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Exploring the role of *Acacia jacquemontii* in mitigating pancreatic β-cell dysfunction via upregulation of Pdx-1 expression

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Abstract: **Background**: The plant *Acacia jacquemontii* is recognized for its traditional medical history, containing many phytochemicals and is used effectively for different ailments. **Objectives**: *Acacia jacquemontii* (A. Jacq.) ethyl acetate extract in the improvement of pancreatic β-cell functioning via upregulation of Pdx-1 was investigated. **Methods**: To evaluate the *in-vitro* antioxidant activity of A. Jacq. hydroxyl (OH) radical scavenging activity and ferric reducing antioxidant power assay (FRAP) were performed. Hyaluronic acid breaking and Lipoxygenase (LOX) enzyme inhibitory activities were measured to determine the in-vitro anti-inflammatory activity. Inhibitory activities against GLP-I cleaving enzyme (DPP-IV) and acid maltase (Alpha-glucosidase) enzymes were evaluated. Pancreatic β-cell functioning was measured by checking levels of HOMA-β, C-peptide, expression of Pdx-1 and insulin genes in the Wistar male rat model. **Results**: Findings showed A. Jacq. possesses OH (52.6 %) and FRAP radical scavenging activity. Additionally, A. Jacq. ethyl acetate extract showed significant in-vitro anti-inflammatory and hypoglycemic effect through inhibition of hyaluronic acid breaking ($p < 0.01$) and LOX ($p < 0.01$) enzymes, GLP-I cleaving (DPP-IV) and acid maltase enzymes. Significant improvement in gluconeogenic status and enhancement in pancreatic β-cell functioning through improvement in HOMA-β, C-peptide and expression of Pdx-1 and insulin genes were observed. **Conclusion**: A. Jacq. ethyl acetate extract showed significant *in-vitro* and *in-vivo* biological activities that indicates its pharmacological importance.

Keywords: Anti-inflammatory; Acid maltase; GLP-I; Hyaluronic acid; Lipoxygenase

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INTRODUCTION

Plant *Acacia jacquemontii* (A. Jacq.) is a xerophytic shrub or small tree that contains brown, smooth, zig-zag branches. Plant also familiar as chota babool, belongs to the family *Fabaceae.* Traditionally, leaves, bark, stem and gum are usually parts of plants from the *Acacia* species used for inflammatory diseases, diarrhea, gastrointestinal disorders and skin diseases. A. Jacq. is commonly present in semi-arid regions around the globe. Plant A. Jacq. possesses valuable phytoconstituents, used commonly in ethnomedicine and pharmaceuticals for the treatment of various diseases (Subhan *et al*., 2018; Ahmed *et al*., 2022; Patel *et al*., 2023).

Since ancient times, various plant preparations have been used for their therapeutic activities in traditional systems (Rahman *et al*., 2022). Due to ease of availability, the least side effects and low cost, plan-derived formulations are a chief player of all the available therapies (Salehi *et al*., 2019). Additionally, several medicinal plants contain various bioactive constituents, which do not possess unwanted side effects but show influential pharmacological activities (Sharifi-Rad *et al*., 2018). Drugs obtained from plants are also a good source to counteract the side effects of synthetic drugs (Rahman *et al*., 2022). The World Health Organization reports stated that approximately 80% of the world’s population is mainly dependent on medicinal formulations for their basic well-being needs (Chirumamilla and Taduri, 2023; WHO, 2023). Traditional medicines are now becoming more popular around the globe due to the increasing belief that medicinal plants provide a limitless source of drugs (Abd El-Wahab *et al*., 2013). Several phytoconstituents are reported for their anti-inflammatory, antioxidant and antidiabetic activities e.g., alkaloids, glycosides, flavonoids, phenolics, resins, coumarins, tannins, essential oils, steroids, polysaccharides, fatty acids (Beg *et al*., 2011; Gaikwad *et al.,* 2014; Alam *et al.,* 2022; Cheng *et al.,* 2024; Muscolo *et al*., 2024).

Diabetes mellitus (DM) is a long-lasting metabolic illness featured by chronic hyperglycemia (Ojo *et al*., 2023). DM prevalence has increased more rapidly in low and middle-income countries, comparable with high-income countries, estimated that roughly 366 million people will have diabetes worldwide in the year 2030. Typically, both types of diabetes, type 1 and type 2, are considered an inflammatory process (Mi *et al.,* 2019; Satyanarayana *et al*., 2022). It has been reported that an increase in plasma glucose level cause oxidative stress. Oxidative stress is an imbalance between antioxidants and free radicals. The relationship between oxidative stress and hyperglycemia contributes to the progression of diabetic complications, like retinopathy, nephropathy, neuropathy and cardiovascular diseases. Peripheral nerve injury and diabetic kidney disease are common clinical challenges

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that are treated by advanced techniques in diabetic patients. So, in order to prevent diabetes and its complications, there is a need for oxidative stress modulation (Zhou *et al.,* 2023; Chen *et al.,* 2024; Chandimali *et al.,* 2025; Xiao *et al.,* 2025).

The enzymes play an important role in glucose homeostasis. The α-glucosidase is a vital enzyme involved in the digestion of carbohydrates, as it breaks oligosaccharides such as maltose and sucrose. Similarly, α-amylase plays a significant role in breaking down starch to facilitate its digestion. The activity of both enzymes results in an increased glucose level. Consequently, inhibiting α-glucosidase and α-amylase has been a research focus to regulate high glucose level in the blood (Mahmud *et al.,* 2023; Loukili *et al.,* 2025). Other enzymes, glucokinase, acts as a glucose sensor, facilitating the phosphorylation of glucose into glucose-6-phosphate (Matschinsky and Wilson 2019). Glucose-6-phosphate dehydrogenase (G6PD) is a rate-limiting enzyme in the pentose phosphate pathway (PPP) (Tiwari, 2017). The glucose 6-phosphatase is an enzyme that catalyzes the hydrolysis of glucose 6-phosphate into a phosphate group and free glucose (Xia *et al.,* 2025). Fructose 1,6-bisphosphatase is a key enzyme in glycolysis and catalyzes the hydrolysis of D-fructose 1,6-bisphosphate to D-fructose 6-phosphate and inorganic phosphate (Cui J and Tcherkez, 2021).

