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Research Article

Assessment of Antidiabetic and Cyto-Regenerative Activity of *Ficus carica* through Gene Expression Analysis in Diabetic Rat Model

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Abstract: Diabetes mellitus is a metabolic disease of the endocrine system, characterized by chronic hyperglycemia resulting from insulin resistance or defective insulin production. Among the complementary and alternative medicines, diet-based approaches are gaining popularity worldwide for the management of it. *Ficus carica*, one of the oldest plants cultivated on the earth, is rich in phytochemicals including anthocyanins, phenolics, flavonoids, and organic acids. The present study was designed to analyze the therapeutic potential of dried fig and extract for their potential against hyperglycemia and related complication in the diabetic rat model. Diabetes was induced by using alloxan monohydrate and divided into five groups including Negative-, Positive-, standard drug- group, treated-I (given extract), and treated-II (given 10 % dried figs). Fig extract was administered through the intragastric tube, and fig paste was mixed in the feed of the experimental group, and then rats were decapitated after 6 weeks to collect the blood and serum. At the end of the study, biochemical analysis such as fasting blood glucose (FBG), serum glucose, and insulin was performed. Histopathological study of the pancreas showed cell deformation in the positive control group whereas damage was reversed in treated groups. The pancreas was also saved for gene expression analysis. The results revealed that the positive control group has lower expression of INS-1, INS-2, Pdx-1, amylin, and GLUT-2 genes. Results revealed that serum glucose and FBG started to normalize after the administration of treatment (Glibenclamide, dry fig, and fig fruit extract), and insulin concentration also started to improve. 10 % dried fig was more effective to control hyperglycemic conditions, which might be due to the presence of fiber. However, the gene expression was more modulated in the group treated with fig extract. The findings of current research suggested the utilization of fig and fig-based products because of their potential to reverse the damage induced by the alloxan or stressors of daily life.

Keywords: Diabetes Mellitus, Pancreases, Regeneration, *Ficus carica*, Genes, Extract, Histopathology

1. INTRODUCTION

Diabetes Mellitus (DM) has become a pandemic in the last few years, if it is not controlled over a long period, it may lead to the development of serious health ailments including retinopathy, blindness, cardiovascular diseases, nephropathy, and neuropathy. However, if proper care is given to patients, these problems can be prevented and delayed [1]. According to the National Diabetes Survey of Pakistan 2016-17 (NDSP), about 26.3 % of individuals are diabetics, of which 7.1 % have been recently diagnosed [2]. Urban people (28.3 %) have more incidence than rural (25.3 %) ones. Unfortunately, nearly 6 % (4.6 million) of people are not aware of their disease. The major risk factors related to DM are aging, obesity, family history, hypertension, and dyslipidemia [2]. According to a recent estimate by IDF, Pakistan has attained the 3rd position, with 33 million people, and is predicted to reach 62.2 million by 2045 [3]. The management of DM and its complications without any marked side effects is still a major challenge. Numerous conventional

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methods such as allopathic medicines, and herbal and medical plants are also in use. The acceptability of complementary and alternative medicines (CAM) has increased many folds in the last few decades. Many surveys have reflected that about 48.5 % of people use at least one or more forms of CAM in Australia and the US including herbal medicine, medicinal plants, and supplements [4]. Globally, almost 30 % of diabetic patients are using CAM, however, in Pakistan, approximately 50 % of diabetic patients rely on these types of medicines, based on personal knowledge and practices. Probiotics**,** vitamins, minerals, and herbs are the major products in these categories. These products are prepared and extensively promoted as dietary supplements. Likewise, numerous plant extracts, pulp, seeds, stems, roots, and sometimes whole fruits, vegetables, and herbs are also used by consumers to treat various diseases. These are famous for being harmless and cost-effective [5].

Ficus carica Linn. belongs to angiosperms and comprises almost 800 species of plants. One of the most consumed and famous fruit and is known by various names such as *Tian* (Arabic), *Anjir* (Urdu), and *Figari* (Hindi),. A few fruits are mentioned in Holy Quran and Ahadith, the fig is one of them. In the Holy Quran, the first verse of Surat At-Tin illustrates the benefits of this fruit as it says ***(I swear), by the fig and the olive*** (Quran, 95:1). Fig fruit is popular for its sweet, delicious taste having nutritional as well as therapeutic benefits. It is consumed by humans as well as by animals in dried and fresh forms. In the Mediterranean region, the fig is known as poor man's food and is extensively consumed in the dried and fresh form [6]. Figs are energy-dense fruit with appreciable amounts of minerals and fiber. It is cholesterol and sodium-free. The fresh fig fruit has nearly 80 % water, 17.3 % carbohydrate, 1.7 % fiber, 1.2 % protein, 0.6 % ash, and 0.3 % fat along with 76 Kcal per 100 g [7]. Fig is rich in hydrocarbons, aliphatic alcohols, volatile compounds, fatty acids, and some other plant metabolites such as coumarins, flavones, triterpenoids, and steroids, [8]. Numerous bioactive components have been isolated in different parts of fig. Phenolic compounds composition is affected by climate conditions, cultivars, processing method, production location, ripening stage, and storage method. These are also popular for their antioxidant potential. Anthocyanins are mainly present in fig varieties having blue, pink, and violet-colored pulp or skin. These bioactive compounds, help to regenerate β-cells and alleviate the symptom of DM [9].

