



RESEARCH ARTICLE

Extract and fraction of cashew nut testa ameliorate the hyperglycemic mice induced by streptozotocin and high-fat diet

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Abstract

Drug strategy is a standard method for treating type 2 diabetes mellitus (T2D), a non-communicable disease with increasing prevalence, which may cause side effects. Therefore, natural compounds with limited adverse effects have come back into vogue for treating T2D. This study aims to evaluate the effects on rehabilitating hyperglycemic mice of cashew nut testa (husk) extract and fraction known as potential bio-substances for improvement in T2D. First, the hyperglycemic mice were induced with a high-fat diet (HFD) for 4 weeks and then were injected with streptozotocin (STZ, dozen for injection was 40 mg/kg/week) for 2 weeks. Next, the confirmed hyperglycemic mice were treated with pioglitazone (HG+PG group), total extract (HG+TE group), and saponin-rich fraction (HG+SRF group) for 3 weeks. Then, the evaluation was based on body mass; blood glucose (BG) level; BG tolerance, lipid profile, pancreatic histology and the expression IRS-1 in the pancreas. The results showed that body mass and BG level significantly increased in hyperglycemic mice. After substance treatment, there was no change in body mass in TE and SRF groups. However, BG level of HG+TE group mice significantly decreased compared to hyperglycemic mice and only BG tolerance of HG+SRF group was improved. Besides, HG+TE and HG+SRF groups modulated the triglyceride, HDL and LDL close to those expressed in normal mice. In addition, histological images of the pancreas revealed the restoration in both HG+TE and HG+SRF groups. Simultaneously, the IRS-1 expression in HG+TE group pancreas was restored to its expression in normal mice. These results demonstrate that the TE and SRF of cashew nut testa could ameliorate BG, lipid profile and pancreatic IRS-1 expression and restore the damaged pancreas and islets in hyperglycemic mice.

Keywords

cashew, nut, testa, streptozotocin, high-fat diet, hyperglycemic, mice

Introduction

Diabetes mellitus is a chronic disease that causes either insulin deficiency or insulin resistance and is characterized by high blood glucose levels (1). This disease is classified into three main types, including type 1 diabetes (T1D), type 2 diabetes (T2D) and gestational diabetes (2). T1D is caused by autoimmune-induced pancreas beta-cell damage, resulting in insulin deficiency (3). There are 5-10% of patients with T1D (3). In contrast,

T2D is the most popular form, accounting for at least 90% of patients with diabetes and characterized by insulin resistance (4). Gestational diabetes only occurs in pregnant stage in women (5). However, there is a high risk of developing T2D later in their life (5). Many studies demonstrated that the leading cause of diabetes is obesity (6). Besides, obesity is known as a main factor causing T2D (7). Abnormal signalling pathways of insulin is supposed to be related to insulin resistance (8). These signals including insulin and insulin-like growth factors I (IGF-I) are transferred to intracellular pathways via insulin receptor (IR). This leads to phosphorylation of IR and insulin receptor substrate 1 (IRS-1) (9). Phosphorylated IRS-1 increase molecules of glucose transporter type 4 (GLUT4) on membrane and glucose uptake from blood into tissue (10). Researches have suggested a down-regulation of IRS-1 in patients of insulin resistance (11, 12).

T2D also causes serious complications, including kidney failure, blindness, heart attacks and stroke (13). However, the popular treatment for hyperglycemia is drug strategy to regulate blood glucose levels. These chemical drugs could cause side effects and lead to drug-dependence. Therefore, using natural bioactive compounds such as tannin, flavonoid and saponin for T2D treatment have been researched to overcome drug therapy limitations. As a result, an animal model is essential for finding new compounds that have potential use in treatment therapy. In an experiment, mice were fed with a high-fat diet and were seen as models of glucose intolerance and T2D (14). C57BL/6J mice were fed a diet of 58% fat, while other mice were given a diet containing 11% fat (normal diet). Body mass of high fat treated mice increased immediately after the first week of feeding. Blood glucose levels increased week by week during the 12 months of the experiment, averaging 1 mmol/L (14). However, these mice took a long time to develop T2D. Streptozotocin (STZ) injection could promote the expression of T2D characterizations, including increased blood glucose level and free fatty acid (15). Therefore, we used mice fed a high-fat diet and injected STZ for our discoveries.

Various studies reported that extracts from cashew components reduced blood glucose in models of diabetes (16-18). The ethanol extract of cashew nut showed hypoglycaemic effects in different fractions, in which polyphenols and flavonoids were identified in most of the fractions (16). These compounds play an important role in metabolic disorders and insulin resistance in T2D via PPAR- γ pathway (16). In addition, bioactive compounds such as tannins, polyphenols, alkaloids and saponins were found in the leaves, bark and roots of cashew nuts (19). Although saponin content accounts for 2% in the cashew bark (20), it was known as a potential compound that decreased blood glucose in T2D (21). Saponin activates glycogen synthesis (22), suppresses activation of disaccharide and gluconeogenesis (23), modulates insulin signalling and stimulates the expression of GLUT4 (24).

