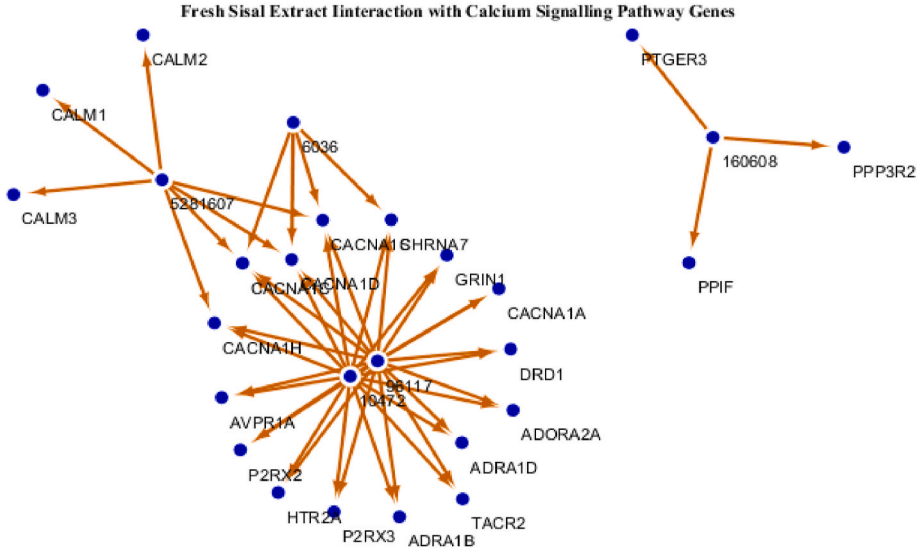

Figure 12. Interaction of the chemical compounds from the freeze-dried extracts from fresh sisal juice with calcium signaling pathway genes.

Other Interaction networks are shown in Figures 12, 13, and 14.

4. Discussion

Although Sisal's popularity as a biodegradable fiber is on the rise globally, its production from *Agave sisalana* leaf generates 95% waste composed of water, parenchymal tissue, short fibers, polysaccharide, inorganic compounds, and secondary metabolites, such as steroidal saponins [7, 14]. The fact that sisal juice, despite its rich content of secondary metabolites, is regarded as waste is a reversal of historical processing practices where it was regarded as a valuable source of hecogenin, which was used in the synthesis of the steroidal

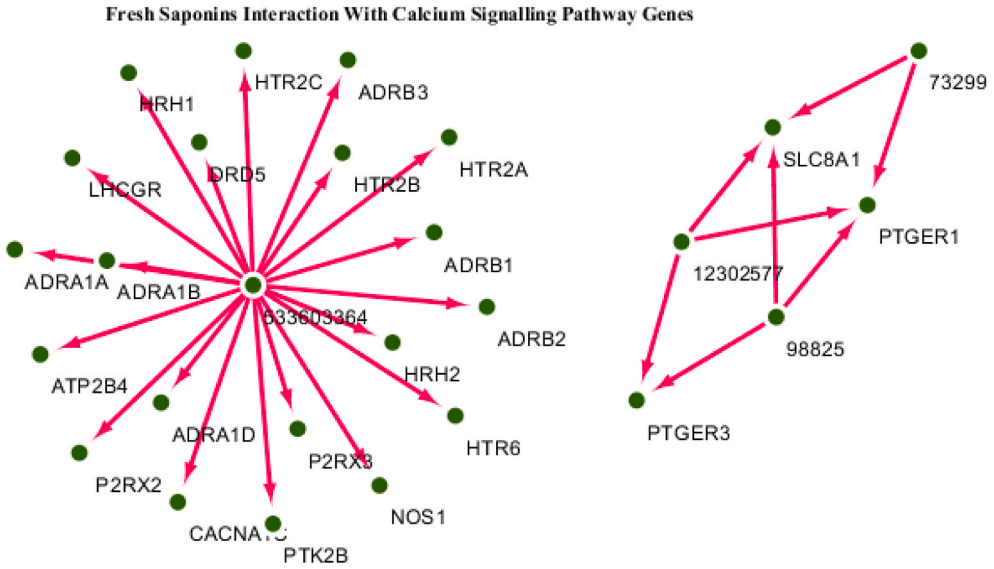

Figure 13. Interactions of the chemical compounds present in the saponin-rich extract from fresh sisal juice with the calcium signaling pathway genes.