In traditional medicines including Siddha, Unani, and Ayurveda, figs are used extensively for the cure and prevention of numerous health ailments. It has been utilized for the treatment of disorders related to the cardiovascular system, endocrine system, gastrointestinal tract (anorexia, colic, diarrhea, indigestion, vomiting, and ulcer), infectious diseases (gonorrhea, scabies, and skin disease), inflammation, liver, reproductive system (menstruation), respiratory system (asthma, bronchial problems, cough, sore throats) and spleen [10, 11]. Fig is also used in a blend with various medicinal herbs, milk, and honey. The fig fruit is used in both dried and fresh forms. Depending on the variety and type, dried figs can be commercialized for diverse uses, such as table consumption or to prepare other commercial products like canned figs and fig paste. Mission variety is used as dried fruit and to make juices and paste, however, Adriatic and Kadota varieties are specifically utilized for paste production. In California, a larger amount of produced fig is used to make energy bars and cookies. Fig is also used in baked products like pastries, pie, and cooked dishes. Low-quality dried figs are used mostly to flavor coffee and to prepare concentrated juices [12]. Fresh unpeeled and peeled figs are used in several ways in bakery products including cakes, fig pies, and puddings. Numerous products like fig ice cream, fig jams, fig marmalade, fig Newtons, fig paste, and fig rolls are commercially available. The addition or filling of fig's paste in wheat and corn flour, along with other ingredients such as oil, syrup, resulting in the production of delicious bakery products. Moreover, sugar syrup from the whole fig is also prepared at the household level [13].

In various efficacy studies, the therapeutic and nutritional potential of fig has been proven. Flavonoids have insulin-mimetic and insulin-secretagogue action, which depends upon the type, quantity, and structure of bioactive compounds. Luteolin, quercetin, and rutin are the prominent flavonoids found in fig and its various components. The bioactive compounds enriched extract of different parts of fig has been evaluated for various

mechanisms such as antioxidant potential and anti-hyperlipidemia. The phenolic-rich extract of fig was evaluated for antioxidant potential and anti-hyperlipidemia in the streptozotocin-induced diabetic rats' model. Indigenous antioxidant enzymes in the heart liver, and kidney has improved after intake of the extract [14]. The c-Jun N-terminal kinase (JNK), Janus Kinase / Signal Transducer and activator of transcription (JAK-STAT), and FOXO1 are the established cell stress and insulin signaling pathways in DM. About 0.2 % dietary intake of cyanidin 3-glucoside helped in the improvement of insulin sensitivity and fasting glucose in high-fat and obese mice. Moreover, the concentration of mRNA of inflammatory cytokines including TNFα and interleukin-6 was also reduced along with repressed infiltration of macrophage in adipose tissue. Insulin signaling transcriptional factors such as FOXO1 are a chief modulator of insulin signaling in β-cells, hepatocytes, and adipocytes. It regulates gluconeogenic enzymes in the fasting state whereas in the fed state reduces gluconeogenesis through insulin-mediated phosphorylation of FOXO1. Cyanidin 3-glucoside down-regulates the enzymes like Glucose-6-Phosphatase and Phosphoenolpyruvate carboxy kinase in the liver and adipose tissues. Moreover, it down-regulates the JNK activation and promoted the phosphorylation of FOXO1 [15]. Keeping this in view, the current experiment is designed as an attempt to explore the antidiabetic and cyto-regenerative ability of fig and fig extract in β-cells and underlying cellular mechanisms.

2. MATERIAL AND METHODS

2.1. Procurement of Materials

Sun-dried Afghani fig required for research was procured from Faisalabad (Pakistan) local market. Alloxan monohydrate and all reagents for biochemical evaluation were procured from Sigma-Aldrich (Sigma Aldrich, Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany). Glibenclamide tablets 5mg (Daonil®) were purchased from Sanofi-Aventis (Pvt.) Ltd., Pakistan. PCR Optical 8-Tube Strip was acquired from the Applied Biosystems™ MicroAmp™ Thermo Fisher Scientific, USA, SYBR® Green qPCR super mixes were procured from BIO-RAD, USA, and TRIzol was bought from Thermo-Fisher Scientific, Massachusetts, USA. Male Sprague Dawley rats were obtained from the National Institute of Health (NIH), Islamabad, Pakistan for bio-efficacy trials.