Based on the urgency of T2D treatment and the available and abundant supply of cashew nut testa in Vietnam, we performed the study to evaluate the role of cashew nut testa extract and fraction in hyperglycemic mice induced by streptozotocin and high-fat diet.

Materials and Methods

Extract preparation

The samples of collected cashew nut testa were cleaned to remove sand and dust. The samples were dried at 60 °C until their weight remained consistent. 78 g of dried testa was crushed extracted using the Soxhlet apparatus with ethanol (25). The high ethanol extract obtained was further subjected to solid-phase extraction with n-hexane, chloroform (CHCl₃), ethyl acetate (EtOAc) and methanol.

Extraction of saponin-rich fraction

Methanol was used to concentrate ethanol extract and foam test was used to detect saponin (26). Methanol was slowly added to 100 mg of the dried total ethanol extract and stirred until the extract completely dissolved. Diethyl ether was the added drop by drop, until precipitation began. The precipitate was filtered using filter paper. The precipitate was collected, dried and weighed.

Experimental animals

All procedures relating to experimental animals were approved by The Animal Care and Use Committee, University of Sciences, Vietnam National University – Ho Chi Minh City (ACCUS), number 699B/KHTN-ACUCUS. The eight-week-old albino mice were provided by Pasteur Institute and divided into four groups:

Eight-week-old mice (*M. musculus*) were purchased at Pasteur TP. HCM. Mice were randomly divided into 2 groups based on weight: the control group (Normal group, n=12) received a normal diet and the diabetic-inducing group (HFD group, n=12) received a high-fat diet.

After 8 weeks of feeding, mice in the HFD group were fasted for 8 hrs and injected with STZ (U-9889) mixed with sodium citrate at a dozen of 40 mg/kg/week, repeated continuously for 2 weeks. At the same time, mice in the Normal group were injected with sodium citrate. After injection, all mice were monitored for 2 weeks. Diabetic mice had blood glucose levels greater than 200 mg/kg.

The diabetic mice were randomly divided into four groups: (1) no treatment (HG group); (2) treatment with total extract (HG+TE group); (3) treatment with saponin-rich ingredient fraction (HG+SRF group) and (4) treatment with pioglitazone (HG+PG group). The drugs were diluted in distilled water and given continuously for 21 days. Throughout the treatment, the batched mice were fed the same diet as during the modelling period (Fig. 1).

Acute toxicity test

The test was performed according to the OECD guidelines (27). The mice were fasted approximately 3-4 hrs or longer before treatment. The test substance was

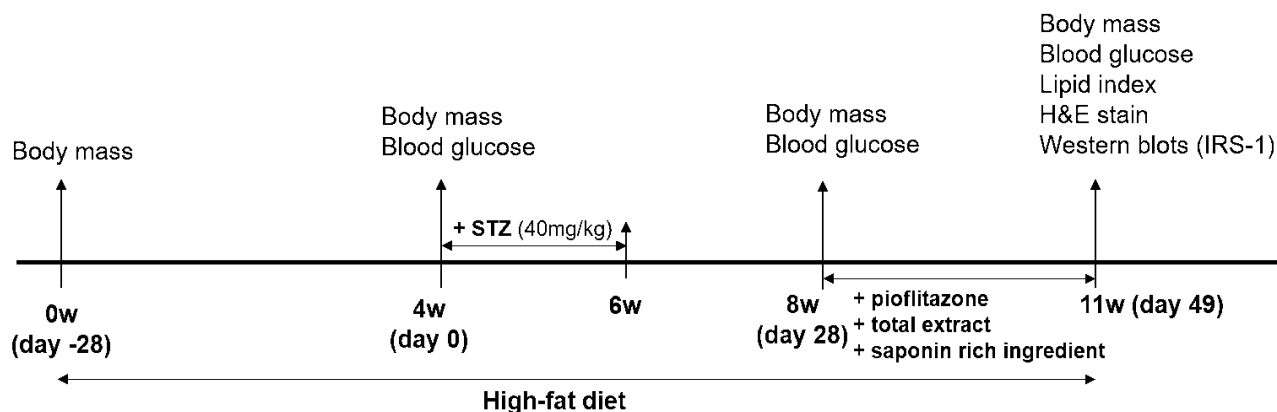


Fig. 1. Experimental arrangement of the in vivo study.