The pancreatic β-cells are responsible for producing insulin, its destruction or dysfunction leads to insulin resistance or insulin deficiency. An attractive cure is to regenerate or restore β-cells within the pancreas. As several approaches have been investigated for β-cells regeneration therapy, e.g. β-cells can be regenerated from pancreatic stem cells, embryonic stem cells and human induced pluripotent stem cell therapies, there are still some challenges to their use (Goode *et al*., 2023; Bourgeois *et al*., 2024). Currently available modern drugs for the treatment of diabetes still have limitations that include high cost, inadequate efficacy and many side effects. In view of the said drawbacks of modern drugs, preparations made from plants have anti-diabetic action have been used as an alternative method in controlling diabetes (Rahman *et al*., 2022). This research aimed to explore the *in-vitro* biological effects of A. Jacq. ethyl acetate extract and to understand the role of the plant under study in the improvement of beta cell functioning. Gene sequencing was not performed due to lack of facilities.

MATERIALS AND METHODS

Plant assortment and preparation of A. Jacq. ethyl acetate extract

Collection of the leaves of *Acacia jaquemontii* was done from the Bhakkar, near the city Bahawalpur, Pakistan. From the Islamia University of Bahawalpur, cholistan institute of desert studies (CIDS) authenticated the plant with # CIDS/ IUB-1901/63.. After the assortment of leaves, the cleaning and drying process was performed. Then, the dried leaves were ground to make a leaf powder. The maceration process was performed by taking powdered sample material of plant under study, dissolved in ethyl acetate for almost one week. Later, this mixture was filtered properly and finally assembled (Singh *et al.,* 2021).

OH scavenging assay of A. Jacq. ethyl acetate extract

The OH radical scavenging action of A. Jacq. ethyl acetate extract was determined by a method as previously described (Muthoni *et al*., 2020). The absorbance was measured at 560 nm using spectrophotometer **(**Shimadzu UV-1800). OH radical scavenging activity was measured as below.

% Radical scavenging activity {Absorbance of control Absorbance of sample/ Absorbance of control} ×100

FRAP assay of A. Jacq. ethyl acetate extract

The FRAP assay of A. Jacq. ethyl acetate extract was performed with formerly defined method (Rahim *et al*., 2017). Firstly, FRAP reagent was prepared by mixing 10 mM TPTZ (tripyridyl triazine) solution, 20 mM $FeCl_3.6H_2O$ solution and 300 mM sodium acetate buffer in a ratio of 1:1:10 in volume. Different concentrations of samples were prepared and mixed with the freshly prepared FRAP reagent (3 mL). Then incubation was performed for 30 min at 37ºC. Finally, an increase in absorbance was taken at 593nm using spectrophotometer.

Hyaluronic acid breaking and LOX enzyme inhibition assay of A. Jacq. ethyl acetate extract

The inhibitory activity of A. Jacq. ethyl acetate extract against Hyaluronic acid breaking enzyme (HYA) was evaluated using a method previously described (Paun *et al.,* 2020). Initially, 50μL of the sample and 100 μL bovine HYA in 0.1 M acetate buffer were mixed and incubation was performed. Later 100μL of CaCl2 was mixed and again incubation was performed for 20 min at 37 °C. Afterward, 250μL of sodium hyaluronate was added to initiate the reaction. Later, 100μL of each potassium borate and sodium hydroxide solution was added. At the end, 3000μL of dimethyl-aminobenzaldehyde was added, and the absorbance was found at 585 nm. The IC_{50} values were measured based on % inhibition. Ibuprofen (IBU) was used as standard. The LOX inhibition assay of A. Jacq. ethyl acetate extract was performed using a method as previously described. Initially, mixture was prepared by mixing 0.2 M borate buffer and 2200 U/mL LOX solution. Then the mixture was incubated for 15 min. At last, linoleic acid was added and absorbance was measured at 234 nm using spectrophotometer. The formation of 13-hydroperoxyocta-decadienoic acid during the reaction caused an increase in the absorbance (234 nm) that is the basis of this process. IBU was used as standard. The IC_{50} values of plant under study and standard were measured based on % inhibition (Paun *et al.,* 2020).

Effect of A. Jacq. ethyl acetate extract on GLP-1 cleaving enzyme (DPP-IV)

The inhibitory activity of A. Jacq. ethyl acetate extract on GLP-1 cleaving enzyme (DPP-IV) was assayed by using previously described method. Vildagliptin was used as a standard drug. Absorbance was measured at 420 nm (Muralidharan *et al*., 2022).

Effect of A. Jacq. ethyl acetate extract on acid maltase enzyme (Alpha-glucosidase)

The inhibitory activity of A. Jacq. ethyl acetate extract against the acid maltase enzyme was assayed by a method as previously described (Rahman *et al.,* 2021). In 96 micro-well plates, each well contained 200 µl of reaction mixture. The reaction mixture contained 10 µl sample, 20 µl enzyme, 130 µl phosphate buffer and 40 µl p-Nitrophenyle- α-D-glucopyranoside (substrate). The blank control contained 200 µl DMSO, while the control contained 10 µl DMSO, 20 µl enzyme, 130 µl PBS and 40 µl alpha PNPG. 10 µl of DMSO and 20 µl of the α-glucosidase enzyme were added to each well. Then 130 µl of PBS and 40 µl of α-PNPG were added to each well. Incubation was done for 15 min at 37 °C and finally percentage inhibition was measured.