2.2. Preparation of Fig Fruit Extracts

The dried fig was freeze-dried with liquid nitrogen and was converted into a fine powder with the motor pestle after. Subsequently, fig fruit extract was obtained through a solvent extraction method by following the modified method of Bucić-Kojić *et al*. [16]. The fig fruit powder (25g), was extracted with 500 mL of ethanol (70 % (v/v)) at 80ºC for 45 mins in the ultrasonic-assisted water bath. Afterward, the sample was placed on the orbital shaker for 3 hrs. at 280 rpm. The obtained mixture was centrifuged at 5000 resolution per minute for 15 minutes, and then polyamide Chromafil disposable filter AO-45/25 was used to filter the supernatant. The rotary evaporator was used to concentrate the acquired solution and freeze-dried. Obtain dried extract was stored in glass vials and stored at -40ºC.

2.3. Bio-efficacy Study

Healthy young albino rats (50), approximately 180-200g weight, were housed in an Experimental Animal House of the Faculty of Food, Nutrition, and Home Science, University of Agriculture, Faisalabad (UAF). The research was performed by observing the Institutional Biosafety Committee (IBC) guidelines, UAF (D. No.: 8856/ORIC, Dated: 28 November 2019). The animals were acclimatized one week earlier to the experimentation under standard conditions. The temperature (23±2°C) and humidity (50±5 %) were provided throughout the period with 12 hrs. light-dark cycle. Rats were given normal feed and water *ad libitum*. The blood glucose level was checked before inducing diabetes in the rats. Afterward, diabetes was induced using a single intraperitoneal injection of alloxan monohydrate (150mg alloxan dissolved in normal saline per kg body weight). The rats were served with dextrose solution (10 %) within the 12 to 24 hrs. of alloxan administration to avoid mortality. Fasting blood glucose level was measured using a glucometer (Vivachek™ Ino BGMS, Biotech Co., Ltd Hangzhou, China) by taking a blood sample from the tail vein after 3, 7, and 10 days for confirmation of diabetes. The rats exhibiting ≥300 mg/dL of fasting blood glucose (FBG), were

divided into the following groups (5 rats per group) for interventional trial:

G_0: Negative control group, rats were fed on the normal diet

G_1: Positive control group, Alloxanized diabetic rats were fed on the normal diet

G_2: Glibenclamide, Alloxanized diabetic rats treated with Glibenclamide (0.6 mg/kg body weight) and fed on the normal diet

G_3: Treated-I, Alloxanized diabetic rats treated with an extract of fig (400mg/kg body weight) and fed on the normal diet

G_4: Treated-II, Alloxanized diabetic rats treated with 10 % dried figs mixed in the normal diet

2.3.1. Biochemical analysis

The rats (3 from each group) were decapitated (after 4 weeks) at the termination of the efficacy trial, to collect blood samples and centrifuged at 4000 rpm for 10-15 mins for the separation of plasma and serum. The sample was stored at -20˚C to use later for biochemical tests using respective methods. With the help of the glucometer, the FBG was measured from the tail vein. A commercially available kit (Bioclin® Glucose Monoreagent diagnostic kit, BioClin Therapeutics, Brazil) was used to measure serum glucose. However, the insulin was determined by using the kit (Insulin ELISA®, Calbiotech Inc, USA).

2.3.2. Histopathological examination

The pancreases of each decapitated rat were preserved in 10 % formalin solution for histopathology whereas, for mRNA extraction, pancreas tissue was stored in Trizol. Histopathology of the pancreas was performed following the methods of Nurdiana *et al*. [17].

2.3.3. Gene expression analysis

RNA isolation was performed from the pancreas by using the TRIzol method (Johnson, 2018), and isolated RNA samples were quantified by using nanodrop. Afterward, isolated RNA was subjected to cDNA synthesis [18] and protein expression was quantified using real-time qPCR. As a housekeeping gene, Beta-actin was used. The expression of underlying cellular mechanisms including insulin signaling pathway (INS-1, INS-2, Pdx-1), calcium signaling pathway (Pias-2, Calm-2, Grk-2), regeneration (IGF-1, FOXA-1, KI67), hormones (amylin, leptin), and glucose transporter (GLUT-2). During PCR following protocol was followed.

Denaturation of the cDNA: for 15 sec at 95 ^{o}C
Annealing temperature: 58 °C for 30 sec
Extension time: 20 seconds at the temperature of 72 ^{o}C for 39 cycles

Afterward, the 2*(- ΔΔct) method was used to analyze the qRT-PCR data.

2.3.4. Statistical Analysis

The recorded results were evaluated statistically by using analysis of variance (ANOVA) and Tukey's HDS test was performed to determine significant differences among the groups as described by Montgomery [19]. Results are presented in Mean values ± standard deviation.