8 weeks- old mice were used for all experiments. Mice were fed with high-fat diet during study. After 4 first weeks of feeding, these mice were injected STZ with dose 40mg/kg for 2 weeks. Then, they were divided groups to treat with pioglitazone, total extract, and saponin rich ingredient for 21 days. Body mass and blood glucose were measured at the specific time points. Besides, lipid index, H&E stain, and Western blots were performed after treatment.

administered by gavage using a stomach tube. The test mice were observed twice a day for the following 72 hrs. The survival signs were recorded daily. Four doses of the substance, including 5, 50, 300 and 2000 mg/kg, were used in this test; each dose was tested on 3 animals.

Hematological biochemistry assessment

Blood glucose level measurement

Blood glucose (BG) level measurements were performed using Accu-Chek[®] Active blood glucose meter (28). Experimental mice were fasted for 8 hrs and put into a mouse-fixing system. The blood was taken from the tail vein using a 1 mL needle. The first drop of blood was discarded, then, the blood was dropped on the test strip. The strip was plugged into the glucose meter and the data was recorded.

Assess blood glucose tolerance (BG tolerance)

The blood glucose tolerance in mice was tested after 21 days of giving drugs. D-glucose was injected into the peritoneal cavity of mice at a dose of 2 g/kg. A glucose meter was used to measure blood glucose levels at 0, 15, 30, 60, 90 and 120 hrs after injection.

Measurement of blood lipid index

Total blood samples from mice were taken after 21 days post treatment. A semi-automatic biochemical analyzer was used to test the total cholesterol, HDL, LDL and triglycerides in the blood serum (29). Protocols were performed according to the manufacturer's instructions.

Histological evaluation

Mice were humanely killed to collect the pancreas after 21 days of giving medication. The pancreas of mice were soaked in formaldehyde for 24 hrs, then dehydrated and soaked in paraffin. Tissue slices were stained with hematoxylin and eosin (30).

IRS-1 expression in pancreas evaluation by Western blot

Tissues were gathered and weighed with a 100 mg reference weight. The tissues were crushed and soaked in

Optiblot LDS sample buffer 4X (ab119196) to isolate protein samples. These samples were seeded into the wells of 4-12% SDS-PAGE gel pads. The gels were electrophoresed in a 50V Optiblot SDS Run Buffer (ab119197, Abcam) for 2 hrs. After electrophoresis, proteins were separated into different lines, transferred to PVDF membrane (ab133411, Abcam) and blocked overnight at 4 °C with blocking buffer (ab 126587, Abcam). Primary antibodies were incubated on the membranes in TBST at a ratio of 4ml antibodies to 1 mL TBST. As controls, primary anti-IRS1 (ab131487) and anti-GAPDH (ab181602, Abcam) antibodies were utilized. Membranes were washed with TBST 3 times for 5 min each time. Membranes were then incubated with Goat anti-rabbit IgG secondary antibody (ab6721; Abcam) for 1 hr. The signal lines were found using the ECL Western blotting Substrate Kit (ab65623; Abcam) and expressed on X-ray film. The signal intensity of the protein bands was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Data processing

Data are expressed as \pm SEM. The data were statistically analyzed using GraphPad Prism 8 software (GraphPad Software, Inc). The data were analyzed by t-test and one-way ANOVA. The results were statistically significant with $p < 0.05$.

Results

The extract from cashew nut testa and saponin-rich fraction

The following corresponding high extracts were obtained: n-hexane extract (1.3 g), CHCl_3 extract (0.2 g), EtOAc extract (7.5 g) and methanol extract (2.0 g) by evaporating the solvents using rotary evaporation.

Table 1. The efficiency of enriching saponin from methanol extract

Methanol extract (mg)	Methanol (ml)	Diethyl ether (ml)	Saponin rich fraction (mg)
100 mg	5	25	104
100 mg	5	25	81
Mean	5	25	92,5 \pm 16,3

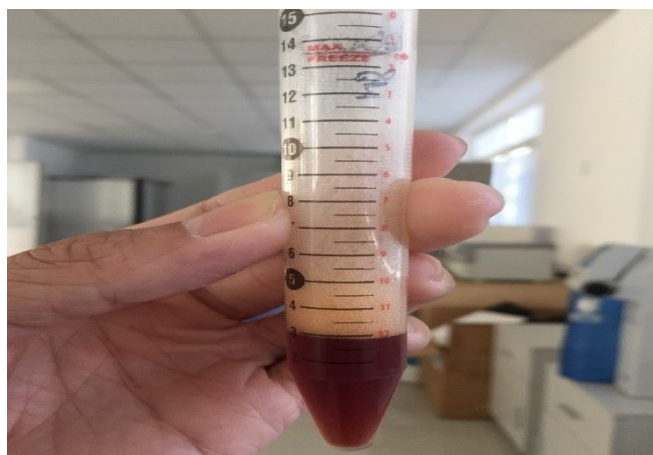


Fig. 2. Foaming of extract fraction. Extract fraction was evaluated using a foam test. Foaming was observed after suspension of fraction.

solvents using rotary evaporation.