Study design and disease induction

The institutional biosafety and bioethical committee of the University of Agricultere, Faisalabad allotted an ethical certificate for approval of experimental protocol (# 1739/ORIC). Thirty-two Wistar rats (150-250g) were restrained in the animal room of IPP, University of Agriculture, Faisalabad. Prior to the experiment, all rats were acclimated to optimal, controlled environmental conditions for 2 weeks. In Aovernight fasted rats, high blood glucose levels were attained by using alloxan monohydrate (145 mg/kg of b.w). After the first week of induction, blood glucose levels were assessed through the tail vein method. Blood glucose levels exceeding 250 mg/dL confirmed the hyperglycemic state (Majeed *et al*., 2021). All rats were assigned to subsequent groups; (8 rats/group), Grp 1: Normal group designated as control group, Grp 2: Diabetic group, Grp 3: Standard group, treated with glibenclamide (10 mg/kg), Grp 4: A.Jacq. ethyl acetate extract treated (250 mg/kg) group. All treatments were administered (orally) daily for 4 weeks. Comparison was done between control and diabetic groups, then diabetic with standard, and A. Jacq. ethyl acetate extract group.

Physical assessment

The body weight of rats from all groups was measured throughout the experimental period on weekly basis at 0, 7th, 14th, 21st and 28th days.

Collection of blood and tissue samples

After completion of trial (4 weeks), all rats were fasted overnight and then sacrificed, followed by administration of anesthesia. Blood samples were collected, serum samples were separated out for further analysis. Pancreatic tissue samples were preserved for gene expression analysis.

Estimation of HOMA−β index

Using serum samples, glucose and insulin levels were measured and the homeostasis model assessment for β cell function (HOMA-β) of ethyl acetate extract of plant under study was determined (Ram *et al*., 2021) by means of the following formula:

HOMA−β=20×Fasting Insulin (U/L) / Fasting Glucose (mmol /L) −3.5

Estimation of C-peptide level

C-peptide is an indicator of insulin secretion in the body. The C-peptide levels were evaluated from serum samples by means of an ELISA kit (# EEL 128) using manufacturer protocol.

Effect of A. Jacq. ethyl acetate extract on enzymes involved in carbohydrate catabolism

The effect of A. Jacq. The ethyl acetate extract on activities of glycolytic enzymes and gluconeogenic enzymes was analyzed using the method as previously described (Saliani *et al.,* 2023). The ELISA kit for glucokinase (MBS765500), G6PD (MBS2702342), G6Pase (ER0967) and FBPase (MBS7227331) were used.

Assessment of expression levels of Pdx-1 and Ins-1 genes

To understand the role of ethyl acetate extract of plant under study in the modulation of insulin secretion, the expression level of Pdx-1 and Ins-1 was evaluated by performing real-time quantitative PCR (iQ5 Bio-Rad machine of Thermo Fisher Scientific). In pancreatic tissues, RNA extraction was done using TRIzol method and then quantified on a Nanodrop. Reverse transcription was done by using RevertAid cDNA synthesis kit. All the data were inspected by the 2-ΔΔ Ct method. Primer sequences used as; Pdx-1; F: TCCCGAATGG AACCGAGACT, R: TTCATCCACGGGAAAGGGAG. Ins-1; F: AGGCTCTGTACCTGGTGTGTG, R: CGGGTCCTCCACTTCACGAC. Beta- actin was used as housekeeping gene.

Statistical analysis

The analysis of variance (one and two way) and the Post Hoc Tukey's test (Graph Pad Prism: 8) were used for data examination. Data presented as mean ± S.E of all all the analyses and performed in triplicate. P value ≤ 0.05 was considered statistically significant.

RESULTS

Effect of A. Jacq. ethyl acetate extract on OH radical scavenging assay

Various concentrations of A. Jacq. ethyl acetate extract showed notable OH radical scavenging activity i.e. 52.6% at a maximum concentration of 1 mg/mL when compared to the standard (Ascorbic acid i.e. Vitamin C) that showed

significant scavenging activity against OH radical (Fig. 1).

Effect of A. Jacq. ethyl acetate extract on FRAP assay

The result displayed that A. Jacq. ethyl acetate extract showed maximum absorbance of 3.2nm; however, standard (Vitamin C) exhibited absorbance of 3.5 nm at a maximum concentration of 1mg/ml (Fig. 2).

Effect of A. Jacq. ethyl acetate extract on hyaluronic acid breaking and lipoxygenase enzymes inhibition

In-vitro anti-inflammatory activity was evaluated by hyaluronic acid breaking enzyme and LOX inhibition assay. From results, A. Jacq**.** ethyl acetate extract showed significant ($p < 0.01$) inhibition for hyaluronic acid breaking enzyme, as compared to IBU, indicated its anti-inflammatory action (Fig. 3). Lipoxygenase is another notable feature of the inflammatory process. Results indicated that A. Jacq. ethyl acetate extract exhibited significant ($p< 0.01$) inhibition for LOX enzyme, as compared to IBU, showed its strong anti-inflammatory activity.

Effect of A. Jacq. ethyl acetate extract on GLP-1 cleaving enzyme (DPP-IV)

In-vitro antidiabetic activity of the A. Jacq. ethyl acetate extract was measured by performing DPP-IV analysis. From the results, concentration dependent inhibition of A. Jacq. ethyl acetate extract against GLP-1 cleaving enzyme was found.. The maximum inhibitory activity of A. Jacq. ethyl acetate extract against GLP-1 cleaving enzyme was 56.5 %, as compared to the standard drug Vildagliptin, showed maximum inhibition 65.3% (Fig. 4

Effect of A. Jacq. ethyl acetate extract on acid maltase inhibition

The inhibitory effect of A. Jacq. ethyl acetate extract against acid maltase (alpha-glucosidase) was evaluated in comparison of acarbose. Fig. 5 shows acid maltase inhibitory activity of A. Jacq. ethyl acetate extract. Results suggested that, A. Jacq. ethyl acetate extract showed concentration dependent inhibition against acid maltase, 62.5% at maximum concentration, when compared to standard acarbose, exhibited 70.3%.

