



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/apjtb.apjtb_11_25

Salsola imbricata attenuates type 2 diabetes mellitus in rats by regulating oxidative stress and insulin signaling

Ifraha Abbas¹, Wafa Majeed^{2✉}, Bilal Aslam¹, Muhammad Naeem Faisal¹¹Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan²Department of Pharmacy, Faculty of Health and Pharmaceutical Sciences, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Objective: To evaluate the antidiabetic effect of *Salsola imbricata* (*S. imbricata*) leaf extract against high-fat-diet/streptozocin-induced alterations in diabetic rats and to explore the underlying mechanisms involved in insulin secretion.

Methods: The total phytochemical content of ethyl acetate extract of *S. imbricata* was analyzed by HPLC. *In vitro* antioxidant and antidiabetic activities were determined. Ethyl acetate extract of *S. imbricata* (250 and 500 mg/kg) was administered to diabetic rats for 21 days. Serum glucose, serum insulin, fasting blood glucose, body weight, and homeostasis model assessment of insulin resistance were measured. The hepatic malondialdehyde and antioxidant enzyme activities including superoxide dismutase, catalase, and glutathione peroxidase were evaluated. Sandwich ELISA kits were used to measure pro-inflammatory cytokines IL-6 and tumor necrosis factor- α (TNF- α) in liver tissue homogenate. *Pdx-1*, *Ins-1*, and *Ins-2* expression levels were assessed using qRT-PCR.

Results: *S. imbricata* had potent antioxidant (IC₅₀=11.75 μ g/mL) and antidiabetic activity (IC₅₀=28.10 μ g/mL). HPLC analysis showed the presence of gallic acid, *p*-coumaric acid, salicylic acid, chlorogenic acid, caffeic acid, and quercetin. *S. imbricata* extract significantly ($P<0.05$) reduced blood glucose levels with marked improvement in insulin sensitivity and reduced insulin resistance. *S. imbricata* leaf extract improved malondialdehyde, superoxide dismutase, catalase, and glutathione peroxidase levels in hepatic tissue. *S. imbricata* leaf extract also decreased levels of TNF- α and IL-6 ($P<0.05$). qRT-PCR revealed the upregulation of insulin signaling transduction genes (*Pdx-1*, *Ins-1*, *Ins-2*).

Conclusions: *S. imbricata* ethyl acetate extract exhibits pronounced antidiabetic activity in rats by improving insulin resistance *via* modulation of insulin signaling pathway and alleviating oxidative stress.

KEYWORDS: Diabetes mellitus; *Salsola imbricata*; Oxidative stress; Insulin resistance; Phytochemicals

1. Introduction

Diabetes mellitus (DM) is a metabolic syndrome marked by impaired metabolism of carbohydrates, fats, and proteins, which is categorized as chronic hyperglycemia due to insufficient synthesis of insulin or its action[1,2]. International Diabetes Federation estimates that 536.6 million adults are suffering from diabetes in 2021. If

Summary

Question: What are the effects and mechanisms of *Salsola imbricata* ethyl acetate extract on diabetes in rats?

Findings: *Salsola imbricata* ethyl acetate extract considerably improved glycemic and antioxidants markers and inflammatory cytokines, and upregulated the expression of *Pdx-1*, *Ins-1*, and *Ins-2* genes involved in insulin signaling pathway.

Meaning: *Salsola imbricata* ethyl acetate extract exerts its antidiabetic activity at least partially by attenuating oxidative stress and modulating insulin signaling proteins and could be further explored for the treatment of diabetes.

✉To whom correspondence may be addressed. E-mail: wafa.majeed@uaf.edu.pk

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2025 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow.

How to cite this article: Abbas I, Majeed W, Aslam B, Naeem Faisal M. *Salsola imbricata* attenuates type 2 diabetes mellitus in rats by regulating oxidative stress and insulin signaling. Asian Pac J Trop Biomed 2025; 15(5): 210-218.

Article history: Received 4 January 2025; Revision 10 February 2025; Accepted 12 May 2025; Available online 22 May 2025

appropriate preventive measures are not implemented, it is predicted that this number will rise to 643 million by 2030 and will further reach 783.4 million by 2045[3].

Persistent hyperglycemia is a major factor in the progression of diabetic complications including insulin resistance, immune function, hypertension, and dyslipidemia[4]. Because hyperglycemia causes oxidative stress, reactive oxygen species (ROS) are produced in excess, and the antioxidant defense mechanism is inactive[5]. ROS has been considered a potential mechanism for diabetes that interrupts PI3K/AKT insulin signaling cascade resulting in development of insulin resistance[6].

Multiple antidiabetic agents are used but long-term usage of oral drugs has undesirable effects on health, including hypoglycemia, drug resistance, edema, lactic acidosis, weight gain, and disturbance of liver and kidney functions[7,8]. This information has created an appetite for the development of novel antidiabetic medications with improved efficacy. Nowadays, traditional folk medicines are more effective for the management of DM[9].

More than 400 medicinal plants have been shown to exhibit varying degrees of hypoglycemic and anti-hyperglycemic activity, as well as valuable medicinal properties in various plant parts[10]. Numerous studies have demonstrated the antidiabetic benefits of different parts of plants containing phenols, coumarins, flavonoids, glycosides, terpenoids, and alkaloids[11].

The genus *Salsola* (commonly known as saltwort) belongs to Amaranthaceae family (formerly Chenopodiaceae). Several therapeutic and pharmacological activities of *Salsola imbricata* (*S. imbricata*) have been acknowledged including antioxidant, tyrosinase inhibitory, oral contraceptive activity, diuretic, and anti-inflammatory[12]. *S. imbricata* has been shown to contain a variety of phytochemicals, including alkaloids, flavonoids, terpenoids, saponins, glycosides, and phenolic compounds[13].

The present study aimed to explore the phytochemical profile (total phenolic and flavonoid content), and *in vitro* antioxidant and antidiabetic activities of ethyl acetate extract of *S. imbricata* (SIEAE), and to investigate antioxidant and antidiabetic potential of SIEAE by measuring antioxidant and gene expression involved in insulin signaling using high-fat diet (HFD)/streptozocin (STZ)-induced diabetic rats.

2. Materials and methods

2.1. Main reagents and apparatus

Metformin (Neodapir) was procured from Sanofi-Aventis (Pvt, Ltd) and STZ from Sigma-Aldrich. Analytical-grade reagents were used in the present study.

2.2. Preparation of *S. imbricata* ethyl acetate extract

Fresh *S. imbricata* aerial parts were amassed from Faisalabad, Pakistan. A taxonomist from Botany Department of University of Agriculture, Faisalabad verified identification of the plant, and a voucher (1512-01-2022) was deposited at the herbarium.

The leaves of the plant were cleaned and shade-dried for one week. After one week, leaves were ground into fine powder using a mechanical grinder and sieved. Then 100 g of powder was weighed and macerated in 75% ethyl acetate for 3 d with constant shaking. The solvent was filtered through Whatman No.1 filter paper. The filtered extract was dried in a rotary evaporator and oven. The dried extract was reserved at 4 °C for advanced analysis[14].

2.3. Qualitative screening of phytochemicals

The phytochemical qualitative tests were conducted in accordance with the method described[15].

2.4. Assessment of total phenolic concentration (TPC) and total flavonoid concentration (TFC) in SIEAE

Total phenolic and flavonoid content of SIEAE was measured by using the reported F-C method and aluminum chloride colorimetric method in accordance with a previously reported method with minor adjustments[1].

2.5. Determination of bioactive constituents in SIEAE using high-performance liquid chromatography (HPLC)

The bioactive constituents in SIEAE were identified by the HPLC system. The stationary phase consisted of a C₁₈ column with 5 µm thickness and 250 cm×4.6 mm dimensions. The combination of acetic acid and acetonitrile was employed as a mobile phase with a flow rate set at 1 mL/min and the injected volume was 20 µL for both standard and test samples. The bioactive constituents were detected with a UV-visible detector at 284 nm[16].