Fractionated extracts were tested for their foaming ability to detect saponin. The extract fractions underwent a foam test. The solution was shaken well, and the foam remained stable for 15 min. Among them, the methanol

Table 2. The results of high-dose toxicity testing of the total ethanol extract from silk shell samples on mice

Dose test (mg/kg)	Number of mice	Live (After 72h)	Survival rate (%)
5	3	3	100
50	3	3	100
300	3	3	100
2000	3	3	100

extract produced the most foam (Fig. 2). The separation efficiency of enriching saponin from the average extract was $92.5 \pm 16.3\%$ (Table 1).

The extracts from cashew nut testa have no toxicity on mice

The mice achieved a 100% survival rate after 72 hrs of oral administration of different concentrations of silk shell sample extract, following the OECD guidelines (Table 2).

The mice remained alive at the tested concentrations, exhibited normal feeding, and engaged in regular activities after 72 hrs of drug administration. Furthermore, no abnormal behaviour was observed in the mice. Therefore, it was not possible to determine the LD50 dose of the ethanol extract from the silk shell sample. The experimental dose used on the mice was 1/10 of the highest toxic dose according to the OECD guidelines. A dose of 200 mg/kg will be used for the subsequent experiments in the study.

High-fat diet increased weight, blood glucose and enhanced the hyperglycemic effect of streptozotocin

Before diet (day -28), mice of equal weight were split into two groups: normal diet-fed (Normal group) weighed 29.16 ± 0.52 g and high-fat diet-fed (HFD group) weighed 29.73 ± 0.93 g. After 28 days of feeding (day 0), the weight of mice in the Normal group reached 31.48 ± 0.92 g and increased insignificantly. In contrast, the weight of mice in the HFD group was considerably raised by 9.33 ± 1.31 g ($p < 0.0001$), and higher than the Normal group ($p < 0.01$). Two groups were fed with respective diets for the next 28

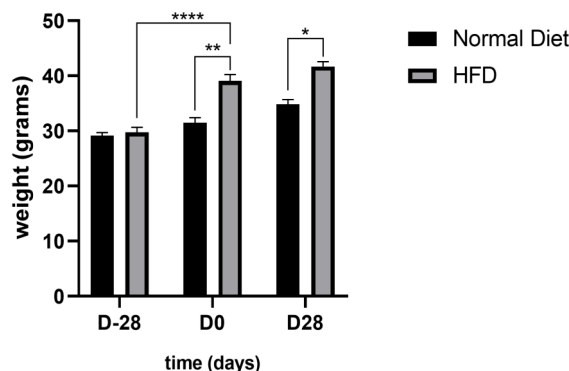


Fig. 3. The change of body weight after 28 days in group of mice with normal diet, and group of mice with high-fat diet and STZ injection (HFD group).

The chart showed that the body weight of mice fed with a regular diet increased but was not statistically significant after 4 weeks and 8 weeks. Whereas, a significant increase in body weight was observed in the group of mice fed with a high-fat diet and STZ injection. The weight of mice in the HFD

days. However, the HFD group was injected with STZ for the first 14 days. The results showed that the weight of both groups changed fractionally comparing day 28 to day 0. On the other hand, the weight of mice in the HFD group was statistically higher than the Normal group ($p < 0.05$). The outcomes demonstrated that a high-fat diet markedly increased the weight of mice ($p < 0.05$) while normal mice slightly raised during the diet. Besides, injecting STZ did not significantly change the weight of mice fed with a high-fat diet which was higher than normal mice (Fig. 3).

At day 0, the mice's plasma was collected to measure the blood glucose (BG) levels. The BG level of the Normal group was 111.5 ± 11.91 mg/dL and the HFD group was 159.9 ± 5.84 mg/dL. Thus, the BG level in the HFD group was significantly higher than in the Normal group at 48.42 ± 15.44 mg/dL; however, neither group's levels exceeded 200 mg/dL. At day 28, the mice's BG level of the HFD group dramatically increased and was higher than the Normal group at 92.88 ± 15.44 mg/dL ($p < 0.0001$). It was also higher than the levels at day 0 at 86.29 ± 9.77 mg/dL

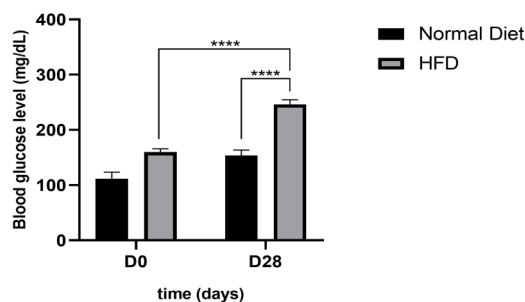


Fig. 4. The level of blood glucose changed when mice were fed with different diets.