Effect of A. Jacq. ethyl acetate extract on body weight

The effect of A. Jacq. ethyl acetate extract on body weight was evaluated. Study found that body weight was noticeably decreased in the diabetic group at different time periods, while a noteworthy ($p<0.001$) increased was observed in A. Jacq. ethyl acetate extract and standard treated rats as compared to the diabetic rats, especially at the last week of the study (Fig. 6).

Effect of A. Jacq. ethyl acetate extract on HOMA- β index

Significant ($p<0.001$) decline was noticed in HOMA- β value in diabetic rats. Later treatment with standard and A. Jacq. ethyl acetate extract β-cell function expressively improved as compared to the diabetic group (Fig. 7).

Effect of A. Jacq. ethyl acetate extract on C-peptide

Results indicated that C-peptide levels in diabetic group were reduced noticeably ($p <0.001$). Subsequently, standard and A. Jacq. ethyl acetate extract treatment, C-peptide levels were increased significantly expresses favorable effects of A. Jacq. (table 1).

Effect of A. Jacq. ethyl acetate extract on enzymes involved in carbohydrate catabolism

Glucokinase and G6PD levels were reduced in the diabetic rats; however, a notable rise ($P<0.01$, $P<0.001$) was detected in the levels of G6Pase and FBPase. The gluconeogenic and glycolytic enzyme levels were improved after standard and A. Jacq. ethyl acetate extract treatment (Fig. 8).

Role of A. Jacq. ethyl acetate extract on expression of Pdx-1 and Ins-1 genes

Results findings established that in the pancreatic tissues, the expressions of Pdx-1 and Ins-1 genes were down-regulated in the diabetic rats compared to the control group. But after administration of standard and A. Jacq. ethyl acetate extract, expression levels of Pdx-1 and Ins-1 genes were raised compared to diabetic rats (Fig. 9).

DISCUSSION

Acacia is an important genus that belongs to the *Fabaceae* family. The plants from the genus *Acacia* possess important pharmacological effects. A. Jacq. is one of the popular multipurpose shrubs (small tree) of arid and semi-arid areas of Pakistan. It is stable in severe climatic conditions. A. Jacq. possesses many medicinal uses for humans as well as livestock. Literature reported antioxidant, antiviral, hepatoprotective and antibacterial activities of A. Jacq. (Amoussa *et al.,* 2020). Drug preparation from medicinal plants plays a vital role in both the prevention and cure of several diseases. Herbal preparation is chief and inexpensive source, especially for patients from low-income countries. Moreover, herbal preparation has gained more attention and fame in developing and developed countries. Some of the currently available drugs e.g. aspirin, quinine and vinblastine were derived from plants. The phytochemicals obtained from plants have valuable effects against several diseases like oxidative stress, inflammation, diabetes, cardiovascular diseases, blood disorders and reproductive disorders, etc. (Roychoudhury *et al.,* 2021). The WHO reported that more than 21000 plants are used effectively for medicinal purposes around the globe (Pandey *et al.,* 2020).

DM is incurable, long-lasting health issue that needs novel tactics for its prevention as well as its management through minimizing its associated complications.

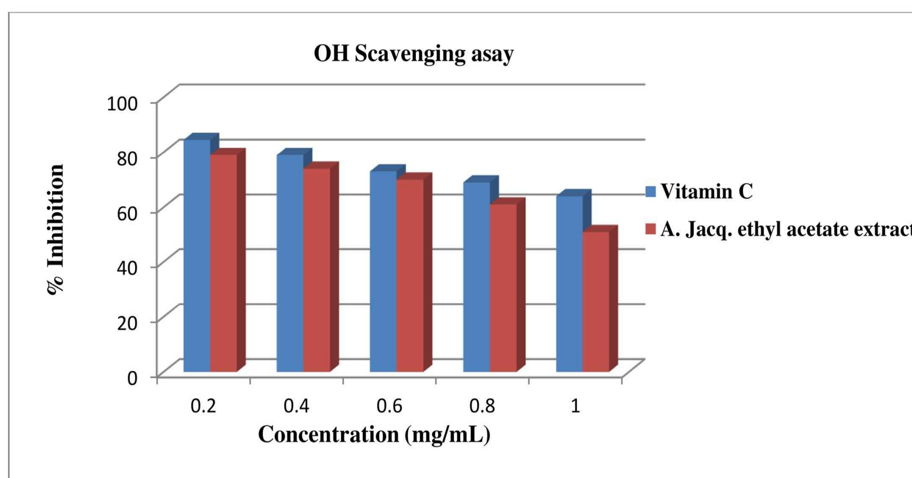

Fig. 1: *In-vitro* OH radical scavenging activity of A. Jacq. ethyl acetate extract and vitamin C. A. Jacq. = *Acacia jacquemontii*