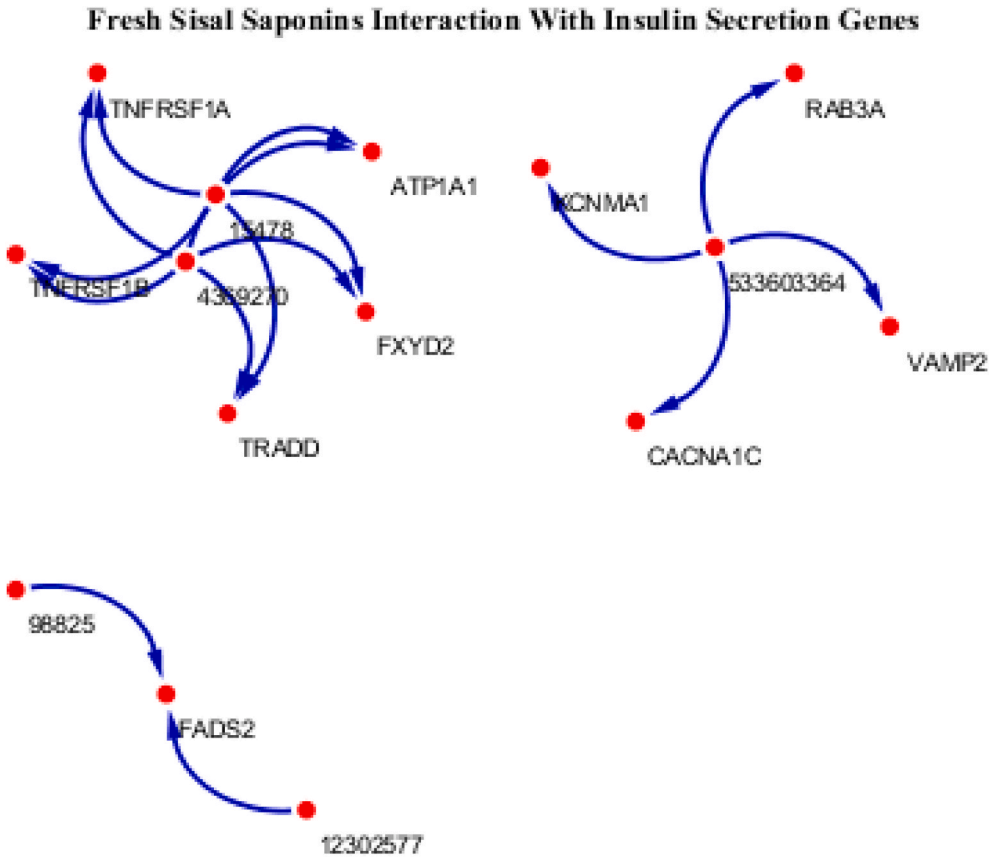

Figure 14. Interactions of the chemical compounds obtained from the saponin-rich extracts obtained from fresh sisal juice with the insulin secretion gene products.

drug cortisone [4, 15]. Consequently, this waste is a source of environmental pollution, underscoring the need to develop mitigation and value addition measures [7, 14, 16].

Various agave genus species, e.g., *Agave tequilana*, *Agave angustifolia,* and *Agave americana* are used in Mexican traditional medicine to treat inflammation-associated conditions [5, 6]. More pertinently, the juice from *Agave sisalana* leaves is used in traditional medicine as a wash for skin diseases and in treating syphilis, pulmonary tuberculosis, diseased liver, and jaundice in Northern Morocco [4].

This study investigated the effects of freeze-dried and fermented saponin-rich extracts prepared from sisal juice on a rodent model of metabolic syndrome. This study was partially inspired by a study showing that the consumption of saponin-rich aguamiel concentrate (A.C.) from *Agave salmiana* extract significantly reduced weight gain. Further, it lowered the fat mass and lowered the serum glucose, insulin, and LDL-cholesterol levels in mice fed a high-fat diet [10].

The metabolic syndrome was induced by administering a high fat and high fructose diet to freshly weaned Sprague-Dawley rats. This model has previously been shown to produce the canonical features of metabolic syndrome, including obesity, hyperglycemia, insulin resistance, and dyslipidemia [17, 18]. All these features were reproduced in the experimental animals belonging to the negative control group in this study, validating this model's use.

