RESEARCH ARTICLE

"*Ficus johannis* Boiss. leaves ethanolic extract ameliorate streptozotocin-induced diabetes in rats by upregulating the expressions of GCK, GLUT4, and IGF and downregulating G6P"

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Abstract

The leaves of *Ficus johannis* Boiss (*F. johannis*), commonly known as Fig tree, Anjir, and Teen, are used by the folk medicinal practitioners in Iran for controlling hyperglycemia in diabetic patients. This study investigated the pharmacological basis for antidiabetic effect of the ethanolic extract of *F. johannis* leaves using in vitro and in vivo experimental models. Qualitative screening of phytochemicals, estimation of total phenolic and flavonoid contents, and in vitro antioxidant and α-amylase inhibition assays were performed. Moreover, the High-performance liquid chromatography (HPLC) quantification, acute toxicity, glucose tolerance, and in vivo antidiabetic effect along with the evaluation of gene expressions involved in diabetes mellitus were carried out. Significant quantities of phenolic (71.208 ± 2.89 mgg^{-1} GAE) and flavonoid (26.38 ± 3.53 mgg^{-1} QE) were present. Inhibitory concentration (IC$_{50}$) of the plant extract exhibited an excellent in vitro antioxidant (IC$_{50}$ = 33.81 µg/mL) and α-amylase (IC$_{50}$ = 12.18 µg/mL) inhibitory potential. The HPLC analysis confirmed the gallic acid (257.79 mgg^{-1}) as main constituent of the extract followed by kaempferol (22.86 mgg^{-1}), myricetin (0.16 mgg^{-1}), and quercetin (3.22 mgg^{-1}). Ethanolic extract displayed glucose tolerance in normo-glycemic rats. Streptozotocin-induced hyperglycemia declined dose dependently in the extract treated rats with improvement in lipid profile and liver and renal function biomarkers. The *F. johannis*-treated groups showed an increase in mRNA expressions of glucose transporter 4 (GLUT-4), glucokinase, insulin growth like factor 1 and peroxisomal proliferator activating receptor gamma in pancreas. However, the Glucose-6-phosphatase was downregulated. Present study suggests that the ethanolic extract of *F. johannis* leaves demonstrates a good anti-diabetic profile by improving insulin sensitivity, GLUT-4 translocation, and carbohydrate metabolism while inhibiting lipogenesis.

Keywords *Ficus johannis* · Antioxidant · Polymerase chain reaction · Streptozotocin · Diabetes mellitus

Abbreviations

ALT	Aalanine aminotransferase
ALP	Alkaline phosphatase
AlCl3	Aluminium chloride
AST	Aspartate aminotransferase
DC	Diabetic control
DM	Diabetes mellitus
DMSO	Dimethylsulfoxide
EDTA	Ethylenediaminetetraacetic acid
F. johannis	*Ficus johannis* Boiss.
FJ 125	*Ficus johannis* 125 Mg/kg
FJ 250	*Ficus johannis* 250 Mg/kg
FJ 500	*Ficus johannis* 500 Mg/kg
FC	Folin-Ciocalteu's
G6P	Glucose-6-Phosphatase
GCK	Glucokinase
GLUT4	Glucose transporter 4
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GAE	Gallic acid equivalent
HDL	High density lipoproteins
H&E	Haematoxylin and eosin
HPLC	High-performance liquid chromatography
IC	Inhibitor concentration
IREC	Institutional Research Ethics Committee
IGF	Insulin growth like factor
LDL	Low density lipoproteins

NIDDM	Non-insulin-dependent diabetes mellitus
NA	Nicotinamide
NC	Normal control
NFJ	Normal + *Ficus johannis*
OGTT	Oral glucose tolerance test
OECD	Organization for Economic and Co-operation and Development
PPAR-γ	Peroxisomal proliferator activating receptor gamma
PC	Positive control
CH3CO2K	Potassium acetate
PCNA	Proliferating cell nuclear antigen
RT-PCR	Reverse transcriptase polymerase chain reaction
PPN	Parts per million
RNA	Ribonucleic acid
Na$_2$CO$_3$	Sodium carbonate
STZ	Streptozotocin
TOP2A	Topoisomerase 2 alpha
TC	Total cholesterol level
TG	Triglyceride level
UV	Ultraviolet
v-LDL	Very low-density lipoproteins
WHO	World Health Organization
DPPH	2,2-Diphenyl-1-picrylhydrazyl

Introduction

Diabetes mellitus (DM) is an endocrinal disorder, acknowledged as long-term hyperglycemia with defective insulin production or its action and metabolic abnormalities in carbohydrates, proteins, and fats (Justino et al. 2018). International diabetes federation estimates that approximately 537 million adults are suffering from diabetes and projected a raise, up to 643 million by the year 2030. It is also estimated that 3 in 4 of these individuals live in low- or middle-income countries. The global public health abnormality is due to the decreased production of insulin or its resistance to liver and adipose tissues (Chaari et al. 2020) (Rossi 2018). Reactive oxygen species cause type 2 DM, owing to unstable nature of free radicals. Non-enzymatic glycation of proteins, glucose oxidation, and augmented lipid peroxidation generates free radicals which further damage enzymes and cellular machinery and develop insulin resistance due to oxidative stress Resistance to insulin is due to insulin receptors which are unable to respond properly to insulin production (Lugrin et al. 2014). Relative insulin deficiency and peripheral insulin resistance leads to type II DM (care 2020). Insulin is responsible for inhibition of glucose production. It does so by suppression of glucose 6 phosphatase (Nakae et al. 2001). Glucose 6 phosphatase