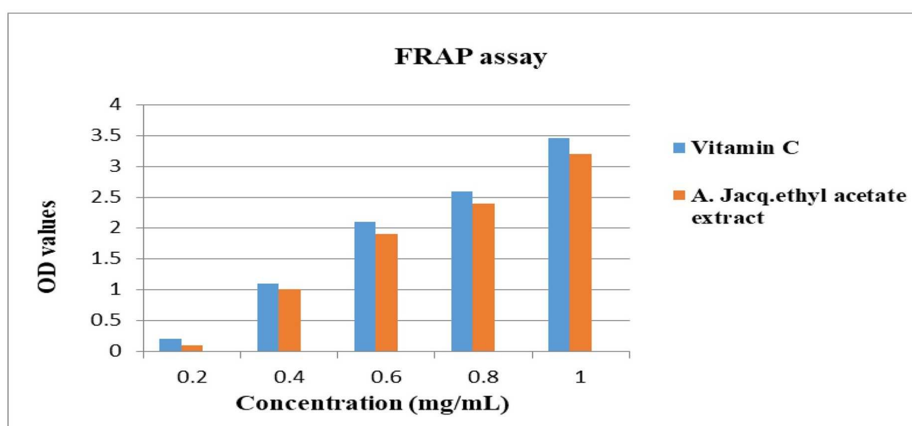

Fig. 2: *In-vitro* FARP assay of A. Jacq. ethyl acetate extract and Vitamin C. A. Jacq. = *Acacia jacquemontii*

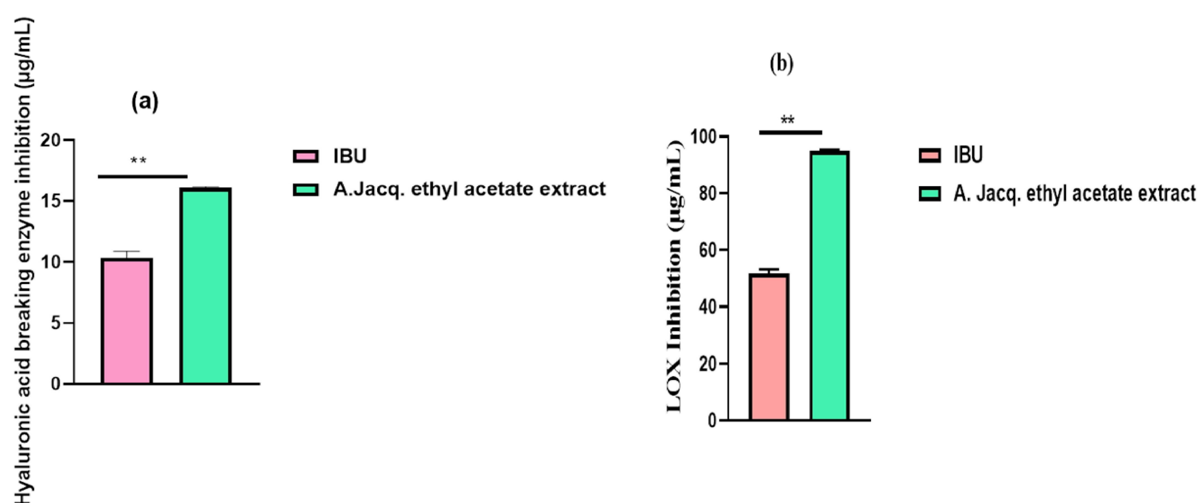

Fig. 3: (a) IC 50 (µg/mL) values of Hyaluronic acid breaking enzyme inhibition of A. Jacq. ethyl acetate extract; (b) IC 50 (µg/mL) values of Lipoxygenase inhibition of A. Jacq. ethyl acetate extract. LOX= Lipoxygenase, A. Jacq. = *Acacia jacquemontii,* IBU=Ibuprofen. Statistical analysis: ** $p < 0.01$.

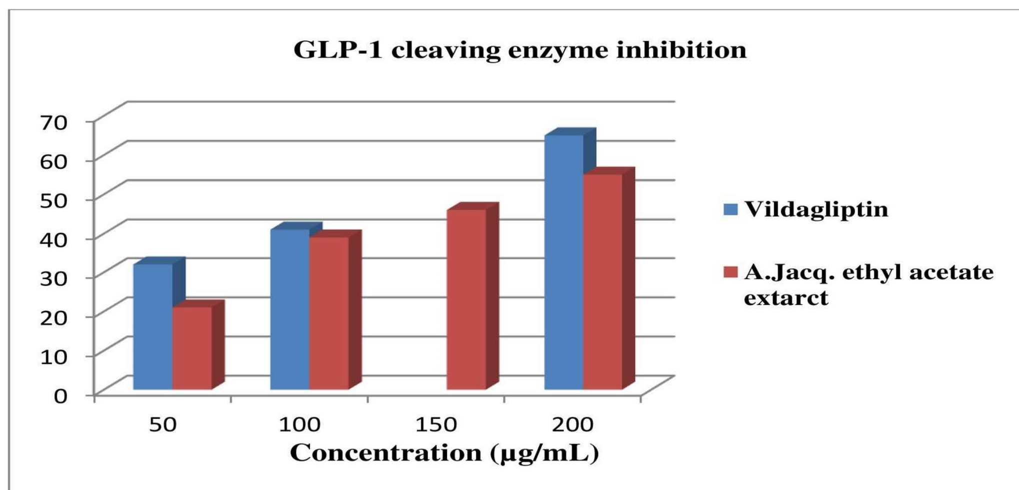

Fig. 4: GLP-1 cleaving enzyme inhibition (%) of A. Jacq. ethyl acetate extract. A. Jacq. = *Acacia jacquemontii*, Vildagliptin = standard.