2.6. *In vitro* antioxidant and antidiabetic activity

Antioxidant potential of SIEAE was estimated by using a DPPH quenching assay with slight modifications of the previous method[17]. The α-amylase inhibitory assay of SIEAE was carried out by modifying the previously described methodology[16].

2.7. *In vivo* study

2.7.1. Animals

Thirty male albino Wistar rats weighing 150-180 g were maintained

under standard environmental conditions at the departmental animal house of University of Agriculture Faisalabad, Pakistan [(27 ± 2) °C temperature with a 12-h dark/light cycle]. The rats were provided with a regular diet and tap water *ad libitum*. They were allowed to acclimate for one week before the start of the experiment.

2.7.2. Ethical approval

The Institutional Biosafety and Bioethical Committee of UAF (1307/ORIC) authorized the experimental protocols, and all animals were treated and cared for in accordance with the Graduate Studies Research Board, UAF Pakistan's regulations.

2.7.3. Animal grouping and treatment

Thirty rats were divided into five groups ($n=6$ rats/group). Four groups were fed with a 35% HFD (20% proteins, 50% carbohydrates, 30% fats) for two weeks while one group continued to be fed with a standard pellet of regular diet (NC). After two weeks, freshly prepared STZ (45 mg/kg) dissolved in citrate buffer was injected intraperitoneally into these rats. Following three days of injection, rats with fasting blood sugar levels equal to or greater than 250 mg/dL were enrolled in the experimental study. Acute toxicity study was performed to adjust the dose of plant extract and symptoms of toxicity and mortality was observed. Experimental rats were allocated as follows: Group 1 (NC): healthy group rats orally received distilled water; Group 2 (DC): diabetic rats orally received distilled water; Group 3 (PC): diabetic rats orally received metformin (250 mg/kg); Group 4 and 5: diabetic rats orally received SIEAE at 250 mg/kg and 500 mg/kg per day for 21 d.

2.7.4. Assessment of glucose tolerance, body weight, and fasting blood glucose (FBG)

The change in body weight of all experimental animals was recorded using a calibrated weighing balance from 1-6 weeks till the end of the trial. FBG was measured using a glucometer *via* tail prick method at the 1st, 2nd, 3rd, and 4th week of the month trial. After overnight fasting at the last week of dose administration, an aqueous solution of glucose (2 mg/kg) was given by oral gavage, and a glucometer was used to measure the glucose concentration in blood at 0, 30, 60, 90, and 120 min for the assessment of oral glucose tolerance test[18].

2.7.5. Sample collection

The experimental animals rats were euthanized by exposure to chloroform. The blood samples were collected by cardiac punctures using a sterile syringe in dry tubes and serum was separated by centrifugation at 3000 rpm for 10 min and stored for further experimental studies. For the investigation of antioxidant enzymes, a part of liver tissue was homogenized using a homogenizer in 2 mL/g tissue of cold phosphate-buffered saline for at least 5 min. After centrifuging the homogenate for 15 min at 4000 revolutions per minute and 4 °C, the supernatant was stored at –80 °C.

2.7.6. In vivo antidiabetic activity and insulin resistance

The serum glucose was determined enzymatically with the use of commercially available kits (Glu1473-3, Bio Spain), and serum insulin was assessed by rat insulin Elisa kit (Ins5628, Calbiotect, USA) using an ELISA reader-plate. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to assess insulin resistance and calculated using the formula: Fasting insulin ($\mu\text{U/mL}$) $\times$ fasting glucose (mmol/L)/22.5[19].

2.7.7. Estimation of markers of oxidative stress

Major markers of oxidative stress which include malondialdehyde (MDA), and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were estimated in liver tissue homogenate by respective assay kits in compliance with the company's directions.

2.7.8. Determination of pro-inflammatory cytokines in hepatic tissue homogenate

Sandwich ELISA kits were used to measure the amounts of pro-inflammatory cytokines IL-6 and tumor necrosis factor- α (TNF- α) in liver tissue homogenate: Catalog No. E0764Ra and E0119Ra, respectively sourced from BT-Lab, bioassay technology laboratory, China, per directions of manufacturer.

2.7.9. Quantitative RT-PCR analyses

Total ribonucleic acid (RNA) was extracted from pancreatic tissues using TRIzol reagent and a nanodrop model was used to quantify the extracted RNA. Reverse transcriptase was used to change the extracted mRNA to cDNA. Maixma SYBER Green/ROX qPCR Master Mix (2 $\times$) was used for qRT-PCR on iQ5 Bio-Rad system. For the amplification of qRT-PCR, denaturation of template was done at 95 °C for 15 s. The annealing step was done at 58 °C for 30 s, and extension time was 20 s at 72 °C. Finally, mRNA expression was calculated by $2^{-\Delta\Delta C_t}$ method, and primers for *Pdx-1*, *Ins-1*, *Ins-2*, and β -*actin* (housekeeping gene) genes were used in compliance with manufacturer's instructions (Supplementary Table 1).

2.8. Statistical analysis

The statistical analysis of experimental data was accomplished using GraphPad Prism (Version 8.0.1). All data was presented as means $\pm$ standard error (mean $\pm$ SE) for animals in each group. One-way Analysis of Variance was used to analyze data followed by *post hoc* test and results were considered significant at $P<0.05$.

3. Results

3.1. Phytochemical screening

The results of phytochemical screening of SIEAE revealed the

presence of phenols, flavonoids, glycosides, sterols, tannins, alkaloids, terpenoids, and proteins in the extract (Supplementary Table 2).

3.2. TPC and TFC of SIEAE

TPC was (403.61 ± 0.04) mg/g per 100 g extract of *S. imbricata* leaves. In addition, TFC was (13.43 ± 0.02) mg/g per 100 g of *S. imbricata* extract.

3.3. Quantitative phytochemical analysis of SIEAE

HPLC was employed for the analysis of bioactive constituents of plant extract. The SIEAE HPLC chromatogram showed phenol compounds (gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid and salicylic acid) and flavonoid (quercetin) at 280 nm with retention periods ranging from 2.727 to 46.993 min, as shown in Figure 1 (Supplementary Table 3).

3.4. In vitro antioxidant and antidiabetic capacity of SIEAE

SIEAE showed 30.39% inhibition at the maximum concentration of 100 μ g/mL with an IC_{50} value of 11.75 μ g/mL in contrast to standard ascorbic acid (IC_{50} of 2.48 μ g/mL). SIEAE also exhibited significant α -amylase inhibition by 76.72% at 100 μ g/mL with IC_{50} =28.10 μ g/mL in contrast to standard acarbose 91.75% (IC_{50} value of 2.15 μ g/mL) (Supplementary Table 4, Supplementary Figures 1-2).

3.5. Effect of SIEAE on body weight

Body weight was measured weekly (Table 1). The weight of diabetic rats was significantly ($P < 0.05$) increased from 1 to 4 weeks after consumption of HFD compared with the NC group. However, the body weight was lower in the diabetic group than in the NC group in the 5th and 6th week. The body weights of the PC, SIEAE 250 mg/kg, and SIEAE 500 mg/kg groups were significantly ($P < 0.05$) greater than that of the diabetic group from the 3rd week.