and glucokinase act in opposite direction. They regulate free glucose intracellularly. Glucokinase activity is decreased in type II DM. Increased ratio of glucose 6 phosphatase to glucokinase, causes efflux of glucose into the blood stream (Haeusler et al. 2015). Similarly, glucose transporter type 4 (GLUT4) expressions are reduced in diabetes mellitus leading to decreased plasma glucose clearance and altering glycemic homeostasis (Passarelli and Machado 2021). Insulin like growth factor interacts with insulin receptors and mediates signaling pathways in different tissues to facilitate carbohydrate metabolism (Lewitt et al. 2014).

A large number of DM sufferers are found obese having large amount of fat in abdominal region (Sabry et al. 2019). Different treatment modalities are available for the management of diabetes. The criteria for the selection of the treatments are based upon disease prevalence, age, gender, and other associated diseases (Peters and Davidson 1996).

Plants play an essential role in the management of a number of diseases like diabetes mellitus, atherosclerosis, and hepatotoxicity (Bano et al. 2023, Barkaoui et al. 2017; Tag et al. 2012; Zhi et al. 2023). These medicinal treatment practices are being used over thousands of years around the globe (Omara et al. 2010). Generally, natural products derived from plants and herbs have low toxicity profiles and high patient compliance (Wang et al. 2017). Most of the herbal medicines and nutraceuticals have some bioactive phytochemicals which are scientifically proven for the treatment of diabetes mellitus (Giovannini et al. 2016; Mousavi et al. 2018). Human diet makes available a large number of phytochemicals with therapeutic potential. Phenolic compounds are the most abundant class of phytochemicals in edible plants, fruits, and beverages (Dias et al. 2017). The demand for natural hypoglycemic agent is increasing day by day (Patel et al. 2012); therefore, plant extracts can be considered an attractive approach for the development of diabetic treatment or disease mitigation (Chaari et al. 2020).

The genus "Ficus" comprises of 850 species, and is widely distributed in tropical and semitropical moderate zones. These plants are a significant source of food for frugivores throughout the year. About 132 species of "Ficus" are reported to be utilized for nutritional consumption. This plant *Ficus johannis* Boiss (*F. johannis*) is grown commonly in different parts of Iran, Pakistan, and Afghanistan. The leaves of *F. johannis* commonly known as Fig tree, Anjir, and Teen are used by the folk medicinal practitioners of Iran for controlling glucose level in diabetic patients (Baharvand-Ahmadi et al. 2016) but no scientific data is reported till now. So, we conducted this study to report its antidiabetic activity in Pakistan. The present study was intended to evaluate the anti-hyperglycemic potential of *F. johannis* leaves by in vitro and in vivo models as its anti-diabetic use is reported in folklore and is used in different regions around the world.

Methods and materials

Chemicals

Streptozotocin (STZ), α-amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, acarbose, and nicotinamide (NA) were purchased from Sigma-Aldrich Chemical Co. (Germany). TRIzol reagent was purchased from Advance Bioscience (Germany). All kits for analysis were purchased from Biolabs and Vivantis Technologies. All chemicals and reagents were used of analytical grade.

Collection of plant material

Fresh *Ficus johannis* Boiss (Moraceae) was collected from Abbottabad, Pakistan. It is located in Khyber-Pakhtunkhwa province between 34°09 ′N latitude and 73°13 ′E longitude at an altitude of 4120 feet (1260 m) (Ijaz et al. 2016). Taxonomic identification for authenticity was confirmed by Dr Zaheer-Uddin, Professor, Department of Botany, Government College University, Lahore using binocular microscope, comparing specimen in the herbarium and consulting (Baharvand-Ahmadi et al. 2016). A voucher was deposited at the herbarium of the university (GC.Herb.Bot.3540).

Preparation of plant extract

The leaves of *F. johannis* were separated from stems and washed with tap water to get rid of the adhering dust and foreign particles. The plant material was sliced into small pieces and shade dried. The dried plant material was powdered (700 g) and macerated in 98% ethanol (2000 mL) for 7 days. The solvent was filtered with whatman no.1 filter paper. The filtrate was concentrated under reduced pressure in a rotary evaporator at 40 °C. The extract was dried in a dry heat oven at 40 °C and stored in a refrigerator at 4 °C for further use in pharmacological evaluation (Mousavi et al. 2016).

Total phenolic content

The total phenolic content of *F. johannis* leaves extract was calculated by Folin-Ciocalteu's (FC) reagent method with slight modifications and stated in mg/g gallic acid equivalent (GAE) of plant extract. *F. johannis* extract was diluted at different concentrations (100, 80, 60, 40, and 20 μg/mL) with methanol. Nine-milliliter distilled water and 1 mL FC reagent were added to dilutions and mixed for 5 min. Ten-milliliter 7% sodium carbonate (Na_2CO_3) and 4 mL distilled water were added and incubated for 90 min at 37 °C. The absorbance was determined by ultraviolet (UV) spectrophotometer at 760 nm. Linear regression model was used to determine the phenolic content using the standard calibration curve of gallic acid (Khan et al. 2020).