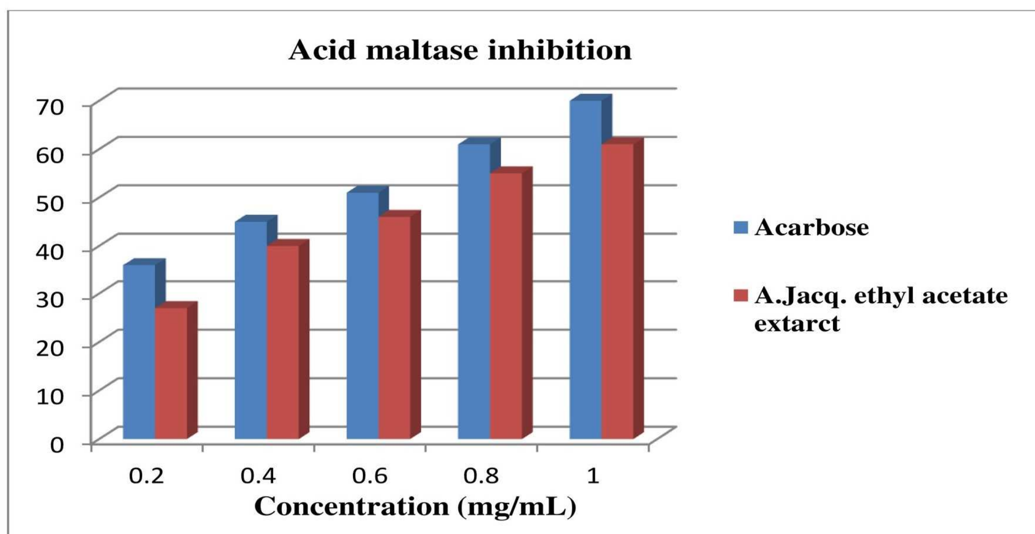

Fig. 5: Acid maltase enzyme inhibition (%) assay of AJ ethyl acetate extract. A. Jacq. = *Acacia jacquemontii*, Acarbose = standard.

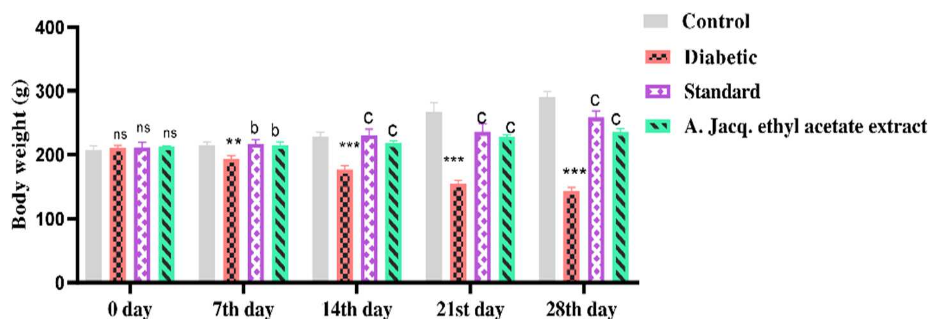

Fig. 6: Graphical representation of mean body weight (g) of control, diabetic, standard, and A. Jacq. ethyl acetate treated groups at different days of treatment. ns = non-significant, ** displays $p < 0.01$, *** displays $p < 0.001$ (comparison between control and diabetic groups), b displays $p < 0.01$, c displays $p < 0.001$ (comparison between diabetic and treatment groups).

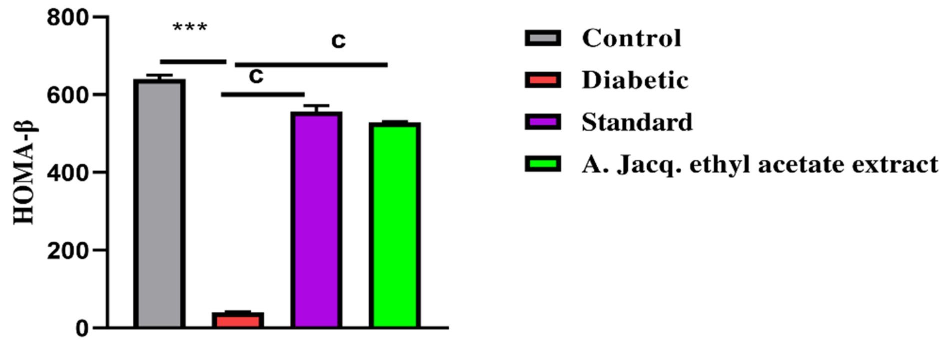

Fig. 7: Graphical representation of Homa-β index in control, diabetic, standard, and A. Jacq. ethyl acetate extract treated groups. *** displays $p < 0.001$ (comparison between control and diabetic groups, c displays $p < 0.001$ (comparison between diabetic and treatment groups).

Table 1: C-peptide level of control, diabetic, standard, and A. Jacq. ethyl acetate extract treated groups.

Groups	C-peptide levels (nmol/L)
Control	1.23±0.03
Diabetic	0.31±0.01***
Standard	1.06±0.08[c]
A. Jacq. ethyl acetate extract	0.73±0.03[b]

*** displays $p < 0.001$ (comparison between control and diabetic groups), b displays $p < 0.01$, c displays $p < 0.001$ (comparison between diabetic and treatment groups).