All the sisal juice extracts (i.e. the saponin-rich extracts from fresh sisal juice, saponin-rich extracts from fermented sisal juice, freeze-dried extracts from fresh sisal juice as well the freeze-dried extracts from fermented sisal juice) at the doses tested possessed significant hypoglycemic effects as shown by the fasting blood glucose levels. These significant hypoglycemic effects were observed after seven weeks (i.e., from day 35). The extracts also possessed significant effects on insulin sensitivity, as shown by the oral glucose tolerance test results on days 28 and 56. This, to our knowledge, is the first time these hypoglycemic effects of sisal juice extracts have been reported in the literature. The results obtained in this study are similar to those obtained by Leal-Diaz et al. The saponin-rich aguamiel concentrate obtained from *Agave salmiana* possessed significant hypoglycemic effects and led to a significant improvement in glucose tolerance [10].

All the various sisal juice extracts (i.e. the saponin-rich extracts from fresh sisal juice, saponin-rich extracts from fermented sisal juice, freeze-dried extracts from fresh sisal juice as well the freeze-dried extracts from fermented sisal juice) possessed significant antiobesity effects at the doses tested. The extracts also prevented the lipodystrophy commonly associated with the metabolic syndrome, as shown by their significant effects on the weights of pericardial, mesenteric, and retroperitoneal adipose tissue are representative of visceral adipose tissue [19, 20].

All the sisal juice extracts at the doses tested possessed significant antidyslipidemic effects, as shown by the reduction in total serum cholesterol, LDL cholesterol, serum triglyceride levels, and the increase in Serum HDL-cholesterol levels. Further, these results are in concordance with results that showed that all the extracts at the doses tested significantly reduced markers of hepatic steatosis/non-alcoholic fatty liver disease (NAFLD), i.e., hepatic triglyceride concentrations, liver weight, and hepatic index (hepatic weight: body weight ratio). It is now accepted that hepatic steatosis is the first manifestation of insulin resistance, a metabolic syndrome component, and precedes dyslipidemia [21, 22].

We then performed LC-MS analysis to determine the various extracts' chemical composition followed by network pharmacology studies to try and identify the possible mechanisms of action underlying the observed pharmacological effects.

LC-MS analysis showed that the freeze-dried extracts contained the largest number of compounds (54), followed by the freeze-dried extracts of fermented sisal juice (43), saponin-rich extracts of fresh sisal juice (27), and saponin-rich extracts of fermented sisal juice (26). The composition of the freeze-dried extracts of fresh sisal juice was similar to that reported in the literature [4, 23], indicating that the process of extract preparation did not meaningfully alter the composition of the sisal juice.

Network pharmacology studies indicated that the chemical components found in sisal juice primarily exert their effects by modulating the voltage-gated calcium channels CACNA1S, CACNA1D, and CACNA1C, in the beta cells of the islets of Langerhans. These voltage calcium channels have been reported to be involved in physiological processes, including but not limited to exocytosis, excitation-contraction coupling, apoptosis, metabolism, and fertilization, among others [24]. These calcium channels play a canonical role in glucose homeostasis via their central roles in beta cell exocytosis. Indeed, animal and clinical studies indicate significant correlations between Ca_v channel down-regulation and glucose intolerance due to decreased insulin release. Consequently, Ca_v channel upregulation in the pancreatic beta cells is a potential target in treating type 2 diabetes mellitus [25]. Therefore, one can reasonably speculate that the sisal extracts may have a role in the up-regulation of Ca_v channels and prevent the development of glucose intolerance characteristic of obesity and metabolic syndrome.

Besides, network pharmacology analysis also indicated that some chemical components of sial juice might interact with AKT1. AKT 1 is a serine/threonine-protein kinase that plays a crucial role in carbohydrate and lipid metabolism. Its impairment in obesity and type 2 diabetes has been shown to lead to reduced insulin secretion [26]. Targeting the A.K.T. signaling pathway has been shown to prevent the development of insulin resistance [27]. Network analysis also showed that some of the chemical moieties in the saponin rich fraction interact with the ADRB3, ADORA1 and ADRB2 genes which are known obesity candidate genes, are involved in lipid lipolysis through catecholamine signaling [28]. Their loss of function is associated with the development of type 2 diabetes and abdominal obesity [29]. These results point to the sisal saponins' potential role in the activation or potentiation of these genes, which could further explain the anti-diabetes effects of the fresh sisal saponins.