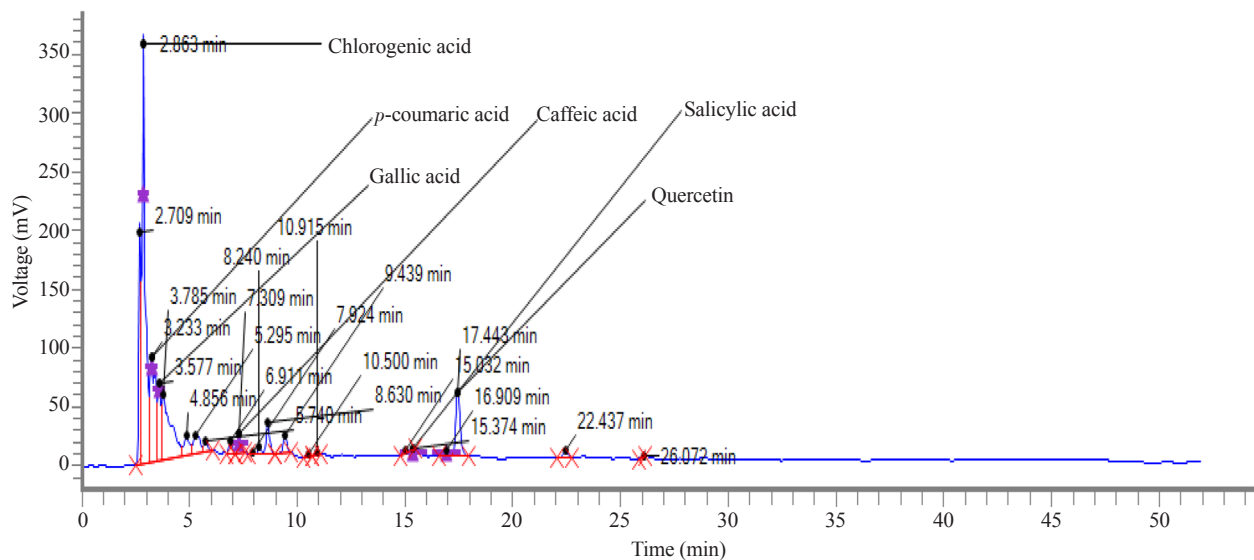


Figure 1. HPLC chromatogram of ethyl acetate extract of *Salsola imbricata* (SIEAE). Stationary phase: C_{18} column. Mobile phase: acetic acid-acetonitrile. HPLC system: Shimadzu Scientific instruments, Japan.

Table 1. Impact of SIEAE administration on body weight in diabetic rats (g).

Weeks	NC	DC	PC	SIEAE 250 mg/kg	SIEAE 500 mg/kg
1	173.06 $\pm$ 1.89	218.38 $\pm$ 0.63 ^a	216.86 $\pm$ 1.39 ^b	217.46 $\pm$ 0.58 ^b	217.20 $\pm$ 0.40 ^b
2	178.85 $\pm$ 2.24	285.41 $\pm$ 1.80 ^a	279.16 $\pm$ 3.21 ^b	283.53 $\pm$ 1.29 ^b	281.10 $\pm$ 1.90 ^b
3	184.91 $\pm$ 2.24	249.05 $\pm$ 1.36 ^a	255.00 $\pm$ 0.81 ^b	266.85 $\pm$ 1.25 ^b	262.50 $\pm$ 1.10 ^b
4	192.00 $\pm$ 2.23	200.16 $\pm$ 0.63 ^a	236.45 $\pm$ 1.31 ^b	255.01 $\pm$ 1.38 ^b	243.50 $\pm$ 1.10 ^b
5	198.83 $\pm$ 2.19	169.96 $\pm$ 1.73 ^a	222.90 $\pm$ 1.64 ^b	243.26 $\pm$ 0.99 ^b	230.90 $\pm$ 0.30 ^b
6	206.66 $\pm$ 2.17	145.60 $\pm$ 1.78 ^a	209.50 $\pm$ 1.87 ^b	231.31 $\pm$ 0.77 ^b	214.10 $\pm$ 0.90 ^b

^a: $P < 0.05$ versus the NC group; ^b: $P < 0.05$ versus the diabetic group; values are shown as mean $\pm$ standard error. NC, DC, PC, and SIEAE represent the normal control, diabetic control, positive control, and *Salsola imbricata* ethyl acetate extract, respectively.

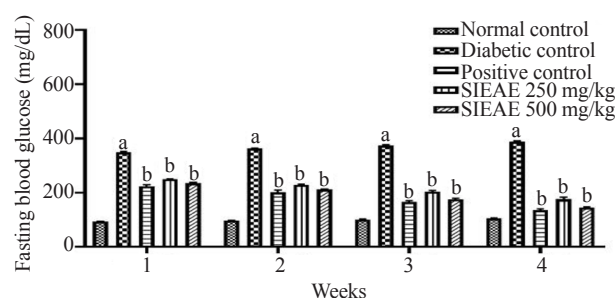


Figure 2. Effect of SIEAE on fasting blood glucose. Values are displayed as mean $\pm$ standard error. ^a: $P<0.05$ versus the NC group; ^b: $P<0.05$ versus the diabetic group.

3.6. Effect of SIEAE on FBG

The results of FBG indicated that diabetic rats had significantly higher FBG levels compared to the normal group ($P<0.05$). Standard medication metformin and SIEAE (250 and 500 mg/kg) could significantly decrease blood glucose levels ($P<0.05$) (Figure 2).

3.7. Effect of SIEAE on oral glucose tolerance test

Blood glucose levels substantially reached maximal values within 30 min in all groups. The glucose tolerance in the diabetic group was significantly ($P<0.05$) impaired compared to the normal group. Rats in the PC and SIEAE (250 and 500 mg/kg) group showed a remarkable glucose tolerance at 60, 90, and 120 min (Figure 3).

3.8. Effect of SIEAE on diabetic serum markers

Figure 4 demonstrates the impact of SIEAE on serum insulin and HOMA-IR. Results showed a significant decline in serum insulin in the diabetic control group. The administration of SIEAE (250 and 500 mg/kg) and metformin (250 mg/kg) significantly raised insulin levels ($P<0.05$), and also improved insulin sensitivity by significantly lowering the HOMA-IR index ($P<0.05$).

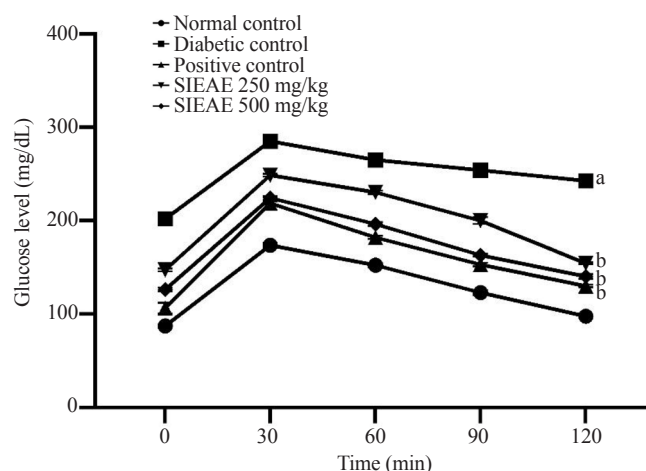


Figure 3. Effect of SIEAE on oral glucose tolerance test in Wistar rats. Values are displayed as mean $\pm$ standard error. ^a: $P<0.05$ versus the NC group; ^b: $P<0.05$ versus the diabetic group.

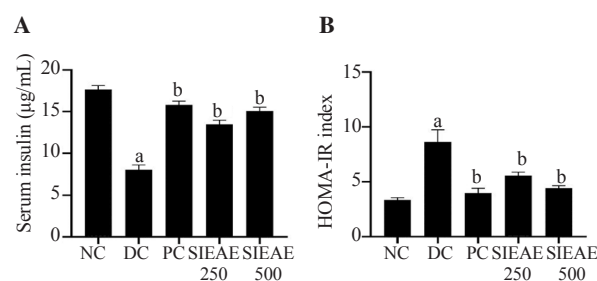


Figure 4. Impact of SIEAE on A) serum insulin and B) homeostasis model assessment of insulin resistance (HOMA-IR). Values are displayed as mean $\pm$ standard error. ^a: $P<0.05$ versus the NC group; ^b: $P<0.05$ versus the diabetic group.

3.9. Attenuation of oxidative stress markers by SIEAE

A marked increase in MDA level was observed in diabetic rats with noticeably decreased levels of SOD, CAT, and GPx. However, SIEAE (250 mg/kg and 500 mg/kg) significantly decreased MDA level and elevated SOD, CAT, and GPx ($P<0.05$) (Table 2).