Total flavonoid content

The total flavonoid content of *F. johannis* leaves extract (100, 80, 60, 40, and 20 μg/mL) was determined by aluminium chloride ($AlCl_3$) colorimetric method with slight modifications. The procedure involves the addition of 200 μL extract dilution, 100 μL $AlCl_3$, 100 μL 1 M potassium acetate (CH_3CO_2K) solution, and 4.6 mL distilled water and incubated at 37 °C for 45 min. Same procedure was done for quercetin. The absorbance of these samples was determined by UV spectrophotometer at 415 nm. Total flavonoid content was estimated utilizing quercetin calibration curve (Zafar et al. 2021). The results were resolved as per quercetin equivalent (mg of quercetin/g of concentrate).

High-performance liquid chromatography (HPLC):

The identification of flavonoid and phenolic in *F. johannis* ethanolic leaves extract was carried out by high-performance liquid chromatography (HPLC). The HPLC system Shimadzu was used with a UV visible detector (280 nm), LC-10AT pump, PDA detector SPD-10AV and column (25 cm × 4.6 mm, 5 μm), and two mobile phases A (H_2O: acetic acid-94:6) and B (acetonitrile 100%) with a flow rate 1 mL/min (Malik et al. 2022).

In vitro antioxidant activity by DPPH scavenging assay

Antioxidant potential of *F. johannis* ethanolic leaves extract was determined by DPPH scavenging assay. Briefly 1 mL of extract solution was incubated with 2 mL of DPPH solution for a time period of 30 min and absorbance was recorded at 517 nm. IC_{50} was calculated at different the concentrations of extract (100, 80, 60, 40, 20, and 10 μg/mL) were used to determine the percentage inhibition and later on IC_{50} was calculated (Abbas et al. 2020). The standard used was ascorbic acid.

$$Inhibition\% = \left[\frac{absorbance(control) - absorbance(sample)}{absorbance(control)} \right] \times 100$$

α-Amylase inhibitory activity

The in vitro hypoglycemic activity of *F. johannis* ethanolic leaves extract was calculated by α-amylase inhibitory assay with slight modifications. Acarbose was used as a standard and dissolved in dimethylsulfoxide (DMSO) (2 mg/mL). 1 mL α-amylase solution and 2 mL 0.02 M phosphate buffer

was added to 1 mL extract dilutions (100, 80, 60, 40, 20, and 10 µg/mL) and incubated at 37 °C for 30 min. 1 mL starch solution (0.5%) was added to previous solution and incubated for 15 min. 1 mL DNS reagent was added and heated at 90 °C in a water bath for 8 min. The absorbance was checked by UV spectrophotometer at 540 nm. IC_{50} was calculated from percentage inhibition by the following formula (Khan et al. 2020a).

$$Inhibition\% = \left[\frac{Absorbance\ (control) - Absorbance\ (sample)}{Absorbance\ (control)} \right] \times 100$$

12 h light/dark cycles. A standard pelleted diet was given to all rats ad libitum with a dietry composition of carbohydrate 58%, fat 5.7%, protein 24%, ash 8%, and fiber 6% and having free access to tap water. The rats were shifted to laboratory 1 week before the experiment to adapt the laboratory environment. The study was approved from the Institutional Research Ethics Committee (IREC-2019–87) of the University of Lahore and the study was executed accordingly (Mousavi et al. 2018).

Acute toxicity study

The acute oral toxicity study of *F. johannis* ethanolic leaves extract was performed according to the Organization for Economic and Co-operation and Development (OECD) guidelines. Twelve albino rats were assigned to 4 groups ($n = 3$) randomly. These rats were fasted over night with free access to water. Group I was given normal saline. Groups II, III, and IV were given 2000, 1000, and 500 mg/kg *F. johannis* extract orally via gastric gavage tube. After administration, the rats were carefully observed for behavioral and

Experimental animals

Colony-bred, adult male Albino Wistar rats were purchased from University of Veterinary and Animal Sciences, Lahore as experimental animals with 170–220 g body weight and aged between 10 and 12 weeks. The selected animals were kept in standard spacious polypropylene cages under standard environmental conditions such as, 25 ± 2 °C temperature with

toxicological symptoms at 0.5, 4, 8, 12, 24, 48, 72 h, and 1 week (Thomson et al. 2015).