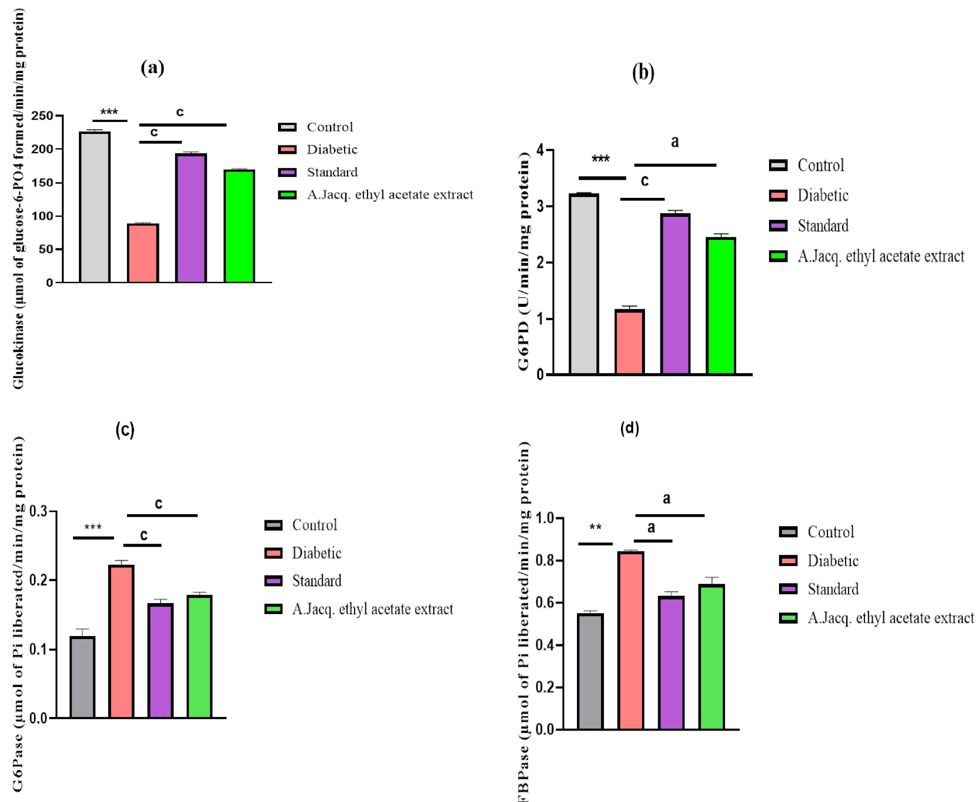

Fig. 8: (a) Graphical representation of glucokinase; (b) Graphical representation of G6PD (Glucose-6-phosphate dehydrogenase); (c) Graphical representation of G6Pase (Glucose-6-phosphatase); (d) Graphical representation of FBPase (Fructose 1-6 biphosphatase), in control, diabetic, standard and A. Jacq. ethyl acetate extract treated groups. ** displays $p < 0 .01$, *** displays $p < 0.001$ (comparison between control and diabetic groups), a displays $p < 0.05$, c displays $p < 0.001$ (comparison between diabetic and treatment groups).

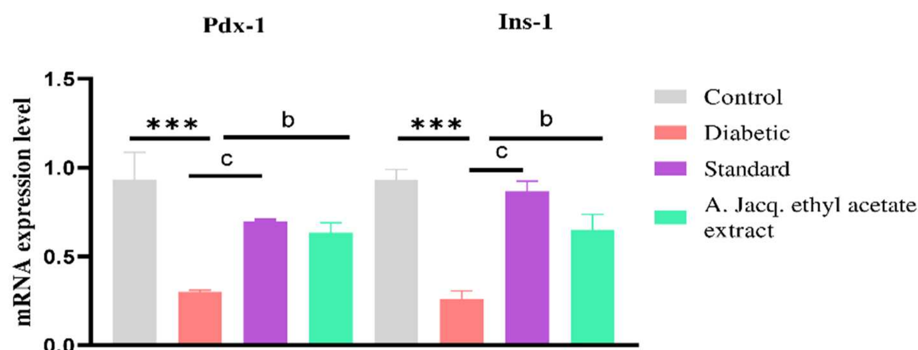

Fig. 9: Graphical representation of Pdx-1 and Ins-1 genes in control, diabetic, standard and A. Jacq. ethyl acetate extract treated groups. *** displays $p < 0.001$ (comparison between control and diabetic groups), b displays $p < 0.01$, c displays $p < 0.001$ (comparison between diabetic and treatment groups).

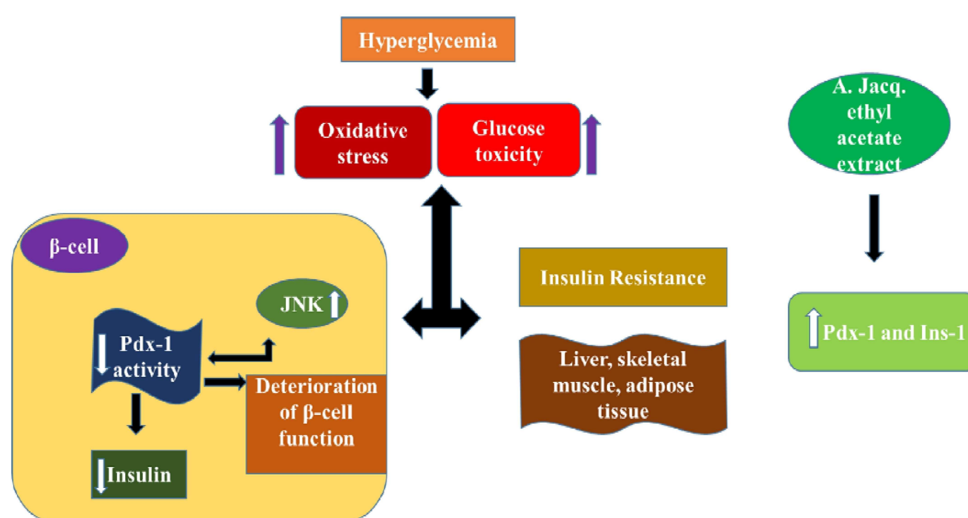

Fig. 10: Effect of A. Jacq. ethyl acetate extract on the Pdx-1and Ins-1 genes

Natural remedies containing different phytochemicals e.g. flavonoids, saponins, phenolic acids and tannins possesses noteworthy activities such as antidiabetic, anti-inflammatory, antioxidant, anticarcinogenic and antimutagenic activities (Albasher *et al.,* 2020; Eweda *et al.,* 2021; Quek *et al.,* 2021).