3.10. Effect of SIEAE on hepatic inflammatory markers

The hepatic tissue of the diabetic control group showed significantly ($P<0.05$) elevated levels of TNF- α and IL-6. SIEAE (250 mg/kg and 500 mg/kg) decreased levels of TNF- α and IL-6 ($P<0.05$) (Figure 5).

Table 2. Attenuation effect of SIEAE on oxidative stress.

Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (nmol/mg protein)
NC	6.92 $\pm$ 0.17	49.45 $\pm$ 0.60	67.82 $\pm$ 1.00	27.70 $\pm$ 0.29
DC	17.85 $\pm$ 0.65 ^a	17.02 $\pm$ 0.46 ^a	19.35 $\pm$ 0.48 ^a	12.91 $\pm$ 0.85 ^a
PC	7.68 $\pm$ 0.19 ^b	45.01 $\pm$ 0.46 ^b	58.86 $\pm$ 0.76 ^b	26.32 $\pm$ 0.37 ^b
SIEAE (250 mg/kg)	9.92 $\pm$ 0.26 ^b	40.01 $\pm$ 0.39 ^b	52.08 $\pm$ 0.44 ^b	23.12 $\pm$ 0.26 ^b
SIEAE (500 mg/kg)	8.28 $\pm$ 0.27 ^b	42.94 $\pm$ 0.37 ^b	55.02 $\pm$ 0.48 ^b	25.51 $\pm$ 0.47 ^b

Values are displayed as mean $\pm$ standard error. ^a: $P<0.05$ versus the NC group; ^b: $P<0.05$ versus the diabetic group. MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, and GPx: glutathione peroxidase.

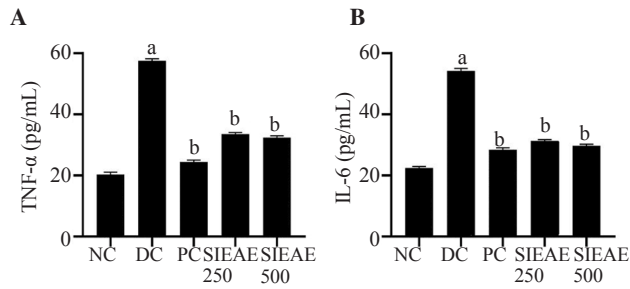


Figure 5. Effect of SIEAE on pro-inflammatory cytokines. A) tumor necrosis factor- α (TNF- α), B) IL-6. Values are displayed as mean $\pm$ standard error. ^a: $P < 0.05$ versus the NC group; ^b: $P < 0.05$ versus the diabetic group.

3.11. Effect of SIEAE on expression of insulin-promoting genes

The results demonstrated that expressions of *Pdx-1*, *Ins-1*, and *Ins-2* were significantly decreased in diabetic rats. SIEAE (250 mg/kg and 500 mg/kg) significantly upregulated *Pdx-1*, *Ins-1*, and *Ins-2* levels (Figure 6).

4. Discussion

Metabolic disorders impact the capacity of the body to break down and distribute macronutrients such as proteins, fats, and carbohydrates[20]. Diabetes is considered a worldwide health issue due to its enormous incidence and ongoing problems[1]. Type II diabetes is closely associated with obesity and insulin resistance as well as oxidative stress and chronic inflammation. Inflammation is a well-acknowledged underlying cause of insulin resistance.

Many scientists are searching for a relatively safe and cost-effective remedy that is equally nontoxic, and relatively long-lasting. Due to the presence of polyphenols and flavonoids, *S. imbricata* is known for its use in folk remedies[21]. In the current investigation, STZ injection combined with HFD was used to establish an experimental

model of DM, and the antidiabetic potential of SIEAE was evaluated.

Phytochemical analysis is essential to assess the possible medical efficacy of herbs and plants[22,23]. *In vitro* phytochemical results showed the presence of alkaloids, flavonoids, phenols, cardioactive glycosides, tannins, terpenoids, gums and mucilage in SIEAE. These phytochemicals have antidiabetic potential, especially alkaloids and flavonoids have the potency to regenerate the damaged β -cells[24].

According to HPLC analysis, quercetin, gallic acid, caffeic acid, chlorogenic acid, salicylic acid and *p*-coumaric acid were found in SIEAE. These metabolites are well known to boost insulin sensitivity, modulate glucose release from the liver, inhibit the digestion and absorption of carbohydrates, and improve lipid profiles to decrease the incidence of metabolic disorders[25,26]. Quercetin, gallic acid, and caffeic acid found in extracts have been reported to have antidiabetic activity by lowering blood sugar, enhancing glucose uptake, and stimulating glucose metabolism[27,28]. Numerous studies have illuminated that phenolic compounds possess remarkable potential to protect against metabolic disorders as potent antioxidants by preventing pancreatic β -cells from oxidative stress.

Compared to synthetic antioxidants, natural antioxidants are more efficient in inhibiting oxidative stress caused by ROS which has harmful effects on biological systems[29,30]. *In vitro* study showed that SIEAE had significant antioxidant potential by inhibiting DPPH, possibly due to the presence of bioactive antioxidant components that can scavenge the free radicals. This study revealed that SIEAE exhibits high DPPH radical scavenger potential ($IC_{50} = 11.75 \mu\text{g/mL}$) as compared to ascorbic acid ($IC_{50} = 2.48 \mu\text{g/mL}$).

In vitro antidiabetic potential was evaluated by alpha-amylase inhibition assay. It was found that SIEAE exhibited 76.72% inhibition of α -amylase activity at 100 $\mu\text{g/mL}$ compared to acarbose control 91.75%. These findings demonstrated that SIEAE might exhibit antidiabetic effects by inhibiting hydrolyzing enzymes. Similar results were reported during the *in vitro* study of *S. imbricata*[31].

High intake of food and beverages rich in energy, fat, and sugar,

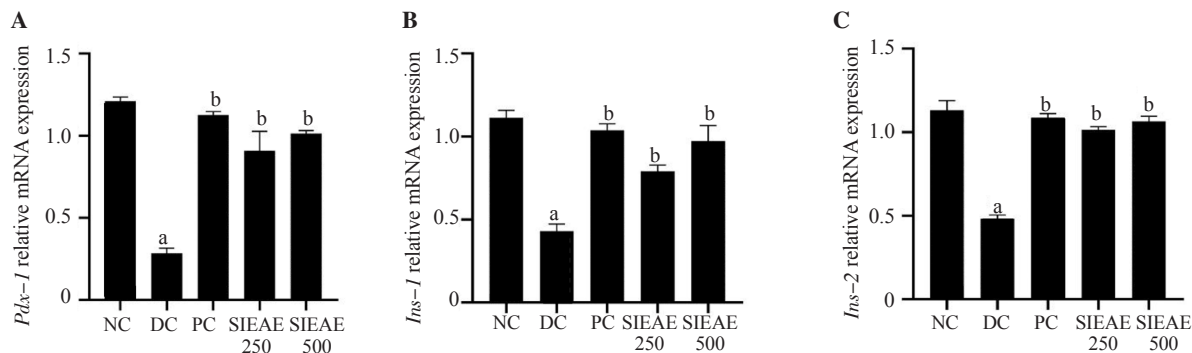


Figure 6. Effect of SIEAE on upregulation of gene expressions of insulin signaling cascade. A: *Pdx-1*; B: *Ins-1*; C: *Ins-2*. Values are displayed as mean $\pm$ standard error. ^a: $P < 0.05$ versus the NC group; ^b: $P < 0.05$ versus the diabetic group.

sedentary lifestyle, genetic predisposition, and perinatal environment have an impact on raising the chances of DM, dyslipidemia, obesity, and other metabolic disorders[32,33]. The etiology of DM is complicated but genetic vulnerability and excessive weight gain have been recognized as a major risk factor for type II diabetes[34]. It has been reported that consumption of HFD for 2-7 weeks in combination with a low dose of STZ can cause insulin resistance[35]. The present study showed that oral administration of SIEAE reversed the change in body weight in diabetic rats. HFD/STZ causes β -cells to be dysfunctional and ultimately impairs insulin secretion and increases blood glucose levels. In addition, the combination of HFD with STZ causes insulin resistance in diabetic rats leading to damage to the downstream hepatic metabolizing process and glucose homeostasis[36]. In the current study, oral administration of SIEAE resulted in a considerable reduction in blood sugar levels under the fasting condition. SIEAE significantly improved serum glucose levels and increased the insulin level in serum.