Oral glucose tolerance test (OGTT)

OGTT was performed on normal rats before the start of study. The normal rats were assigned to five groups and each group consisted of five animals ($n = 5$). The group I, control group was fed with normal saline. The group II was fed metformin 100 mg/kg, used as a positive control. The groups III, IV, and V were fed orally with 3 different doses of *F. johannis* leaves extract 125, 250, and 500 mg/kg, respectively. All treatments were given to the rats, 5 min before loading glucose (2 g/kg). After 0, 0.5, 1, 1.5, 2, 2.5, 3 h blood samples were collected from tail vein. The blood glucose levels were measured by (Accu-Check glucometer, Roche, Switzerland) (Malik et al. 2022). The hypoglycemic potential of the plant was determined by calculating AUC on GraphPad Prism 5.0.

$$\% \text{ Hyperglycemia inhibition factor} = \frac{AUC\ (control\ group) - AUC\ (treatment\ group)}{AUC\ (control\ group)} \times 100$$

Experimental design

The experimental animals were randomly assigned to seven groups, each containing five rats ($n = 5$). The division of groups was in the following manner:

- Group I: Normal Control (NC), the normal rats given normal saline oral gavage tube for 21 days.
- Group II: Positive Control (PC), the STZ induced diabetic rats received metformin 100 mg/kg as a standard drug orally, by oral gavage tube once daily for 21 days.
- Group III: Diabetic Control (DC), the STZ induced diabetic rats given normal saline by oral gavage tube for 21 days.
- Group IV: Normal + *Ficus johannis* (NFJ500), the normal rats received 500 mg/kg plant extract by oral gavage tube once daily for 21 days.

- Group V: *Ficus johannis* 125 mg/kg (FJ125), the STZ induced diabetic rats received 125 mg/kg plant extract by oral gavage tube once daily for 21 days.
- Group VI: *Ficus johannis* 250 mg/kg bw (FJ250), the STZ treated diabetic rats received 250 mg/kg plant extract by oral gavage tube once daily for 21 days.
- Group VII: *Ficus johannis* 500 mg/kg bw (FJ500), the STZ induced diabetic rats received 500 mg/kg plant extract by oral gavage tube once daily for 21 days (Hassan et al. 2015).

Induction of diabetes

Type II diabetes was induced in 12 h fasted rats. A single dose of NA (120 mg/kg) dissolved in 0.5 mL normal saline was administered intraperitoneal. After 15 min, STZ (60 mg/kg) in a single dose, freshly dissolved in 0.5 mL 0.01 M citrate buffer

(pH 4.5) was administered via intraperitoneal route (Rouhi et al. 2017). Five percent dextrose solution was administered to overnight fasted rats followed by STZ administration to prevent hypoglycemic shock (Soni et al. 2018). Glucometer was used to access the levels of blood glucose. Rats with blood glucose levels above 250 mg/dL were considered diabetic and inducted in the study (Thomson et al. 2015).

In vivo anti-diabetic activity

Post induction the rats were kept under observation for 21 days. During this period, rats were administered with the respective doses mentioned in experimental design via oral gavage tube daily. The blood glucose level was determined at days 1, 7, 14, and 21 under fasting conditions with glucometer (ACCU-CHEK, Roche, Switzerland). Moreover, we also monitored feed and water intake along with their body weights (Zafar et al. 2021).

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total ribonucleic acid (RNA) was isolated from pancreas using Trizol reagent. The RNA was reverse transcribed to single stranded complementary deoxy ribonucleic acid cDNA according to the protocol of cDNA synthesis kit (Cat no. 4368814). Species specific primers were synthesized. The cDNA was amplified in thermal cycler by given protocol of PCR kit (Cat no. 32161000). Ten-microliter PCR products, stained with ethidium bromide, were loaded in 2% agarose gel in 1×Tris Acetate Ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.3–8.5) (Irudayaraj et al. 2016; Kim et al. 2010; Stalin et al. 2016). The electrophoretic picture was quantified by the ImageJ software and GraphPad Prism 5.0.

Biochemical analysis

At the end of the study period (21 days), rats were anesthetized using diethyl ether. Cervical dislocation was performed and blood was collected in sterile tubes through cardiac puncture. Blood sampling was performed in EDTA tubes to study the hematological variables. To study the biochemical parameters centrifugation of the blood was performed at 1500 g for a time period of 15 min at temperature 4 °C. The sera samples were separated and used to analyze triglyceride contents (TG), cholesterol contents (TC), High density lipoproteins level (HDL), low density lipoproteins level (LDL), very low-density lipoproteins level (v-LDL), alanine aminotransferase level (ALT), alkaline phosphatase level (ALP), aspartate aminotransferase level (AST), urea concentration, and creatinine contents. The samples were analyzed at the diagnostic Lab of the University of Lahore (Bano et al. 2023, Toma et al. 2015).

Histopathological investigation

After the 21 days' study, the rats were sacrificed and their liver, kidney, and pancreas were removed. The histopathological examination was carried out by washing each tissue with ice-cold 0.9% saline and fixing the tissue sections in 10% neutral buffered formalin. The tissue sections were processed in graded series of ethanol and embedded in paraffin wax. Tissue sections were cut (5 μm thick) and stained with haematoxylin and eosin (H&E). Later, the microscopic examinations were carried out and photographed (Toma et al. 2015). Field views were selected randomly. Light microscope (optika vision lite 2.1) was used to view the histology slides for any signs of toxicity. A pathologist who was un aware of the treatment groups accessed the slides. A scoring system was devised (Table 1) to access the level of damage to the tissues. We devised a total of five grades. The grades were scored on the basis of the level of the damage ranged from 0 to 4. Hepatic alterations include inflammation, sinusoidal space dilation, and hydropic swelling ballooning hepatocytes whereas kidney injury includes fibrosis, dilation of renal tubule, and space of the glomerular capsule. For the severity of the pancreatic injury, we used the same parameters as we have accessed in one of our previous studies.