Results showed, that A. Jacq. ethyl acetate extract has strong potential against OH and FRAP radicals indicating its antioxidant ability. Inflammation is a biological response and occurs due to several factors. One of the key factors is protein denaturation in the tissues. Henceforth, in this study, the anti-inflammatory activity of A. Jacq. ethyl acetate extract against hyaluronic acid breaking and LOX enzymes was found. LOX is involved in inflammatory illnesses e.g. inflammatory bowel diseases, allergic rhinitis, rheumatoid arthritis and several types of cancer. In the reaction medium, peroxy radicals are made during the activity of LOX, which serves as a basis of free radicals. So, antioxidants that hunt free radicals might be responsible for acting as LOX inhibitors. Hyaluronic acid breaking enzyme is involved in several physiological processes, and literature suggests that hyaluronidase inhibitors are potential candidates that serve as anti-inflammatory, antitumor, and anti-allergic agents, as well as used for the treatment of several bacterial infections and arthritis (Neagu *et al.,* 2023).

DPP-IV and acid maltase inhibitors are now extensively used and appear excellent therapeutic options for the treatment of DM. DPP-IV inhibitors promotes glucose homeostasis by inhibiting the enzyme DPP IV, responsible for the degradation of two vital metabolic hormones i.e. GIP and GLP-1 (Amin *et al.,* 2019). From the results, significant inhibitory activity of A. Jacq. ethyl acetate extract against both enzymes, i.e., acid maltase and DPP-IV was found, suggests its anti-diabetic activity. Inhibitory activity against acid maltase is one of the therapeutic approaches for reduction of postprandial hyperglycemia (Abd El-Wahab *et al*., 2013).

The homeostasis model HOMA- β is surrogate indicator for the measurement of pancreatic β-cell functioning in humans and rodents (Ekakitie *et al*., 2021). From results,

HOMA- β values were reduced in diabetic group, comparable with control group, signifying decreased β-cell functioning. Current results demonstrate the anti-hyperglycemic role of A. Jacq. ethyl acetate extract treatment via improvement in HOMA-β and C-peptide levels.

Deficiency of glucokinase and G6PD causes the formation of ROS. A. Jacq. ethyl acetate extract remarkably improved the level of these enzymes. FBPase and G6Pase enzymes are responsible for the assembly of glycogen in the liver (Ramu *et al*., 2016; Njume *et al.,* 2019). In disease-induced animals, these enzymes were noticeably increased. However, A. Jacq. ethyl acetate extract treatment, significantly regulated the activity of these enzymes.

For the growth, regeneration and proper functioning of beta cells, pancreatic and duodenal homeobox 1(Pdx-1) is vital gene in the pancreas. Pdx-1 is a vital transcription factor for controlling beta cells function, particularly regulating the normal glucose-insulin secretion. It is accountable for the regulation and transcription of various genes, including glucose-activated insulin genes i.e. Ins-1, Ins-2, glucose transporter 2, glucokinase and islet amyloid. Pancreatic endocrine and exocrine cells development depends on Pdx-1 and it is the first transcription factor that is produced in the growing pancreas. The absence of factor Pdx-1 results in diabetes mellitus (Bahrami *et al.,* 2020; Jara *et al*., 2020; Leenders *et al.,* 2021; Anbumani *et al*., 2025). In response to glucose stimulation the transcription factor Pdx-1 localize to the nucleus and activates the transcription of INS mRNA. Previous studies reported Pdx-1 expression was decreased in diabetic patients. Pdx-1 is upregulated by several key factors, including glucose and GLP-1. Both activate the PI3-kinase (PI3K) pathway. Oxidative stress triggers the JNK pathway that results in the inhibition of insulin gene expression (Fig.10) and inhibition of activity of transcription factors, Pdx-1 and Mafa (Huang *et al*., 2015; Babaiedarzi *et al*., 2022; Usher and Showalter, 2022; Zhang *et al*., 2022). Previous studies showed several plant exhibited β-cell protective effects, for e.g. Curcuma longa (Damame *et al*., 2021), Panax ginseng (Yin *et al*., 2024), Aloe vera, Momordica charantia (Deora and Venkataraman, 2023), Teucrium polium, Tamarindus indica and Myrica rubra (Wickramasinghe *et al.,* 2021). In this study, Pdx-1 and Ins-1 gene expression were suppressed in diabetic rats and after administration of A. Jacq. ethyl acetate extract, Pdx-1 and Ins-1 gene expressions were considerably improved, results in β-cell regeneration and augmented insulin release. In the present study, for the first time, we have explored the mechanism of A. Jacq. ethyl acetate extract involved in the improvement of beta cell functioning via improvement in beta cell count, as shown by HOMA-β index and significant regulation of Pdx-1 expression, responsible for improved insulin release.

CONCLUSION

The ethyl acetate extract of plant A. Jacq. exhibited significant *in-vitro* biological activities, including the reduction of oxidant status and inflammation. The current study revealed the significant role of A. Jacq. ethyl acetate extract on GLP-1 cleaving and acid maltase enzymes inhibition. Additionally, A. Jacq. ethyl acetate extract exhibited significant in-vivo hypoglycemic activity via improvement in the levels of HOMA-β, C-peptide, gluconeogenic enzyme, as well as Pdx-1 and insulin genes. Improvement in the HOMA-β and Pdx-1 levels suggested significant role of A. Jacq. in restoration of β-cell function and insulin release.

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Authors' contributions
Ambreen Mehmood Awan and Wafa Majeed: Designed the project and analyzed the data; Anas Sarwar Qureshi and Muhammad Naeem Faisal: Generated the data.

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Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on request.

Ethical approval
The institutional biosafety and bioethical committee of the university allotted an ethical certificate for approval of experimental protocol (# 1739/ORIC). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The researchers declare no conflict of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/05/SUP1779524160.pdf

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