Diabetes-induced hyperglycemia is an important contributing factor for production of reactive oxygen species resulting in oxidative stress. It is reported that the production of hydroxyl ions was increased due to STZ-induced damage to the liver[4]. The present study showed a significantly elevated hepatic oxidative stress marker MDA and reduced antioxidant markers SOD, CAT as well as GPx. SIEAE (250 mg/kg and 500 mg/kg) significantly elevated hepatic antioxidant levels and reduced MDA levels. The amelioration of oxidative stress may be attributed to the flavonoids *i.e.* quercetin found in plant extract[37].

Diabetes-induced hyperglycemia increases ROS generation, which in turn causes a surge in pro-inflammatory cytokines release; hence, factors associated with inflammation are important in the progression of diabetes-associated complications[38]. Rats in the diabetic control group showed significantly higher hepatic concentrations of pro-inflammatory markers (TNF- α , and IL-6) that is correlated to previous studies[39]. Our findings showed that treatment with SIEAE significantly reduced the concentrations of hepatic pro-inflammatory cytokines (TNF- α and IL-6). It has been reported that increased release of pro-inflammatory cytokines leads to degradation to β -cells associated with insulin resistance[40,41].

PDX-1 is an important transcription factor in maintaining the proliferation and survival of β -cells. It could regulate the expression of insulin genes (*Ins-1* and *Ins-2*) activated by glucose[42]. In the current study, treatment with SIEAE increased the expression level of genes (*Pdx-1*, *Ins-1*, and *Ins-2*), which are implicated in the insulin signal transduction cascade. The upregulation of *Pdx-1*, *Ins-1*, and *Ins-2* expression levels in pancreatic tissue could be attributed to the amelioration of insulin secretion due to the presence of flavonoids and phenolic compounds detected in SIEAE.

This study has some limitations. It relied upon an animal model and might not accurately represent human physiological change. Also, more research on molecular pathways is needed. Due to the short

experiment period, possible long-term effects are still unknown. Variability in the concentrations of bioactive compounds may potentially affect clinical applicability and reproducibility. Therefore, more experiments are required to understand the mode of action of antidiabetic effect of isolated bioactive compounds of *S. imbricata*.

In conclusion, our experiment demonstrated that *S. imbricata* possesses potential antidiabetic activity *via* modulation of insulin resistance, oxidative stress, and inflammatory biomarkers. Flavonoids and phenols found in *S. imbricata* are considered to be responsible for the antidiabetic effect. *S. imbricata* can be used as a suitable therapeutic alternative/nutrition supplement to alleviate disorders associated with DM.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

The authors highly acknowledge the Endowment Fund Secretariat, University of Agriculture, Faisalabad, Pakistan and Institute of Physiology & Pharmacology, University of Agriculture, Faisalabad, Pakistan for providing the technical support and resources.

Funding

The authors received no extramural funding for the study.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Coneptulization and design were performed by IA and WM. Data collection, analysis and interpretation were done by BA and MNF. The first draft was written by IA. The final manuscript were read and approved by IA, WM, BA and MNF.

References

- [1] Giri BR, Baral R, Bhatt H, Khadka A, Tamrakar R, Timalina G, et al. Phytochemical screening, free-radical scavenging activity, *in vitro* alpha-