Blood glucose levels were recorded in different groups of mice. Blood glucose level was maintained below 200mg/dL in normal mice during the experiments. Prior to STZ injection (day 0), the BG levels of mice fed with a high-fat diet were 159.91 ± 28.6 mg/dL, higher than normal mice (111.5 ± 29.17 mg/dL). After STZ injection, at day 28, BG levels of HFD mice strongly increased, gained 246.62 ± 40.62 mg/dL, and were significantly higher than those at day 0 and blood glucose levels of normal mice (****):

($p < 0.0001$). In the control group, the BG level did not substantially change and maintained below 200 mg/dL during experiments. The results indicated that a high-fat diet combined with STZ injection caused an increase in mice's blood glucose levels (Fig. 4).

The findings showed that mice's weight and blood glucose levels significantly increased as a result of a high-fat diet and STZ injection. These hyperglycemic mice were used in the subsequent treatment investigations on model of high-glucose mice.

Extract and fraction of cashew nut testa

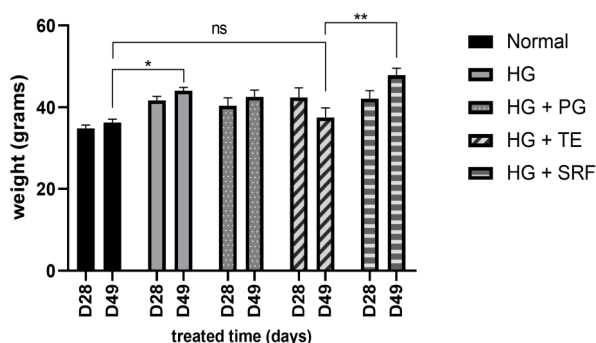


Fig. 5. The treatment affected weight in hyperglycemic (HG) mice. There was no change of weight in all groups after treatment 21 days. HG mice's weight increased and was higher than normal mice. HG mice injected pioglitazone, saponin rich fraction slightly increased in weight and was respective with non-treated mice. In contrast, the weight of HG+total extract mice decrease after injecting and was significantly lower than HG+saponin rich ingredient mice (* $p < 0.05$, ** $p < 0.01$, ns: no significant).

ameliorates the hyperglycemic mice

The change of weight after treatment

The weight change was observed after 21 days of treatment (day 49). The weight of the HG group, HG+PG, and HG+SRF group continued to increase, while the weight of the HG+TE group slightly decreased compared to day 28. Besides, no difference in weight was found between the HG, HG+PG and HG+SRF groups, and the weight of these groups was significantly higher than both the HG+TE and CT group, whereas the weight of the HG+TE group was equal to the CT group (Fig. 5).

Improvement blood glucose level

After 21 days of treatment (day 49), the BG level of the HG+PG, HG+TE and HG+SRF groups dramatically decreased compared to day 28 ($p < 0.0001$) and was lower than the HG group. The recorded weights were

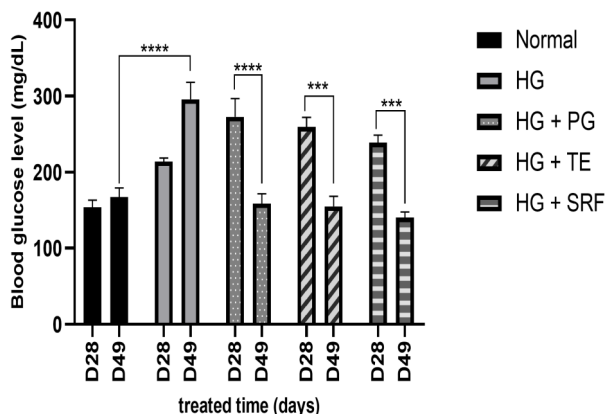


Fig. 6. The level of blood glucose was modulated in HG mice after treatments. Blood glucose of HG mice sustained at level of >200 mg/dL. While the level markedly decreased in mice treated with pioglitazone, total extract, and saponin rich ingredient compared with day 0 (** $p < 0.001$, **** $p < 0.0001$). Their blood glucose level was equal with normal mice.

158.83 \pm 31.82 mg/dL; 154.83 \pm 33.25 mg/dL; 140 \pm 18.71 mg/dL; 295.67 \pm 22.67 mg/dL, respectively. According to these results, saponin-rich ingredient effectively decreased the blood glucose in HG mice and was better than pioglitazone and total extract. On the other hand, the difference was not significant between the treated groups and the Normal group, while the BG level of the HG group was higher than the HG+PG 1.86 times, HG+TE 1.91 times, HG+SRF 2.1 times and the Normal group 1.76 times (Fig. 6). These results demonstrated that extract and fraction of cashew nut testa significantly improved the blood glucose level in HG mice.