3. RESULTS

3.1. Biochemical Analysis

Mean squares showed significant variation in the FBG, serum glucose, and serum insulin among the different experimental groups (Table 1). The highest FBG (345.74±12.77 mg/dL) was observed in the positive control group (G_1), followed by the group treated with Glibenclamide (G_2) (284.42±10.41 mg/dL). The rat groups treated with dried fig fruit extract (G_3) and 10 % dried fig fruit supplemented diet (G_4) showed significant downregulation in FBG levels.

The recorded values for FBG in G_3 and G_4 were 261.23±11.19, and 210.91±7.71 mg/dL, respectively. The highest serum glucose (278.85±10.30 mg/dL) was observed in the positive control group (G_1) followed by the group treated with Glibenclamide (G_2) (227.67±8.02 mg/dL). The groups treated with fig-based interventions showed a significant reduction in serum glucose levels. The group G_3 and G_4 reported 210.69±9.03 and 169.91±5.95 mg/dL glucose levels, respectively. The highest insulin (24.80±0.45 µIU/mL) was observed in the negative control group (G_0),

Table 1. Effect of treatments on fasting blood glucose, serum glucose, and serum insulin in the experimental rats

Experimental groups	Parameters		
	Fasting Blood Glucose (mg/dL)	**Serum Glucose (mg/dL)**	**Serum Insulin (µIU/mL)**
G_0	96.32±8.27^d	85.79±4.99^d	24.80±0.45^a
G_1	345.74±12.77^a	278.85±10.30^a	15.28±0.79^c
G_2	284.42±10.41^b	227.67±8.02^b	22.69±0.88^b
G_3	261.23±11.19^b	210.69±9.03^b	24.33±0.79^a
G_4	210.91±7.71^c	169.91±5.95^c	22.58±0.23^b

Mean ± SD; Mean values within a column, bearing a different superscript are significant
G_0: Negative control group, **G_1:** Positive control group, **G_2:** Glibenclamide, **G_3:** Treated-I, **G_4:** Treated-II

followed by the G3 (24.33±0.79 µIU/mL), then G2 (22.69±0.88 µIU/mL), and G4 (22.58±0.23 µIU/mL). The hyperglycemic condition started to normalize after the administration of treatment (Glibenclamide, dry fig, and fig fruit extract), and insulin concentration also started improving.

3.2. Histopathological Examination

Uncontrolled inflammatory processes and hyper generation of free radical moieties make cells susceptible to damage and injuries. The histopathological examination assists in evaluating the type and degree of organ damage. In this research, a histopathological assessment of the pancreas was done to predict the efficiency of figs (extract and feed) in comparison to alloxan and Glibenclamide. The histological examination of pancreatic tissues is presented in Figure 1. In the negative control group (G_0), the acinar cells and β-cells of the pancreas were present in the normal proportion, showing complete, and regular pancreatic volume. The cytoplasm of acinar cells was stained light with prominent dark-stained nuclei which are organized in lobules. The β-cells are seen surrounded by acinar cells and by a fine capsule. Neither necrosis nor inflammatory cell infiltration nor any microscopic lesion around the islet tissues and the alveolar cells was observed in G_0. In positive control rats (G_1), administration of alloxan leads to the inflammation and destruction of the β-cells, alongside the steatosis of the acinar cells. The necrosis of the islet tissues leads to a reduction in the number and size of pancreatic islets. The