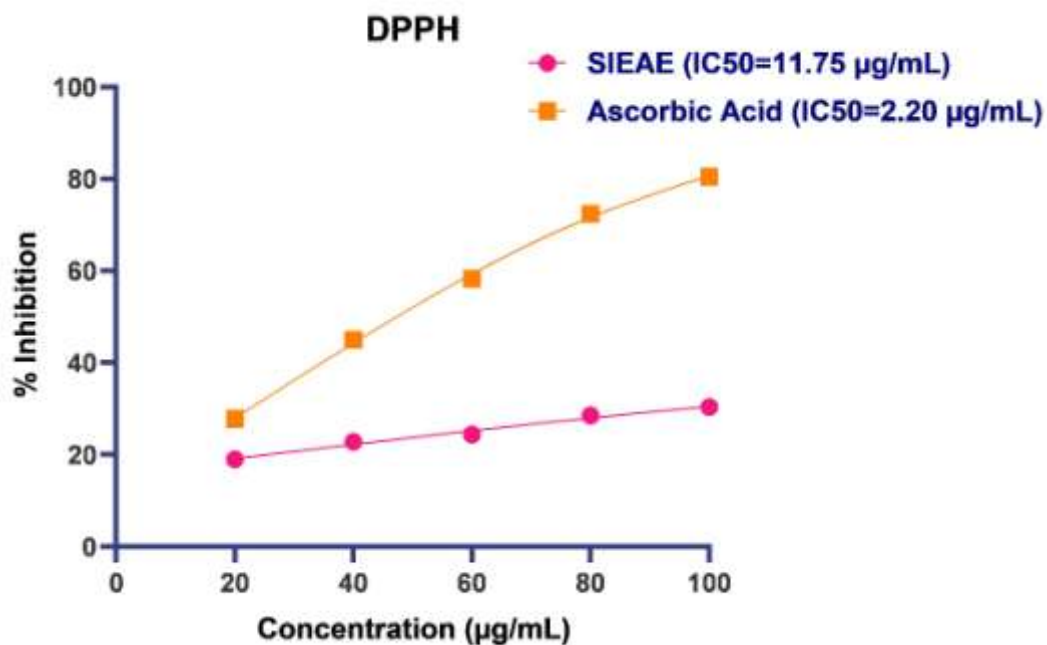
- amylase inhibitory activity, and *in vivo* hypoglycemic activity studies of several crude drug formulations based on selected medicinal plants of Nepal. *Pharm Chem J* 2023; **56**(10): 1369-1378. doi: 10.1007/s11094-023-02799-z.
- [2] Abdou HM, Hamaad FA, Ali EY, Ghoneum MH. Antidiabetic efficacy of *Trifolium alexandrinum* extracts hesperetin and quercetin in ameliorating carbohydrate metabolism and activating IR and AMPK signaling in the pancreatic tissues of diabetic rats. *Biomed Pharmacol* 2022; **149**. doi: 10.1016/j.biopha.2022.112838.
- [3] Amare YE, Dires K, Asfaw T. Antidiabetic activity of mung bean or *Vigna radiata* (L.) wilczek seeds in alloxan-induced diabetic mice. *Evid Based Complement Altern Med* 2022; **2022**. doi: 10.1155/2022/6990263.
- [4] Yari beygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxid Med Cell Longev* 2020; **1**(2020). doi: 10.1155/2020/8609213.
- [5] Shah AS, Zeitler PS, Wong J, Pena AS, Wicklow B, Arslanian S, et al. ISPAD clinical practice consensus guidelines: Type 2 diabetes in children and adolescents. *Pediatr Diabetes* 2022; **23**: 872-902. doi: 10.1111/pedi.13409.
- [6] Matthews J, Villegas S, Herat L, Schlaich M, Matthews V. Implications of ADAM17 activation for hyperglycaemia, obesity and type 2 diabetes. *Biosci Rep* 2021; **41**. doi: 10.1042/BSR20210029.
- [7] Sharma P, Hajam YA, Kumar R, Rai S. Complementary and alternative medicine for the treatment of diabetes and associated complications: A review on therapeutic role of polyphenols. *Phytomed Plus* 2022; **2**. doi: 10.1016/j.phyplu.2021.100188.
- [8] Shurrah NT, Arafa ESA. Metformin: A review of its therapeutic efficacy and adverse effects. *Obes Med* 2020; **17**. doi: 10.1016/j.obmed.2020.100186.
- [9] Ardakanian A, Rahbardi MG, Omidkhoda F, Razavi BM, Hosseinzade H. Effect of alpha-mangostin on olanzapine-induced metabolic disorders in rats. *Iran J Basic Med Sci* 2022; **25**: 198. doi: 10.22038/IJBMS.2022.58734.13047.
- [10] Sivakumar T, Deepa B. A critical review on antidiabetic potential of herbal plants and its their bioactive components. *J Uni Shanghai Sci Technol* 2023; **25**: 303-314.
- [11] Ullah M, Mehmood S, Khan RA, Ali M, Fozia F, Waqas M, et al. Assessment of antidiabetic potential and phytochemical profiling of *Viscum album*, a traditional antidiabetic plant. *J Food Qual* 2022; **2022**(1). doi: 10.1155/2022/5691379.
- [12] Ajaib M, Shah S. Chemical characterization and pharmacology of *Salsola imbricata* forssk. 1st ed. In: *Bioactives and pharmacology of medicinal plants*. New York: Apple Academic Press; 2022, p. 323-331.
- [13] Amer HA, Elalem SF, Al-Askar AA, Sharaf OA, Gaber MA, Kowalczewski P, et al. Antioxidant and antimicrobial activities of *Salsola imbricata* methanolic extract and its phytochemical characterization. *Open Life Sci* 2024; **19**(1): 20221011.
- [14] Islam MS, Sana S, Haque ME, Rahman SM, Samad A, Al Noman A, et al. Methanol, ethyl acetate and n-hexane extracts of *Tragiain volucrata* L. leaves exhibit anxiolytic, sedative and analgesic activity in Swiss albino mice. *Heliyon* 2021; **7**(1). doi: 10.1016/j.heliyon.2020.e05814.
- [15] Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. *Int J Chem Stud* 2020; **8**(2): 603-608. doi: 10.22271/chemi.2020.v8.i2i.8834.
- [16] Rawal P, Pandey B, Yadav RK, Panta S. Antioxidant, alpha-amylase inhibitory and hypoglycemic activity of *Smallanthus sonchifolius* leaves from Nepal: An integrated *in vitro*, *in vivo*, and *in silico* approach. *Food Sci Nutr* 2025; **13**(1): e4672.
- [17] Mogole L, Omwoyo W, Mtunzi F. Phytochemical screening, antioxidant activity and α -amylase inhibition study using different extracts of loquat (*Eriobotrya japonica*) leaves. *Heliyon* 2020; **6**(8). doi: 10.1016/j.heliyon.2020.e04736.
- [18] Sun K, Ding M, Fu C, Li P, Li T, Fang L, et al. Effects of dietary wild bitter melon (*Momordica charantia* var. abbreviate Ser.) extract on glucose and lipid metabolism in HFD/STZ-induced type 2 diabetic rats. *J Ethno pharmacol* 2023; **306**. doi: 10.1016/j.jep.2023.116154.
- [19] Abdelghffar EA, Mostafa NM, El-Nashar HA, Eldahshan OA, Singab AN. Chilean pepper (*Schinus polygamus*) ameliorates the adverse effects of hyperglycaemia/dyslipidaemia in high fat diet/streptozotocin-induced type 2 diabetic rat model. *Ind Crops Prod* 2022; **183**. doi: 10.1016/j.indcrop.2022.114953.
- [20] El-Dawy K, Saad S, Hussein MM, Yahia R, Al-Gamal M. Naturally based nano formulation in metabolic and reproductive disorders: A review. *Int J Vet Sci* 2023; **12**: 7-17. doi: 10.47278/journal.ijvs/2022.142.
- [21] Radjai I, Chakali G. Extraction and identification of some metabolites from three Algerian Sahara medicinal plants. *Ukr J Ecol* 2022; **12**: 57-65. doi: 10.15421/2022_354.
- [22] Khalid W, Maqbool Z, Arshad MS, Kousar S, Akram R, Siddeeq A, et al. Plant-derived functional components: Prevent from various disorders by regulating the endocrine glands. *Int J Food Prop* 2022; **25**: 976-995. doi: 10.1080/10942912.2022.2070643.
- [23] Alawfi BS. A review on the use of phytochemicals for the control of zoonotic Giardiasis. *Pak Vet J* 2024; **44**(3): 592-598. doi: 10.29261/pakvetj/2024.251.
- [24] Rahmawati N, Kusmiyati DK, Afifah DN. Antioxidant total and HOMA-IR of diabetic rats given *Crocotum piper* and *Andrographis paniculata* leaf extracts. *J Biomed Transl Res* 2021; **7**: 56-61. doi: 10.14710/jbtr.v7i2.11524.
- [25] Elbakry M, Elremaly W. Antidiabetic activity of some common medicinal plants. *Biol Biomed J* 2023; **1**: 1-16. doi: 10.21608/bbj.2023.321880.
- [26] Gupta A, Jamal A, Jamil DA, Al-Aubaidy HA. A systematic review exploring the mechanisms by which citrus bioflavonoid supplementation benefits blood glucose levels and metabolic complications in type 2 diabetes mellitus. *Diabetes Metab Syndr Clin Res Rev* 2023; **17**(11). doi: 10.1016/j.dsx.2023.102884.
- [27] Shreelakshmi SV, Chaitrashree N, Nazareth MS, Kumar SS, Shetty NP, Giridhar P. Bioactive compounds and antioxidant activity during ripening of *Malpighia glabra* fruits. *J Food Sci Technol* 2024; **61**: 717-726. doi: 10.1007/s13197-023-05872-3.
- [28] Kalai FZ, Boulaaba M, Ferdousi F, Isoda H. Effects of isorhamnetin on