Statistical analysis

The data was presented by taking mean ± SD. GraphPad Prism 5.0 was used to determine IC_{50} using non-linear regression model. Furthermore, one-way analysis of variance (ANOVA) was also used to analyze the data followed by Bonferroni's multiple comparison test.

Results

Percentage yield

The percentage yield of *F. johannis* ethanolic leaves extract was calculated to be 6.36%.

Preliminary phytochemical screening

The phytochemical screening confirmed the presence of some essential bioactive compounds including cardiac glycosides, flavonoids, alkaloids, phenolics, carbohydrates, proteins, saponins, and fixed oils (supplementary data Table 1).

Table 1 Histopathological scoring system for hepatic, renal, and pancreatic changes

Sr no	Organ	Item	Score	Grading
1	Pancreas	Inflammation	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
		Degeneration of acinar cells	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
		Degeneration of beta cells	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
2	Liver	Inflammation	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
		Sinusoidal space dilation	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
		Hydropic swelling ballooning hepatocytes	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
3	Kidney	Fibrosis	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
		Dilation of renal tubule	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4
		Space of the glomerular capsule	<1%	0
			1 to 25%	1
			26 to 50%	2
			51 to 75%	3
			>75%	4

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of *F. johannis* ethanolic leaves extract (100 µg/mL) was calculated as 71.208 ± 2.89 mgg^{-1} GAE and 26.38 ± 3.53 mgg^{-1} QE, respectively.

HPLC analysis

The HPLC investigation of *F. johannis* ethanolic leaves extract demonstrated the presence of kaempferol (22.86 mgg^{-1}), gallic acid (257.79 mgg^{-1}), myricetin (0.16 mgg^{-1}), and quercetin (3.22 mgg^{-1}) (Table 2).

DPPH radical scavenging activity

F. johannis ethanolic leaves extract showed the highest percentage inhibition at 100 µg/mL (77.98%) with IC$_{50}$-33.81 µg/mL (Fig. 1A). Ascorbic acid showed IC$_{50}$-20.19 µg/mL with a percentage inhibition (94.62%).

α-Amylase inhibitory assay

Concentration dependent inhibition was exhibited by *F. johannis* ethanolic leaves extract which was the highest at maximum dose 100 µg/mL. The percentage inhibition of *F. johannis* was 82.43% (IC$_{50}$-28.49 µg/mL) (Fig. 1B) which was close to that of Acarbose 97.23% (IC$_{50}$-12.18 µg/mL).

Acute oral toxicity

The *F. johannis* ethanolic leaves extract was found to be non-lethal even at highest orally administered dose 2000 mg/kg body weight. The rats were monitored for 1 week. No mortality was observed and rats did not exhibit any signs of toxicity (Table 3). The findings suggest the safety of the extract to use in experiments further.

Oral glucose tolerance test (OGTT)

Remarkable hypoglycemic activity was observed by *F. johannis* ethanolic leaves extract compared to metformin. FJ500 exhibited (21.49%) (Fig. 2) inhibition factor which is comparable to standard drug (27.53%).

Anti-hyperglycemic potential of *F. johannis*

F. johannis ethanolic leaves extract exhibited significant hypoglycemic potential in STZ-NA loaded rats. The glucose levels of FJ500 and PC on day 1 were 338 ± 11.2 and 345 ± 9.45 mg/dl respectively. These levels declined significantly ($p < 0.05$) to 133 ± 6.62 and 120 ± 7.44 mg/dl on day 21. The results were compared with disease control group (Fig. 3). However, the glucose level of DC on day 1 and day 21 was 350 ± 7.42 and 394 ± 6.96 mg/dl, respectively.

Effect on dietary intake and weight alterations

Weight gain, feed, and water intake were observed on days 1, 7, 14, and 21. Significant improvement in weight gain was

Table 2 Quantification of phyto-constituents in the *F. johannis* ethanolic leaves extract by HPLC

Compound	Retention time (min)	Area (mAU*s)	Height (mAU)	Amount (mgg^{-1})
Gallic Acid	3.080	498.329	31.476	257.79
Myricetin	4.172	14.420	1.216	0.16
Quercetin	5.808	25.877	2.236	3.22
Kaempferol	8.432	1639.505	97.601	22.86

Fig. 1 Percentage inhibition of *F. Johannis* leaves extract in **A** alpha amylase assay and **B** DPPH assay
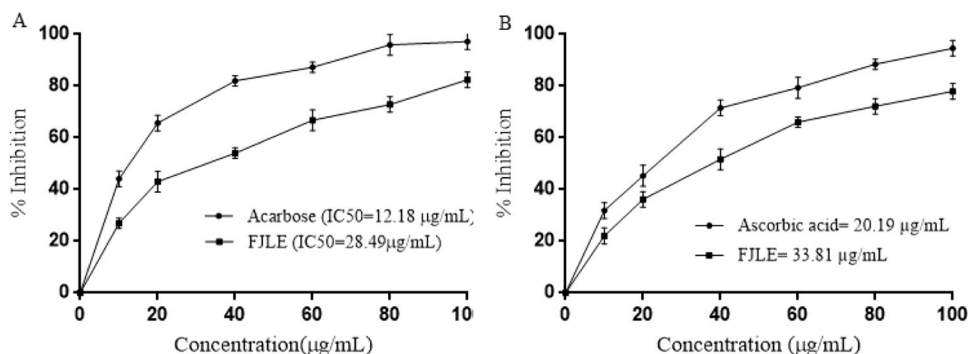
Table 3 Observation of behavioral changes during acute toxicity study