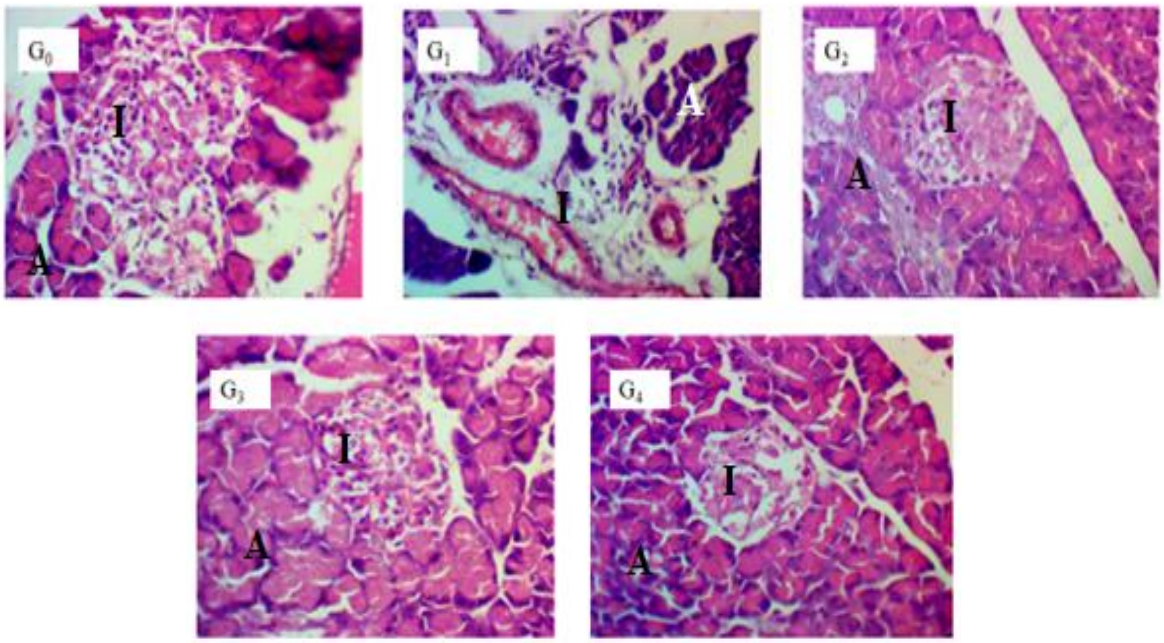

Fig. 1. Histopathological indications of pancreas tissues
A= Acinar cells I= Islet of Langerhans, H&E stain, Magnification= 40x, Sacle bar= 100 µm

acinar cells around the β-cells are arranged loosely and the nucleus occupies a large area, which does not look normal.

In glibenclamide-treated rats (G_2), the disruption of the β-cells and the acinar cells steatosis along with infiltration of inflammatory cells was observed. It also showed moderate destruction of alveolar cells. Compared with the normal group, G_2 showed improvement in pancreatic structure, volume, and the number of pancreatic cells, with compact cytoplasm, and reduced vacuolar degeneration. In the group treated with fig fruit extract (G_3), significant protection of β-cells and acinar cells has been seen. The acinar cells were seen as normal with only mild disruptions. The islets have a substantial proportion of β-cells with scanty inflammatory cell infiltration. Similarly, the group fed on a 10 % figs fruit-supplemented diet (G_4), showed normal proportions of the acinar cells and β-cells. The islet cells were surrounded by acinar cells and the fine capsule. Slight necrosis and inflammatory cell infiltration were observed. The characteristic interlobular and intralobular ducts were also seen.

3.3. Gene Expression Analysis

To probe the effect of dietary interventions on the insulin production capacity and cyto-regenerative ability of β-cell, underlying cellular mechanisms including insulin signaling pathway (INS-1, INS-2, Pdx-1), calcium signaling pathway (Pias-2, Calm-2, Grk-2), and regeneration genes (IGF-1, FOXA-1, KI67), were studied through gene expression analysis. Moreover, genes of amylin, leptin (hormones), and glucose receptor GLUT-2 were also analyzed and presented in Figure 2. The expression level of insulin signaling genes is shown in Figure 2 (a, b, c). In the G_1, the expression level of INS-1, INS-2, and Pdx-1 genes were significantly down-regulated as compared to the negative control group (G_0; 2.45±0.07, 2.67±0.02, and 2.39±0.03, respectively). Glibenclamide and fig fruit extract treated groups and the group fed on 10 % figs fruit-supplemented feed, exhibited significantly higher expression levels for INS-1, INS-2, and Pdx-1 genes in comparison to G_1. The expression levels of Pias-2, Calm-2, and Grk2 were significantly upregulated (4.31±0.14, 4.90±0.14 and 4.34±0.11 respectively) in G_1 as compared to G_0 (1.19±0.01, 1.09±0.01, and 1.23±0.00 respectively) as shown in Figure 2 (e, f, g). G_2 and fig fruit-based interventional groups exhibited significantly lower expression levels of Pias-2, Calm-2, and Grk2 genes in comparison to G_1. The result of the current study demonstrates the positive effect of fig-based interventions in regulating glucose homeostasis. The expression level of IGF-1 (3.12±0.01), FOXO-1 (2.78±0.06), and Ki-67 (5.22±0.10) were significantly upregulated in the positive control group when compared to the negative control group (G_0; 1.34±0.09, 1.49±0.01, and 1.56±0.03 respectively) as shown in Figure 2 (g, h, i). Treated groups such as G_2, G_3, and G_4 exhibited a significantly lower expression level of the IGF-1 gene in comparison to G_1. In the positive control group, the expression level of amylin and GLUT-2 was significantly downregulated (0.67± 0.01 and 0.44±0.01) as compared to G_0 (1.23± 0.04 and 1.99±0.06) respectively. However, in the positive control group, the expression level of leptin was significantly upregulated (1.78±0.06) as compared to G_0 (0.56±0.02) as shown in Figure 2 (j, k, l). The results depicted that fig extract and feed supplementation have the potential to modulate the expression of this gene.