- diabetes and its associated complications: A review of *in vitro* and *in vivo* studies and a post hoc transcriptome analysis of involved molecular pathways. *Int J Mol Sci* 2022; **23**. doi: 10.3390/ijms23020704.
- [29] Celep NA, Erbaş E, Kara A, Kara H. Silymarin reduces paclitaxel-induced lung damage *via* down-regulating P2X7R expression and inhibiting inflammation and apoptosis in the rats. *Pak Vet J* 2024; **44**(1): 169-175. doi: 10.29261/pakvetj/2024.129.
- [30] Pammi SS, Suresh B, Giri A. Antioxidant potential of medicinal plants. *J Crop Sci Biotech* 2023; **26**: 13-26. doi: 10.1007/s12892-022-00159-z.
- [31] Al-Omar MS, Mohammed HA, Mohammed SA, Abd-Elmoniem E, Kandil YI, Eldeeb HM, et al. Anti-microbial, anti-oxidant, and α -amylase inhibitory activity of traditionally-used medicinal herbs: A comparative analyses of pharmacology, and phytoconstituents of regional halophytic plants' diaspora. *Molecules* 2020; **25**. doi: 10.3390/molecules25225457.
- [32] Abdulllah AM, Ahmed AE, Bajaber MA, Alalwiat AA. Evaluation of the antidiabetic effects of methanolic extracts of Neem (*Azadirachta indica*) seeds on the streptozotocin-induced Wistar rats. *Pak Vet J* 2023; **43**: 792-798. doi: 10.29261/pakvetj/2023.106.
- [33] Kumari D, Gautam J, Sharma V, Gupta SK, Sarkar S, Jana P, et al. Effect of herbal extracts and Saroglitzar on high-fat diet-induced obesity, insulin resistance, dyslipidemia, and hepatic lipidome in C57BL/6J mice. *Heliyon* 2023; **9**(11). doi: 10.1016/j.heliyon.2023.e22051.
- [34] Prasad M, Rajagopal P, Devarajan N, Veeraraghavan VP, Palanisamy CP, Cui B, et al. Comprehensive review on high-fat diet-induced diabetes mellitus: An epigenetic view. *J Nutr Biochem* 2022; **107**. doi: 10.1016/j.jnutbio.2022.109037.
- [35] Vatandoust N, Rami F, Salehi AR, Khosravi S, Dashti G, Eslami G, et al. Novel high-fat diet formulation and streptozotocin treatment for induction of prediabetes and type 2 diabetes in rats. *Adv Biomed Res* 2018; **7**. doi: 10.4103/abr.abr_8_17.
- [36] Zhang Y, Li L, Chai T, Xu H, Du Y, Jiang HY. Mulberry leaf multi-components exert hypoglycemic effects through regulation of the PI-3K/Akt insulin signaling pathway in type 2 diabetic rats. *J Ethnopharmacol* 2024; **319**. doi: 10.1016/j.jep.2023.117307.
- [37] Jin Y, Arroo R. The protective effects of flavonoids and carotenoids against diabetic complications—A review of *in vivo* evidence. *Front Nutr* 2023; **10**. doi: 10.3389/fnut.2023.1020950.
- [38] Chen Z, Tian R, She Z, Cai J, Li H. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radic Biol Med* 2020; **152**: 116-141. doi: 10.1016/j.freeradbiomed.2020.02.025.
- [39] Hussein JS, Medhat D, Abdel-Latif Y, Morsy S, Gaafar AA, Ibrahim EA, et al. Amelioration of neurotoxicity induced by esfenvalerate: Impact of *Cyperus rotundus* L. tuber extract. *Comp Clin Path* 2021; **30**: 1-10. doi: 10.1007/s00580-020-03182-0.
- [40] Tarfeen N, Nisa KU, Ahmad MB, Waza AA, Ganai BA. Metabolic and genetic association of vitamin D with calcium signaling and insulin resistance. *Indian J Clin Biochem* 2023; **38**: 407-417. doi: 10.1007/s12291-022-01105-0.
- [41] Ishtiaq SM, Khan JA, Muhammad F, Shahid M. Peroxisome proliferator-activated receptor gamma agonists modulate high-fat diet-and carbon tetrachloride-induced non-alcoholic fatty liver disease pathophysiology and transcriptional expression of inflammatory markers in a murine model. *Pak Vet J* 2022; **42**: 292-299. doi: 10.29261/pakvetj/2022.017.
- [42] Hassan F, Aslam B, Muhammad F, Faisal MN. Hypoglycemic properties of *Sphaeranthus indicus* and *Nigella sativa* in alloxan induced diabetes mellitus in rats; a new therapeutic horizon. *Pak Vet J* 2022; **42**: 141-146. doi: 10.29261/pakvetj/2022.011.

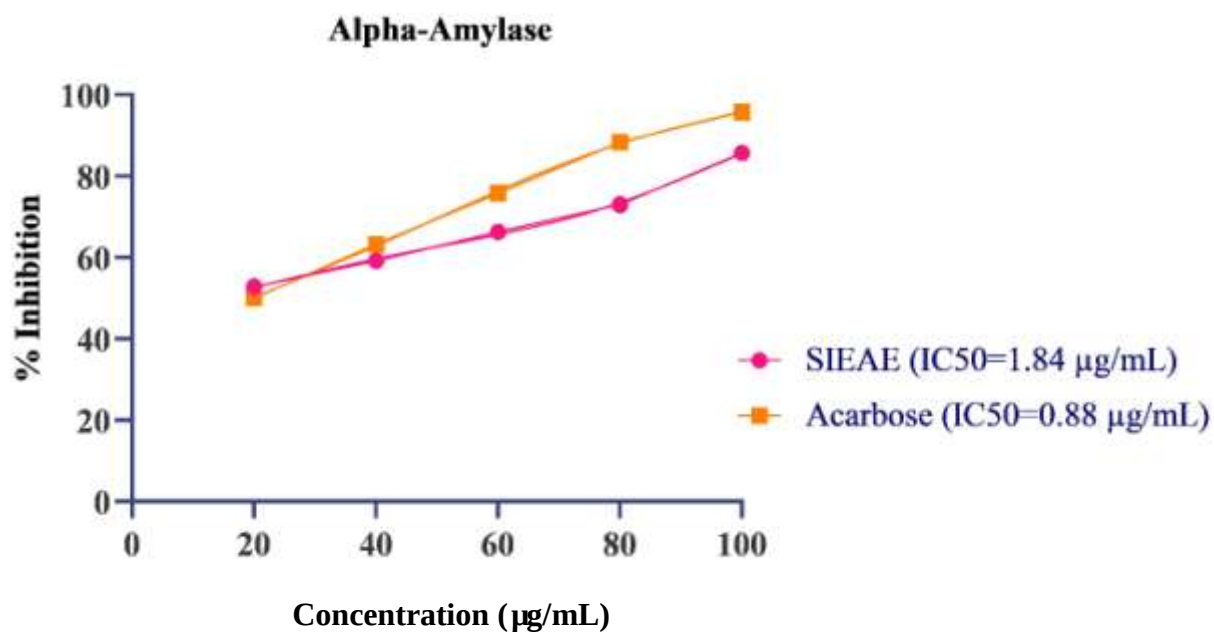
Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Tan BJ, Liang Q



Supplementary Figure 1. DPPH radical scavenging activity of *Salsola imbricata* ethyl acetate extract.



Supplementary Figure 2. Alpha-amylase inhibitory activity of *Salsola imbricata* ethyl acetate extract

Supplementary Table 1. Primer pairs for quantitative RT-PCR.

Genes	Sense (5'→3')	Antisense (5'→3')
<i>Pdx-1</i>	TCCCGAATGGAACCGAGACT	TTCATCCACGGGAAAGGGAG
<i>Ins-1</i>	AGGCTCTGTACCTGGTGTGTG	CGGGTCCTCCACTTCACGAC
<i>Ins-2</i>	GGAGCGTGGATTCTTCTACACA	AGTGCCAAGGTCTGAAGGTCAC
<i>β-actin</i>	CGAGTACAACCTTCTTGCAGC	TATCGTCATCCATGGCGAACTG

Supplementary Table 2. Qualitative phytochemical constituents of ethyl acetate extract of *S. imbricata* (SIEAE).

Phytochemicals	SIEAE
Phenols	+
Flavonoids	+
Cardioactive glycosides	+
Steroids	+
Quinones	-
Tannins	+
Saponins	-
Terpenoids	+
Alkaloids	+
Gums and mucilage	+
Proteins	+

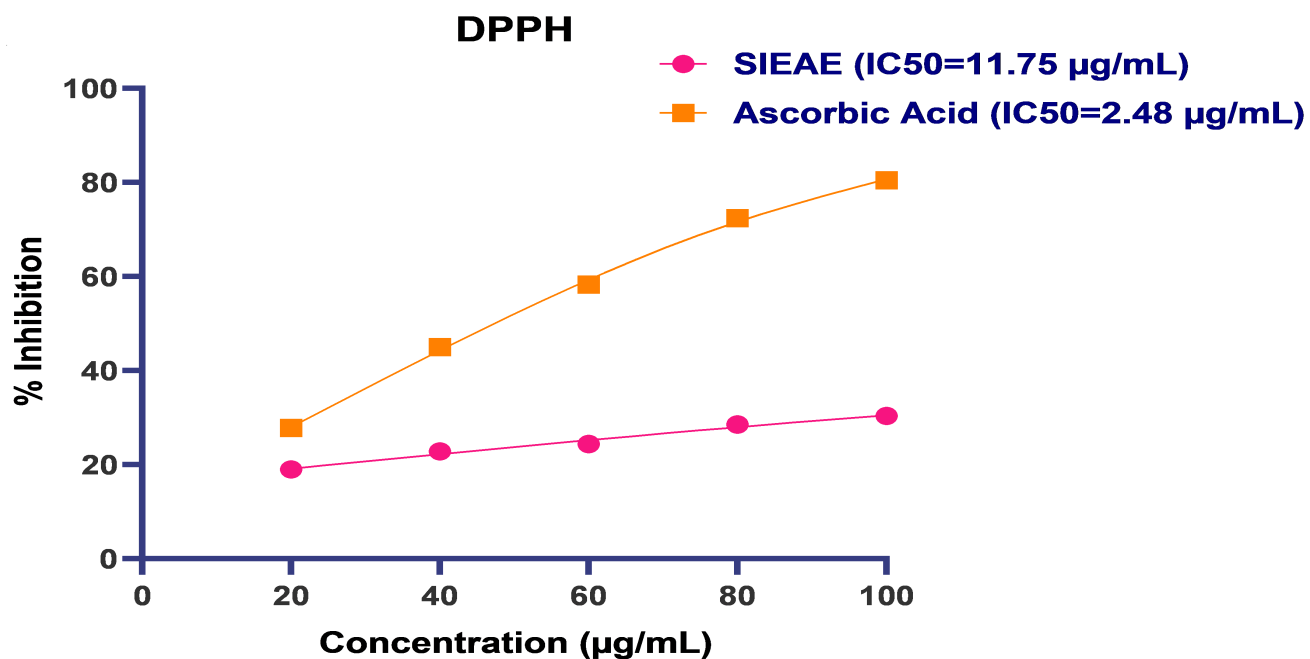
+ = Present, - = Absence.