Behavioral changes	Groups			
	Group I (NS)	Group II (FJ 2000 mg/kg)	Group III (FJ 1000 mg/kg)	Group IV (FJ 500 mg/kg)
Straub tail	Absent	Absent	Absent	Absent
Sedation	Absent	Absent	Absent	Absent
Excitation	Absent	Absent	Absent	Absent
Analgesia	Absent	Absent	Absent	Absent
Abnormal gait	Absent	Absent	Absent	Absent
Pilo erection	Absent	Absent	Absent	Absent
Aggressiveness	Absent	Absent	Absent	Absent
Mydriasis	Absent	Absent	Absent	Absent
Myosis	Absent	Absent	Absent	Absent
Hyperthermia	Absent	Absent	Absent	Absent
Hypothermia	Absent	Absent	Absent	Absent
Skin color	Normal	Normal	Normal	Normal
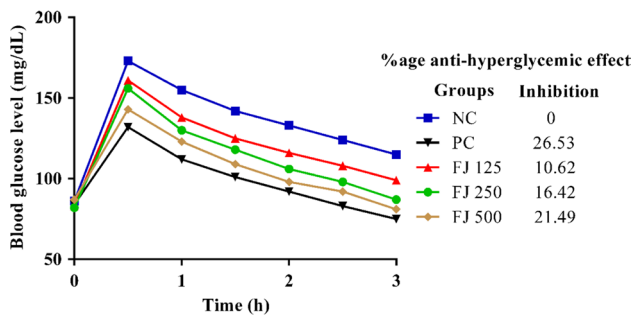
Fig. 2 Effect of the treatments on glucose tolerance level in Wistar rats. OGTT was performed on overnight fasting animals ($n = 5$). Here NC, PC, and FJ represent the normal control, positive control, and *F. johannis* respectively

observed ($p < 0.05$) compared to the normal group (Table 4). Diabetic rats consumed higher amounts of feed and water. The administration of *F. johannis* ethanolic leaves extract significantly reduced ($p < 0.05$) the quantity of food and water consumed by diabetic rats (Tables 5, 6).

Evaluation of proliferation

Gene expression of KI67 decreased in the disease control group, upon treatment with NFJ500 expression of KI67 increased. Similarly, peroxisomal proliferator activating receptor gamma (PPAR-γ), topoisomerase 2 alpha (TOP2A), and proliferating cell nuclear antigen (PCNA) gene expressions are also increased in dose-dependent manner compared to disease control group. Post treatment, the levels of gene expression were found in the following descending order NFJ500 > NC > PC > FJ500 > F

Fig. 3 Effect of the treatments on the animal blood glucose levels during anti-hyperglycemic study. Data is presented as mean ± SD. The level of significance was estimated by one-way ANOVA followed by Fisher's LSD test and is expressed as "a" ($p < 0.05$), "b" ($p < 0.01$), and "c" ($p < 0.001$) when compared to the disease control group. Abbreviations: NC; normal control, PC; positive control, NFJ; normal rats treated with *F. johannis*, FJ; *F. johannis*; and DC; diabetic control
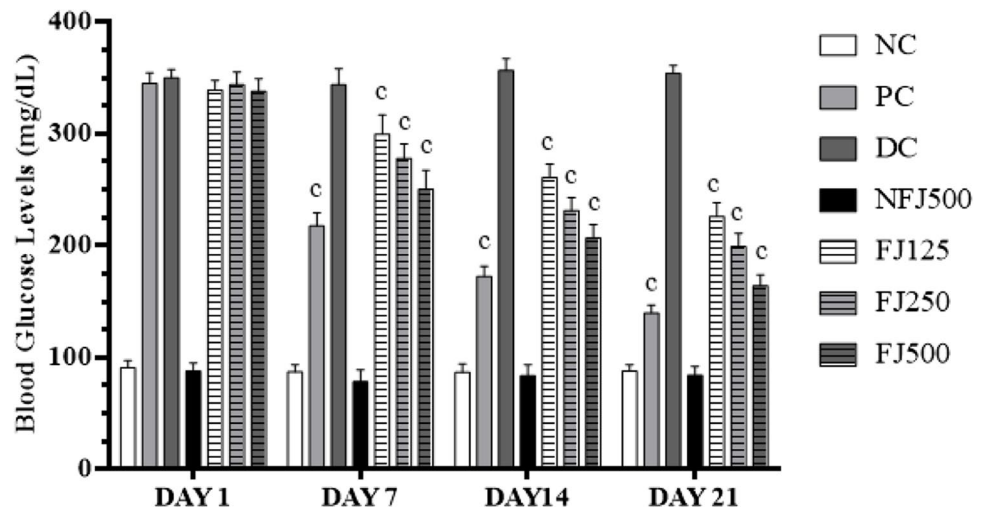
Table 4 Effect of *F.johannis* ethanolic leaves extract and metformin on body weight gain