Although mRNA analysis or protein expression studies are often performed as a means of verifying/corroborating target prediction and network construction, this was not done in this study due to resource constraints and this is a therefore a limitation of the study.

5. Conclusion

Extracts of *Agave sisalana* juice prepared by a variety of methods i.e., lyophilization, fermentation, and saponin extraction possessed

significant antihyperglycemic, antiobesity and antidyslipidemic effects in a rodent model of metabolic syndrome which appear to mediated primarily through the modulation of insulin secretion by the pancreatic beta cells. This indicates that's the sisal plant juice which is commonly discarded may be processed to isolate compounds that can be used in the management of metabolic syndrome associated conditions. Future studies will involve the optimization of the extraction of the compounds implicated in these important pharmacological activities as well as elucidating the exact mechanism of this modulation of the calcium channels.

Declarations

Author contribution statement

Boniface Mwangi Chege: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data.

Nelly Murugi Nyaga, Prabjot Sehmi Kaur: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Wycliffe Odhiambo Misigo: conceived and designed the experiments; performed the experiments; contributed reagents, materials, analysis tools or data; wrote the paper.

Nelson Khan, Wycliffe Chisutia Wanyonyi: conceived and designed the experiments; performed the experiments; contributed reagents, materials, analysis tools or data.

Peter Waweru Mwangi: conceived and designed the experiments; analyzed and interpreted the data; wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] K. Reynolds, J. He, Epidemiology of the metabolic syndrome, in: American Journal of the Medical Sciences, Lippincott Williams and Wilkins, 2005, pp. 273–279.
[2] M.G. Saklayen, The global epidemic of the metabolic syndrome, Curr. Hypertension Rep. 1 (2018).
[3] S.M. Grundy, A constellation of complications: the metabolic syndrome, Clin. Cornerstone 7 (2005) 36–45.
[4] D. Tewari, Y.C. Tripathi, N. Anjum, Agave sisalana : a plant with high chemical diversity and world, J. Pharmaceut. Res. 3 (2014) 238–249.
[5] L. Santos-Zea, A. Leal-Diaz, E. Cortes-Ceballos, J. Gutierrez- Uribe, Agave (agave spp.) and its traditional products as a source of bioactive compounds, Curr. Bioact. Compd. 8 (2012) 218–231.
[6] N. Monterrosas-Brisson, M.L. Arenas Ocampo, E. Jiménez-Ferrer, A.R. Jiménez-Aparicio, A. Zamilpa, M. Gonzalez-Cortazar, et al., Anti-inflammatory activity of different agave plants and the compound Cantalasaponin-1, Molecules 18 (2013) 8136–8146.
[7] J.D.G. Santos, I.J.C. Vieira, R. Braz-Filho, A. Branco, Chemicals from agave sisalana biomass: isolation and identification, Int. J. Mol. Sci. 16 (2015) 8761–8771.
[8] J.D.G. Santos, A.F. Espeleta, A. Branco, S.A. De Assis, Aqueous Extraction of Pectin from Sisal Waste, Carbohydr Polym. 92 (2013) 1997–2001.
[9] Exploring Technological Strategies for Valorization of Solid Sisal Waste: A Research Review | Kivaisi | Tanzania Journal of Science [Internet]. [cited 22 Dec 2019]. Available: https://www.ajol.info/index.php/tjs/article/view/155769.
[10] A.M. Leal-Díaz, L.G. Noriega, I. Torre-Villalvazo, N. Torres, G. Alemán-Escondrillas, P. López-Romero, et al., Aguamiel concentrate from Agave salmiana and its extracted saponins attenuated obesity and hepatic steatosis and increased Akkermansia muciniphila in C57BL6 mice, Sci. Rep. 6 (2016).
[11] E. Barrett-Connor, The oral glucose tolerance test, revisited, Eur. Heart J. 23 (2002) 1229–1231. Available: http://www.ncbi.nlm.nih.gov/pubmed/12175655.
[13] Z. Liu, F. Guo, Y. Wang, C. Li, X. Zhang, H. Li, et al., BATMAN-TCM: a Bioinformatics analysis tool for molecular mechANism of traditional Chinese medicine, Sci. Rep. 6 (2016), 21146.
[14] A.C. Apolinário, E.M. de Carvalho, B.P.G. de Lima Damasceno, P.C.D. da Silva, A. Converti, A. Pessoa, et al., Extraction, isolation and characterization of inulin from Agave sisalana boles, Ind. Crops Prod. 108 (2017) 355–362.
[15] G. Santos Cerqueira, G. Dos Santos E Silva, E. Rios Vasconcelos, A.P. Fragoso De Freitas, B. Arcanjo Moura, D. Silveira MacEdo, et al., Effects of hecogenin and its possible mechanism of action on experimental models of gastric ulcer in mice, Eur. J. Pharmacol. 683 (2012) 260–269.