Supplementary Table 3. Identified phytoconstituents of SIEAE at 280 nm by HPLC.

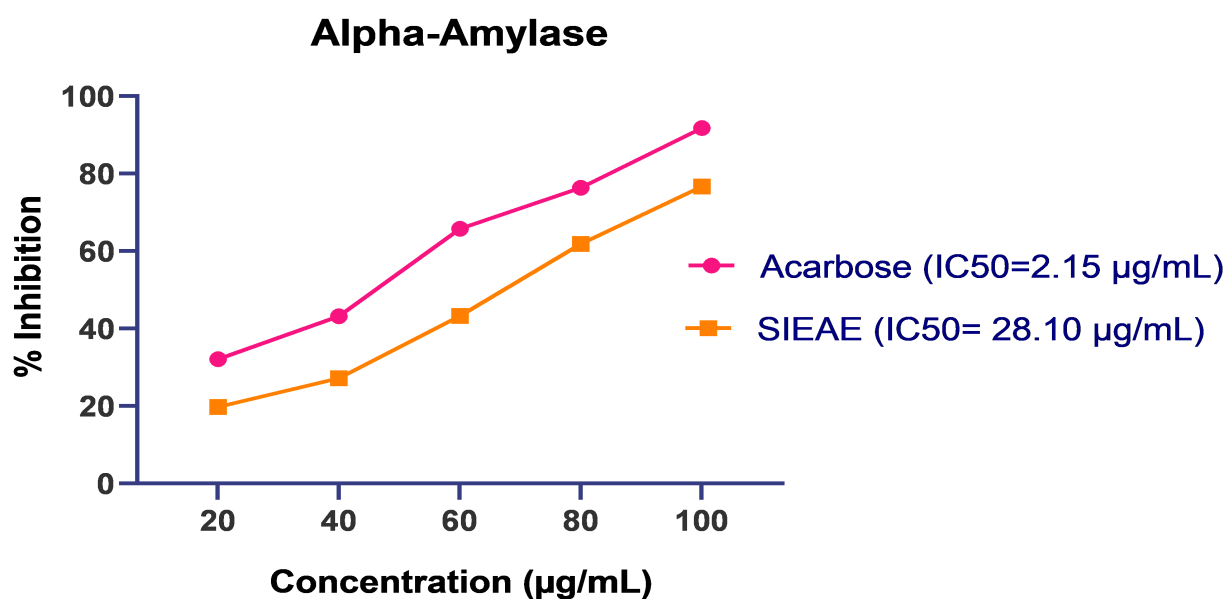
Identified constituents	Retention time (min)	Relative area (%)
Chlorogenic acid	2.863	3.00
<i>P</i> -Coumaric acid	3.233	10.12
Gallic acid	3.577	5.86
Caffeic acid	7.039	1.54
Salicylic acid	15.374	0.26
Quercetin	17.443	13.59

Supplementary Table 4. Inhibitory percentage of DPPH and α -amylase analysis of SIEAE.

Concentrations(μ g/mL)	Ascorbic acid	SIEAE	Acarbose	SIEAE
20	27.81 $\pm$ 0.01	19.06 $\pm$ 0.05	32.13 $\pm$ 0.02	19.82 $\pm$ 0.02
40	43.01 $\pm$ 0.01	22.86 $\pm$ 0.02	43.24 $\pm$ 0.03	27.22 $\pm$ 0.01
60	61.28 $\pm$ 0.01	24.35 $\pm$ 0.03	65.83 $\pm$ 0.02	43.27 $\pm$ 0.02
80	72.41 $\pm$ 0.02	28.55 $\pm$ 0.01	76.39 $\pm$ 0.01	61.86 $\pm$ 0.04
100	80.52 $\pm$ 0.02	30.39 $\pm$ 0.01	91.75 $\pm$ 0.03	76.72 $\pm$ 0.01
IC₅₀	2.48	11.75	2.15	28.10



Supplementary Figure 1. DPPH radical scavenging activity of *Salsola imbricata* ethyl acetate extract.



Supplementary Figure 2. Alpha-amylase inhibitory activity of *Salsola imbricata* ethyl acetate extract

Supplementary Table 1. Primer pairs for quantitative RT-PCR.

Genes	Sense (5'→3')	Antisense (5'→3')
<i>Pdx-1</i>	TCCCGAATGGAACCGAGACT	TTCATCCACGGGAAAGGGAG
<i>Ins-1</i>	AGGCTCTGTACCTGGTGTGTG	CGGGTCCTCCACTTCACGAC
<i>Ins-2</i>	GGAGCGTGGATTCTTCTACACA	AGTGCCAAGGTCTGAAGGTCAC
<i>β-actin</i>	CGAGTACAACCTTCTTGCAGC	TATCGTCATCCATGGCGAACTG

Supplementary Table 2. Qualitative phytochemical constituents of ethyl acetate extract of *S. imbricata* (SIEAE).

Phytochemicals	SIEAE
Phenols	+
Flavonoids	+
Cardioactive glycosides	+
Steroids	+
Quinones	-
Tannins	+
Saponins	-
Terpenoids	+
Alkaloids	+
Gums and mucilage	+
Proteins	+

+ = Present, - = Absence.

Supplementary Table 3. Identified phytoconstituents of SIEAE at 280 nm by HPLC.

Identified constituents	Retention time (min)	Relative area (%)
Chlorogenic acid	2.863	3.00
<i>P</i> -Coumaric acid	3.233	10.12
Gallic acid	3.577	5.86
Caffeic acid	7.039	1.54
Salicylic acid	15.374	0.26
Quercetin	17.443	13.59

Supplementary Table 4. Inhibitory percentage of DPPH and α -amylase analysis of SIEAE.

Concentrations(μ g/mL)	Ascorbic acid	SIEAE	Acarbose	SIEAE
20	27.81 $\pm$ 0.01	19.06 $\pm$ 0.05	32.13 $\pm$ 0.02	19.82 $\pm$ 0.02
40	43.01 $\pm$ 0.01	22.86 $\pm$ 0.02	43.24 $\pm$ 0.03	27.22 $\pm$ 0.01
60	61.28 $\pm$ 0.01	24.35 $\pm$ 0.03	65.83 $\pm$ 0.02	43.27 $\pm$ 0.02
80	72.41 $\pm$ 0.02	28.55 $\pm$ 0.01	76.39 $\pm$ 0.01	61.86 $\pm$ 0.04
100	80.52 $\pm$ 0.02	30.39 $\pm$ 0.01	91.75 $\pm$ 0.03	76.72 $\pm$ 0.01
IC₅₀	2.48	11.75	2.15	28.10