Days	Body weights (g)						
	NC	PC	DC	NFJ 500	FJ 125	FJ 250	FJ 500
1	189 ± 4.93	189 ± 5.76	188 ± 3.94	190 ± 5.24	191 ± 6.19	187 ± 6.53	184 ± 4.34
7	194 ± 4.76	192 ± 5.13	184 ± 3.96	195 ± 6.80	189 ± 5.86	189 ± 6.30	186 ± 4.64
14	201 ± 5.94	196 ± 4.32	177 ± 3.96	202 ± 6.44^{c}	187 ± 5.66	191 ± 6.27	190 ± 5.07
21	212 ± 5.41	201 ± 4.44	170 ± 3.90	211 ± 6.14	184 ± 6.06^{a}	192 ± 6.35^{b}	193 ± 4.66^{c}

The level of significant difference was estimated by one-way ANOVA followed by Fisher's LSD test. Data is presented as mean $\pm$ standard deviation; [a]represents $p < 0.05$, [b]represents $p < 0.01$, and [c]represents $p < 0.001$ when compared to disease control group. Abbreviations: *NC*, normal control; *PC*, positive control; *NFJ*, normal rats treated with *F johannis*; *FJ*, *F johannis*; *DC*, diabetic control

Table 5 Effect of *F. johannis* ethanolic leaves extract and metformin on food intake

Days	Food intake (g)						
	NC	PC	DC	NFJ 500	FJ 125	FJ 250	FJ 500
1	16.4 ± 1.14	26.4 ± 1.14^{c}	27.8 ± 1.78^{c}	17.0 ± 1.87	29.4 ± 1.51^{c}	28.8 ± 1.92^{c}	28.0 ± 1.22^{c}
7	16.2 ± 0.83	29.6 ± 1.14^{c}	31.8 ± 1.92^{c}	16.6 ± 1.81	30.6 ± 1.14^{c}	29.6 ± 1.14^{c}	29.0 ± 1.58^{c}
14	16.8 ± 0.83	20.8 ± 1.78^{b}	32.0 ± 1.58^{c}	15.4 ± 1.14	25.8 ± 1.30^{c}	23.6 ± 1.67^{c}	21.2 ± 1.78^{b}
21	14.8 ± 0.83	18.0 ± 1.22^{a}	30.0 ± 1.58^{c}	15.2 ± 0.83	24.6 ± 1.51^{c}	21.2 ± 1.48^{c}	17.8 ± 1.30^{a}

The level of significant difference was estimated by one-way ANOVA followed by Fisher's LSD test. Data is presented as mean $\pm$ standard deviation; [a]represents $p < 0.05$, [b]represents $p < 0.01$, and [c]represents $p < 0.001$ when compared to normal control group. Abbreviations: *NC*, normal control; *PC*, positive control; *NFJ*, normal rats treated with *F johannis*; *FJ*, *F johannis*; *DC*, diabetic control

Table 6 Effect of *F.johannis* ethanolic leaves extract and metformin on water intake

Day	Water intake (mL)						
	NC	PC	DC	NFJ 500	FJ 125	FJ 250	FJ 500
1	29.6 ± 6.06	78.2 ± 5.45^{c}	82.6 ± 4.82^{c}	28.6 ± 7.79	74.8 ± 7.91^{c}	74.0 ± 5.00^{c}	75.6 ± 5.72^{c}
7	28.2 ± 8.23	59.6 ± 5.22^{c}	83.2 ± 5.17^{c}	28.0 ± 7.48	68.4 ± 4.34^{c}	67.4 ± 5.13^{c}	62.2 ± 4.97^{c}
14	31.6 ± 4.98	48.6 ± 4.62^{c}	88.2 ± 4.02^{c}	31.8 ± 7.29	62.6 ± 5.55^{c}	58.4 ± 6.3^{b}	52.6 ± 5.41^{a}
21	29.0 ± 5.24	41.6 ± 4.34^{a}	81.6 ± 5.13^{c}	28.8 ± 7.53	56.6 ± 4.98^{b}	52.6 ± 5.98^{a}	48.4 ± 5.03^{a}

The level of significant difference was estimated by one-way ANOVA followed by Fisher's LSD test. Data is presented as mean $\pm$ standard deviation; [a]represents $p < 0.05$, [b]represents $p < 0.01$, and [c]represents $p < 0.001$ when compared to normal control group. Abbreviations: *NC*, normal control; *PC*, positive control; *NFJ*, normal rats treated with *F johannis*; *FJ*, *F johannis*; *DC*, diabetic control

J250 > FJ125 > DC (Fig. 4). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as internal control and remained insignificant for all groups.

Evaluation of apoptosis

Gene expression of apoptotic markers exhibited upregulation in diseased control group. Expression levels of BCL2 associated X (BAX) and P53 genes were decreased upon treatment with extracts of *F. johannis*. Post treatment, the levels of gene expression were found to be in the following order NC < NFJ500 < PC < FJ500 < FJ250 < FJ125 < DC (Fig. 5). GAPDH served as internal control and remained insignificant for all groups.