[16] M. Debnath, M. Pandey, R. Sharma, G.S. Thakur, P. Lal, Biotechnological intervention of Agave sisalana: a unique fiber yielding plant with medicinal property [Internet], J. Med. Plants Res. (2010) 177–187. Available: https://www.cabdirect.org/cabdirect/abstract/20103080394.
[17] S.K. Panchal, W.-Y. Wong, K. Kauter, L.C. Ward, L. Brown, Caffeine attenuates metabolic syndrome in diet-induced obese rats, Nutrition 28 (2012) 1055–1062.
[18] B.M. Chege, M.P. Waweru, B. Frederick, N.M. Nyaga, The freeze-dried extracts of Rotheca myricoides (Hochst.) Steane & Mabb possess hypoglycemic, hypolipidemic and hypoinsulinemic on type 2 diabetes rat model, J. Ethnopharmacol. 244 (2019), 112077.
[19] S. K Panchal, H. Poudyal, A. Iyer, R. Nazer, A. Alam, V. Diwan, et al., High-carbohydrate, high-fat diet–induced metabolic syndrome and cardiovascular remodeling in rats, J. Cardiovasc. Pharmacol. 57 (2011) 611–624.
[20] K.L. Stanhope, J.M. Schwarz, N.L. Keim, S.C. Griffen, A.A. Bremer, J.L. Graham, et al., Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans, J. Clin. Invest. 119 (2009) 1322–1334.
[21] N. Katsiki, D.P. Mikhailidis, C.S. Mantzoros, Non-alcoholic fatty liver disease and dyslipidemia: an update, Metabolism 65 (2016) 1109–1123.
[22] H. Kitade, G. Chen, Y. Ni, T. Ota, Nonalcoholic Fatty Liver Disease and Insulin Resistance: New Insights and Potential New Treatments, Nutrients 9 (2017).
[23] Agave (Agave sisalana) | Plant Profiler | Sigma-Aldrich [Internet]. [cited 26 Sep 2020]. Available: https://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler/agave.html.
[24] G.W. Zamponi, J. Striessnig, A. Koschak, A.C. Dolphin, The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential, Pharmacol Rev. 67 (2015) 821–870.
[25] S.N. Yang, P.O. Berggren, The role of voltage-gated calcium channels in pancreatic β-cell physiology and pathophysiology, Endocrine Rev. (2006) 621–676.
[26] Q. Huang, L. Chen, H. Teng, H. Song, X. Wu, M. Xu, Phenolic compounds ameliorate the glucose uptake in HepG2 cells' insulin resistance via activating AMPK: anti-diabetic effect of phenolic compounds in HepG2 cells, J. Funct. Foods 19 (2015) 487–494.
[27] X. Huang, G. Liu, J. Guo, Z.Q. Su, The PI3K/AKT pathway in obesity and type 2 diabetes, Int. J. Biol. Sci. (2018) 1483–1496.
[28] L.F. Saliba, R.S. Reis, R.C. Brownson, A.A. Hino, L.V. Tureck, C. Valko, et al., Obesity-related gene ADRB2, ADRB3 and GHRL polymorphisms and the response to a weight loss diet intervention in adult women, Genet. Mol. Biol. 37 (2014) 15–22.
[29] A. Takenaka, S. Nakamura, F. Mitsunaga, M. Inoue-Murayama, T. Udono, B. Suryobroto, Human-specific SNP in obesity genes, adrenergic receptor beta2 (ADRB2), beta3 (ADRB3), and PPAR γ2 (PPARG), during primate evolution, PLoS One 7 (2012).