Effect on blood glucose tolerance

The BG tolerance of all the experimented groups was the same trending, with the BG levels increasing to the highest at the time point 15 mins and decreasing at the following time points (Fig. 7). More detailed, although the HG+PG group's BG level slightly reduced from 30 to 60 mins and strongly decreased to 120 mins, it sustained above 200

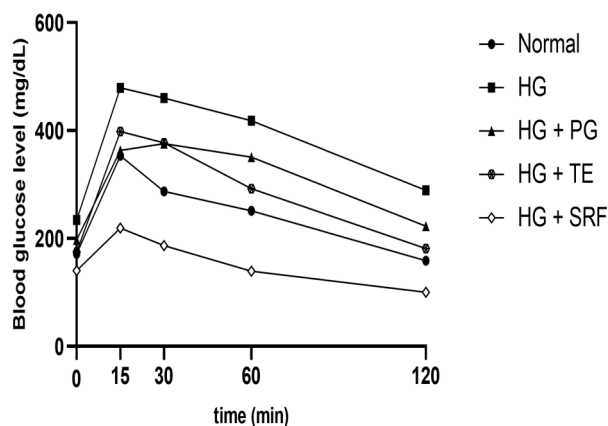


Fig. 7. The chart of glucose tolerance in normal mice, HG mice and treated mice. The blood glucose reached the highest level after 15 minutes in all groups. Then the level decreased after 120 minutes. The blood glucose of HG + saponin rich ingredient was lower than normal mice during test. While HG mice, HG+pioglitazone mice, and HG+total extract mice were higher than normal group.

mg/dL and was higher than the control group. In comparison, the HG+TE group's BG level was clearly improved and respective with the control group at 120 mins. Similarly, the BG level of the HG+SRF group was decreased to below 200 mg/dL at 30 mins and continuously decreased at the following time points. On the other hand, at all the time points, the BG level of the HG group was highest compared to others and exceeded 200 mg/dL, while the the BG levels of HG+SRF group was lowest. These results showed that total extract and saponin-rich fraction ameliorated glucose tolerance in HG mice.

Improvement of blood lipid index

The tests of blood lipid index showed that HG mice treated with pioglitazone, total extract, saponin rich ingredients were strongly remodelled when compared with HG mice. The total cholesterol was insignificantly different in the HG+PG, HG+SRF and HG groups. However, it was found to be higher than in the Normal group. Besides, the

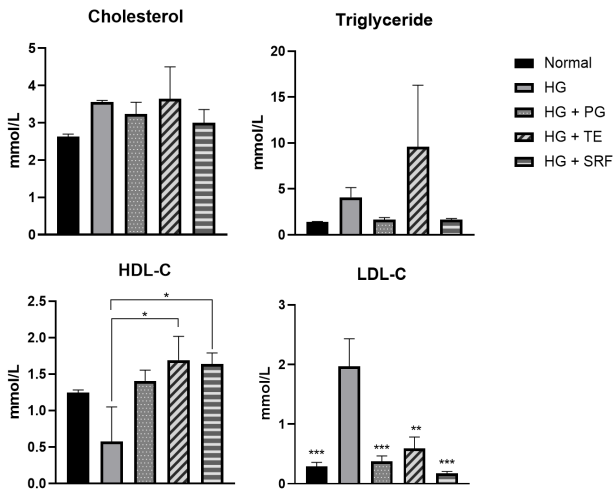


Fig. 8. The treatment improved the blood lipid index in HG mice. In HG mice, cholesterol, triglyceride, and LDL-C were higher dramatically than normal mice. HDL-C of the group was lower than normal mice but not statistical. Treatment of pioglitazone and saponin rich ingredient markedly improved blood lipid indexes. These indexes were approximate with normal group. In mice injected with total extract, cholesterol, triglyceride, HDL-C were not different while LDL-C were significantly lower than HG treated mice (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

triglyceride level was the first highest in the HG+TE group and was the second in the HG group, whereas it was approximately in HG+PG, HG+SRF and Normal groups and lower than in the HG group. In comparison, HDL-C index showed a different trend. It was lowest in the HG group, less than 1 mM/L; and was significantly higher in the treated groups than in the HG and Normal groups. In contrast, LDL-C index in HG mice was the highest and folded respectively HG+TE 3.31 times, HG+SRF 11.34 times and HG+PG 5.22 times, whereas there was no difference between the treated groups and the Normal group (Fig. 8).