4. DISCUSSION

4.1. Biochemical Analysis

Biochemical analysis can reveal the necessary information that is required for accurate diagnosis of diseases and effect of treatments.

Plasma glucose is a key prognostic factor for the diagnosis of DM. The results of the current study are well backed by the previous findings. In a study, the methanolic extract of fig fruit exhibited a dose-dependent decline in FBG levels at 250 and 500 mg/Kg of the extract, *i.e.,* 165 and 50 mg/dL, respectively [20]. Purified fig fruit extracts containing abscisic acid were administered at two different doses, to investigate their effect on the glycemic index and insulinemic index in humans. The higher doses of abscisic acid (1200 mg) reduced insulinemic and glycemic reactions by ~25 %. This indicates that fig fruit extract supplementation is a good intervention for glycemic response management [21]. In another study, hexane, ethyl acetate, ethanol, and aqueous extracts of fig fruit were investigated for their

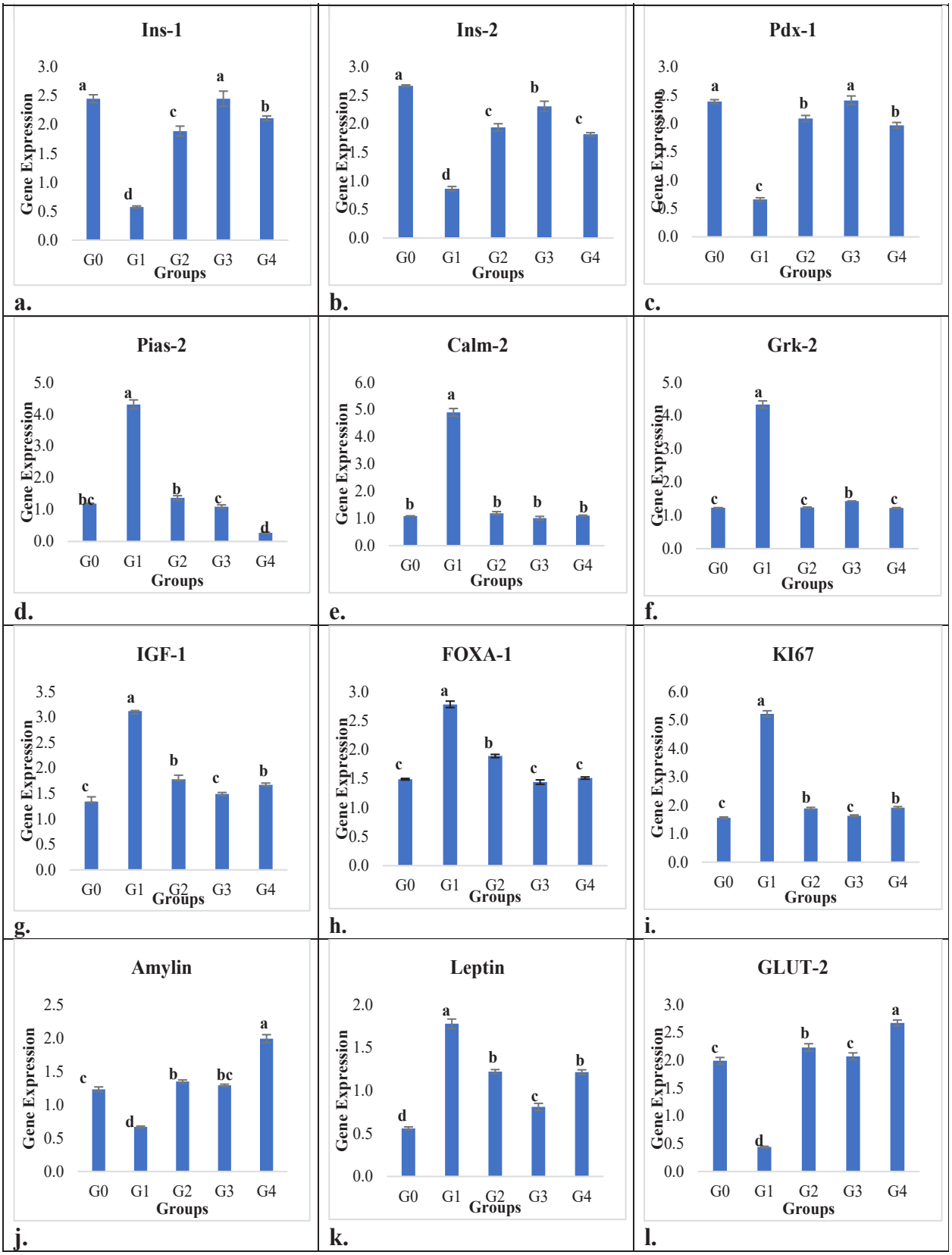

Fig. 2. The expression level of the insulin signaling pathway (a. INS-1, b. INS-2, c. Pdx-1), calcium signaling pathway (d. Pias-2, e. Calm-2, f. Grk-2), regeneration (g. IGF-1, h. FOXA-1, i. KI67), hormones (j. amylin, k. leptin), and glucose transporter (l. GLUT-2) in the pancreas of the experimental rats Mean values, bearing a different superscript are significantly different from each other ($p < 0.05$)

potential to hinder α-amylase and α-glucosidase. The IC50 values indicated the better potency of the ethanolic extract to inhibit α-amylase (315.89 mg/mL), and α-glucosidase (255.57 mg/mL) [22]. The consumption of ethyl acetate fig leaf extracts reduced the overall glucose concentration to 129.14 mg/dL which was comparable to the glucose level (131.32 mg/dL) in the group treated with Glibenclamide [23]. In another study, the effect of ficusin on serum insulin levels in diabetic rats was evaluated and the findings suggested significant improvement in the levels of insulin (22.77 μIU/mL) in a dose-dependent manner [24].

In a subsequent study, fig fruit was analyzed for its anti-hyperglycemic potential at different concentrations *i.e.,* 50, 100, and 150 mg/Kg in STZ diabetic rats over 14 days. Results showed that fig extract was able to reduce blood glucose from 379 to 87 mg/dL at the dose of 150 mg/Kg in 14 days. However, positive control remained hyperglycemic, and blood glucose increased from 361 to 505 mg/dL [25]. In the current research, the group fed on a 10 % figs fruit-supplemented diet showed better glycemic control among the interventional groups. This may be attributed to the dietary fiber content in fig fruit which hinders glucose absorption, along with regulation of the lipid levels without disturbing the gastrointestinal tract (GIT) system [26]. The reduction of hyperglycemic conditions in the experimental group fed on dried fig fruit and extract might be done to better control glucose levels in serum due to the presence of bioactive components in fig fruit that have insulin-mimetic properties [27]. Comparable results are reported by El-Shobaki *et al*. [28], that diabetic rats showed drastically higher serum glucose (228 mg/dL) compared to normal rats (94 mg/dL) and diabetic rats treated with 5, 10, and 20 % fig fruit supplemented diet (198, 198, and 177 mg/dL, respectively). 4, 6, and 8 % fig leave supplemented diet (158, 138, and 131 mg/dL, respectively).

4.2. Histopathological Examination

Exhaustion of β-cells resulted in insulin deficiency. Insulitis is commonly seen in islets containing residual β-cells [29]. In a study, the pancreas of normal rats exhibited embedded β-cells in the exocrine portions. However, the diabetic group showed pathological changes in the exocrine and endocrine parts of the pancreas along with a noticeable reduction in the β-cells [30]. The alloxan-induced diabetic rats showed necrosis of the islet tissues with the destruction of the alveolar cells [31]. Moreover, alloxan administration resulted in the infiltration of inflammatory cells in both β-cells and acinar cells [32]. STZ-induced diabetes decreased insulin-immunoreactive expression along with defects in insulin action in the peripheral tissues which resulted in hyperinsulinemia that destroyed the cell integrity and functional ability of the β-cells. However, the fig-treated diabetic rats in the study conducted by Irudayaraj *et al*. [23] showed increased insulin-immunoreactive expression indicating the cytoprotective (β-cells) role of the fig extract. Similarly, fig leaf extract showed a protected effect on β-cells in diabetic rabbits [33].

4.3. Gene Expression Analysis

INS-1 and INS-2 are genes encoded for precursor protein that undergoes proteolytic cleavage and produces insulin which is stored in secretory granules of β cells of the pancreas [34]. Pdx-1, another gene, encodes a protein that is a transcriptional activator of various genes, including glucokinase, glucose transporter type 2 (GLUT2), somatostatin, and insulin. This nuclear protein plays a key role in the glucose-dependent regulation of insulin gene expression. The defects in this gene cause pancreatic agenesis and Type-2 diabetes [34]. The mechanism involved might be the alloxan-mediated generation of free radicals *i.e.* ROS inside pancreatic β-cell leading towards the decreased expression of the PDX-1 gene, which in turn causes the suppression of other genes involved in the insulin signaling pathway including INS-1 and INS-2 [35]. Plant extracts with the ability to inhibit the enzymes are useful in DM care. Numerous studies have reported that phytocompounds such as rutin, quercetin, and cyanidin alter the digestion as well as metabolism and absorption of carbohydrates in the gut [36]. Insulin resistance is a major problem in the management of DM. Defects in insulin receptors, transport of glucose, oxidation of glucose, metabolism of fatty acid, and synthesis of glycogen lead to insulin resistance. Tissues of adipose, liver and skeletal muscle are the most resistant to insulin in Type-2 DM. GLUT4 is active in adipose tissue and muscle and regulates the uptake of glucose [37]. Augmented expression of these receptors

facilitates glucose storage as glycogen. The results of current research showed that fig extract and feed supplementation have the potential to modulate the expression of insulin signaling pathway genes. This may be attributed to the bioactive moieties of figs that reverse the damage induced by the alloxan. In a study, the treatment of insulinoma cells with resveratrol upregulated the expression of some key genes such as GLUT2, Pdx1, and INS1 through regulating SIRT1 [38].