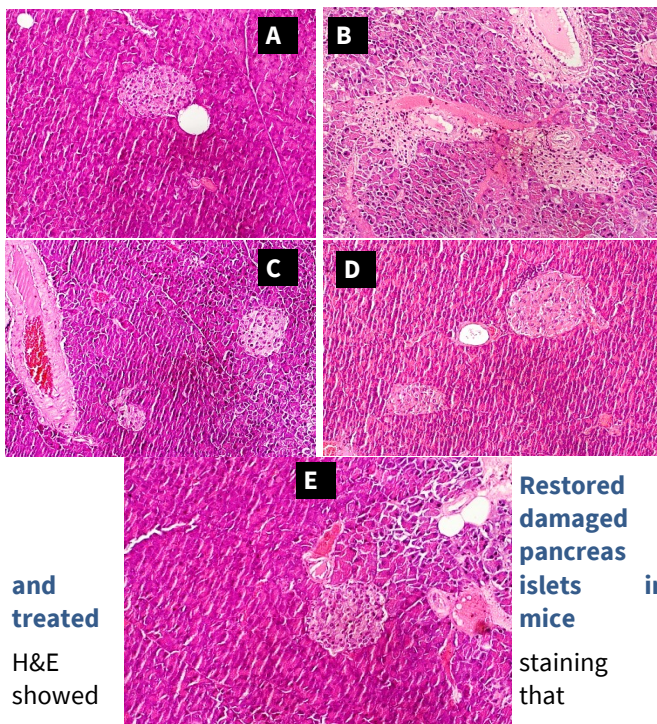


Fig. 9. Extract and fraction remodelled the pancreas and islets in HG mice. Damaged pancreas and islets were found in HG mice. These damages were remodelled after treatment of pioglitazone, total extract, and saponin rich ingredient: elliptical shape was restored, and the number of islets increased although their size was small (A: Normal; B: HG; C: HG + PG; D: HG + TE; E: HG + SRF).

pancreas and islets were markedly restored in HG mice injected extract and fraction of cashew nut testa and pioglitazone. In the HG group, the structure of pancreas tissue was incoherent, and islets were not held elliptical-shaped. In comparison, the treated groups were remodelled in morphology and number of islets. Despite the small size, their islets increased and had a clear boundary. In addition, the structure of the surrounding tissue was restored. Besides, no difference was found

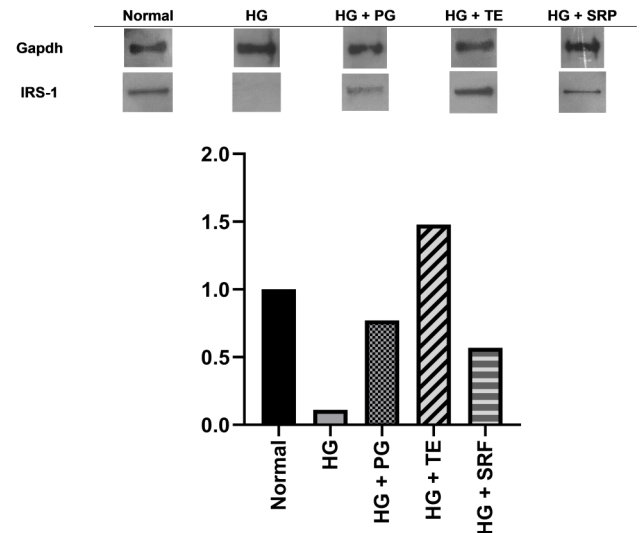


Fig. 10. Expressing IRS-1 of HG mice after treatment. After treatment with various drugs, IRS-1 protein was found by western blots while this was not observed in non-treated mice.

between HG+PG, HG+TE and HG+SRF groups (Fig. 9).

IRS-1 expression in mice treated with cashew nut testa extract and fraction

To investigate the mechanism of extract and fraction of cashew nut testa in HG mice, expression of IRS-1 protein was performed by Western blots. The results indicated that IRS-1 did not express in the HG group. In contrast, the protein was found in HG+PG, HG+TE and HG+SRF groups, which was the same as the Normal group (Fig. 10).

Discussion

Diabetes is a chronic disease that causes either insulin deficiency or insulin resistance, characterized by hyperglycemia (1). One of the main causes of type 2 diabetes is obesity (31). Multiple models of obesity-induced type 2 diabetes and multiple low-dose STZ injections have been performed in mice (32-34). The high-fat diet, which has 45%-60% fat, leads to obesity and induces inflammation as well as glucose and insulin resistance in mice (35). High-fat diet-induced type 2 diabetic mice have characteristics such as weight gain, hyperglycemia, hypertension, insulin resistance, secretion

of inflammatory cytokines and ectopic lipid accumulation (36). Markers of β -cell senescence were increased in these mice (37). Streptozotocin is a toxin with a sugar-like structure that is preferentially transported via the GLUT2 (38). STZ induces DNA breakage, selective destruction of β -cells and cause insulin-dependent diabetes mellitus (39).