Pias-2 gene encodes a member of the protein inhibitor of the activated STAT family, which modulates several cellular processes including cell proliferation, innate immune system, inflammation cascade, and DNA damage. It is also suspected to modulate insulin resistance pathogenesis [39]. The Calm-2 gene is a member of the calmodulin gene family. It is a calcium-binding protein that plays a role in signaling pathways, cell cycle progression, and proliferation. Calm-2 gene has been identified to be associated with a risk of Type-2 diabetes [40]. The Grk2 gene plays an essential role in regulating insulin signaling and insulin-mediated glucose homeostasis in diabetic animals and its inhibition can restore insulin sensitivity in metabolically active tissues [41]. The result of the current study demonstrates the positive effect of fig-based interventions in regulating glucose homeostasis. The protein encoded by the IGF-1 gene is like insulin in function and structure and involved in facilitating growth and development [42]. The FOXO gene family plays a vital role in the development and maintenance of the endocrine pancreas. The Ki-67 gene encodes a nuclear protein that is associated with cell proliferation [43]. In the present study, the Ki-67 was augmented in positive control as beta cell damage (due to Alloxan) increase the expression of this gene.

It is apparent from the present results that fig extract and fruit have the potential to modulate the expression of the above analyzed genes, due to the presence of some bioactive components that reverse the damage induced by the alloxan. The results of the present study are supported by previous studies where different bioactive components from plants were tested for their potential to modulate gene expressions. In a previous study, the higher concentration of ROS resulted in increases in IGF-1 mRNA expression and protein in the rats [44]. Moreover, a higher level of ROS in the G_1 was also observed in the current study which might result in the overexpression of the IGF-1 gene. It is evident from the results that fig extract and feed supplementation have the potential to restore the antioxidative potential of the body by modulating the expression of this gene. Figs have several potential bioactive components such as flavonoids, phenolics, and anthocyanins that reverse the damage induced by the alloxan. In another study, polyphenols from tomato and soy help to downregulate the expression level of IGF-1 [45]. In rat pancreatic cells, supplementation with epigallocatechin gallate for two hours increased the expression of Pdx-1 and FOXO1 resulting in augmented β-cell viability and insulin secretion [36].

Amylin plays an important role in satiety regulation by controlling (slower) gastric emptying, which controls the spikes in post-prandial glycemic load [46]. The average surge in the amylin gene expression in G_4 compared to G_0 and other groups might result in better control of blood, and serum glucose (discussed in sections 3.1. and 4.1.). Leptin has been understood to have a main role in energy balance. It plays a role in mediating energy balance and food intake suppression which leads to weight loss. It can conclude from the result that fig extract and feed supplementation have the potential to modulate the expression of this gene. It can also interpret that fig has some bioactive components that reverse the damage induced by the alloxan.

5. CONCLUSION

Recently, for managing diabetes-related complications, a growing interest has been noted in developing natural antidiabetic drugs, especially from plant sources. Recent studies have reported that the crude extracts and active compounds from various Ficus species exhibit antidiabetic properties under various in vitro and in vivo models. In conclusion, the present work has demonstrated that 10 % dried *Ficus carica* incorporated in feed and its ethanolic extract have the potential to normalize oxidative stress, induce regeneration in cells, and reverse the damage induced by alloxan. It helped to normalize the blood and serum glucose and improved insulin concentration. Moreover, it was able to modulate the expression of genes of the

insulin signaling pathway (INS-1, INS-2, Pdx-1), calcium signaling pathway (Pias-2, Calm-2, Grk-2), regeneration pathway (IGF-1, FOXA-1, KI67), hormones (amylin, leptin), and glucose transporter (GLUT-2). INS-1, INS-2, amylin, and GLUT-2 gene expression were downregulated in diabetic rat control, where these genes showed more expression in treated groups.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors have no conflict of interest.

8. ETHICAL STATEMENT

The study was conducted in an Experimental Animal House of the Faculty of Food, Nutrition, and Home Science, University of Agriculture, Faisalabad (UAF). The research was performed by observing the Institutional Biosafety Committee (IBC) guidelines provided by the institution (UAF). The ethical approval number is D. No.: 8856/ORIC, Dated: 28 November 2019.

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10. DECLARATION

I declare that the results are original, and the same material is neither published nor under consideration elsewhere. The approval of all authors has been obtained and in case the article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Science.