In our study, the weight and blood glucose level significantly increased in mice fed with a high-fat diet 28 days. These indexes continuously rose after injection STZ, markedly blood glucose level reached up 200 mg/dL. This was known as the first characteristics of hyperglycemia in T2D. After that the raise of lipid indexes, glucose intolerance and injured islets were detected during experiments. The results were found in which rats fed with a high-fat diet increased body weight, plasma glucose level, cholesterol, triglyceric (40). In the study by Jiao et al., rats were fed a high-fat diet consisting of 12% lard, 18% sugar and 70% of a normal diet for 28 days. Then mice were injected with STZ at a dose of 40 mg/kg. After 72 h, the fasting blood glucose of rats was above 11.1 mmol/L and remained above 20 mmol/L after 7 weeks of modelling (41).

To treat T2D, pioglitazone was known as antidiabetic drugs and popularly used for T2D patients (42, 43). Pioglitazone plays a role as ligand for a nuclear receptor - the peroxisome proliferator-activated receptor (PPAR γ) (43). Pioglitazone-induced PPAR γ activates expression of several genes related to glucose and lipid metabolism such as lipoprotein lipase, fatty acid transporter protein, GLUT4 glucose transporter (44, 45). This results in reducing insulin resistance via increase of GLUT4 expression, TNF- α inhibition to reduce serum FAA, enhance IRS expression and insulin sensitivity (44, 46). In our study, the treated outcomes showed that effects of pioglitazone and saponin-rich ingredient were the same on body weight, blood glucose level, lipid indexes, restoring islets and expression of IRS-1. Their body mass was higher than day 0 and insignificantly changed to compared with HG mice. The blood glucose level strongly improved and reduced to be equal with normal mice. However, glucose tolerance of HG+saponin-rich ingredient mice was better than HG+pioglitazone mice. Besides, lipid indexes indicated that pioglitazone and saponin-rich ingredient affected to decrease cholesterol, triglyceride, LDL-C and to increase HDL-C and was respective with normal mice. The equivalence of islets restoring and expression of IRS-1 were shown. In another study, cashew nutshells were shown to contain saponins (47). Saponins have also been shown to improve diabetes in rats (48). In a study, total saponin at concentrations of 0.025 g/kg and 0.1 mg/kg was used to treat diabetic rats induced by high-fat diet and by injection STZ within 8 weeks (48). The results showed that total saponin reduced blood glucose and lipid levels in rats (48). Total saponin increases GLUT4 expression and decreases G6P expression in the insulin signaling pathway (48). In addition, saponin also increased expression of GLUT4 in muscle tissue and PPAR- γ in adipose tissue (48). At the same time, in a study, saponins have been shown to stimulate increased insulin secretion

through the PI3K/Akt/FoxO1 pathway (49). Based on treated mechanism and these results demonstrated that saponin-rich ingredient of cashew nut testa could reduce hyperglycemia, remodel injured pancreas and islets and raise insulin sensitivity.

After extraction, the highest dose of ethanol extract 2000 mg/kg did not cause toxic reactions in mice. Treatment results showed that total extract of cashew nut testa could decrease body mass, blood glucose level, LDL and enhance IRS-1 expression to remodel damaged pancreas. Additionally, mice injected with total extract showed an improvement in their glucose tolerance as compared with HG mice. However, their cholesterol and triglyceride were dramatically higher than normal mice and mice injected saponin-rich ingredient, pioglitazone. Although injured pancreas was restored, islet mass of the group was bigger than other groups. Therefore, effect of total extract was less than saponin-rich ingredient and pioglitazone.

Conclusion

Our study built hyperglycemic model in mice by high-fat diet and streptozotocin injection, extracted total extract and saponin-rich ingredient from cashew nut testa. After treatment, total extract and saponin-rich ingredient ameliorated blood glucose, lipid index and restored injured pancreas, islets by increasing IRS1-expression and improving glucose tolerance. Especially, saponin-rich ingredient of cashew nut testa had similar effect with pioglitazone.

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Authors' contributions

Conceptualization: HDV and LTTD; methodology: HDV, LTTN, NLCP, LTL and LTTD; investigation and data curation: HDV, NTNN, HTNN and LTTD; writing-original draft: HDV, LTL, TTTD, NTNN and LTTD; writing-review and editing: HDV, TTTD, NTNN and LTTD. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: Experimental animals were approved by The Animal Care and Use Committee, University of Sciences, Vietnam National University – Ho Chi Minh City (ACCUS), number 699B/KHTN-ACUCUS.

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