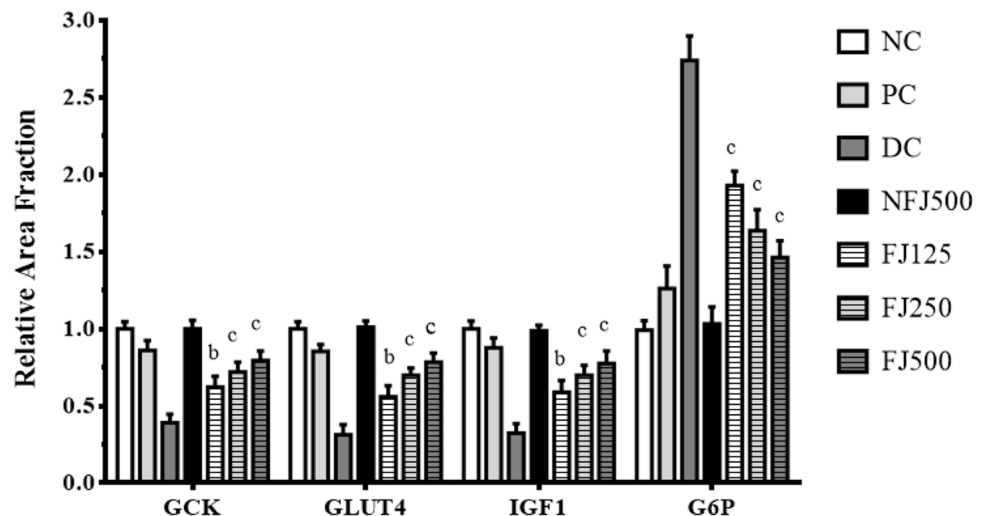## Evaluation of functional genes

Glucose-6-Phosphatase (G6P) expression in diabetic control were increased compared to normal control. Gene expressions of G6P were significantly decreased when the rats were treated with *F. johannis* extracts. The results were comparable to positive control (STZ + 100 mg/kg metformin). On the contrary, the expression of glucokinase (GCK), glucose transporter 4 (GLUT4), and insulin growth like factor (IGF) was decreased in the diseased control groups. Treatment with NFJ500 (normal + 500 mg/kg extract) resulted in increased expressions of the abovementioned functional genes of diabetes (Fig. 6). GAPDH served as an internal control and remained significant in all groups (Table 7).

Fig. 4 Effect of the treatments on relative mRNA expression of genes related to cell proliferation. Data is presented as mean ± SD. The level of significance was estimated by one-way ANOVA followed by Fisher's LSD test and is expressed as "a" ($p < 0.05$), "b" ($p < 0.01$), and "c" ($p < 0.001$) when compared to the disease control group. Abbreviations: NC; normal control, PC; positive control, NFJ; normal rats treated with *F. johannis*, FJ; *F. johannis*; and DC; diabetic control
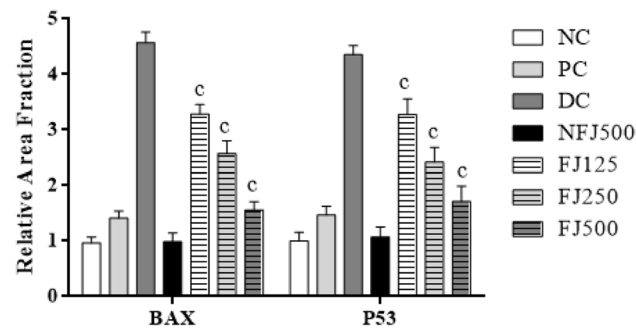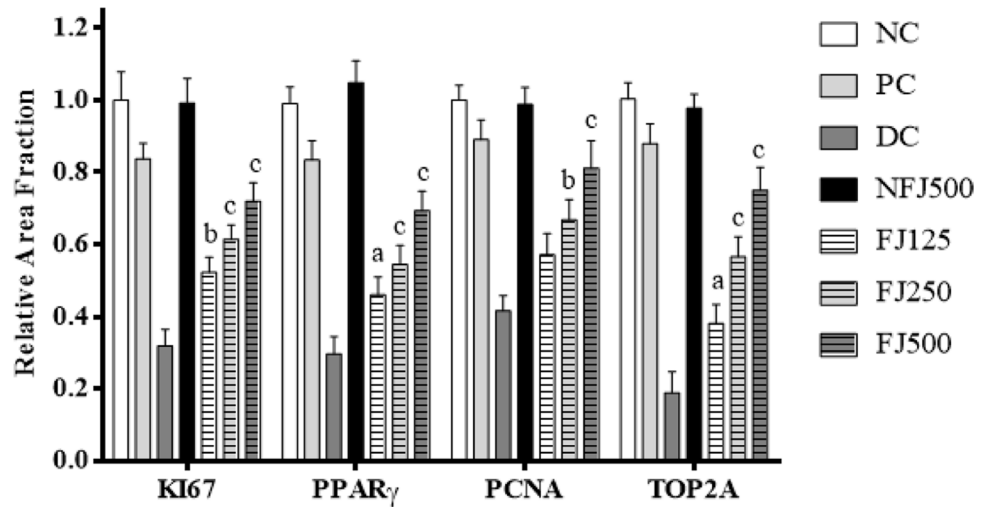
Fig. 5 Effect of treatments on relative mRNA expression of genes related to apoptosis. Data is presented as mean ± SD. The level of significance was estimated by one-way ANOVA followed by Fisher's LSD test and is expressed as "a" ($p < 0.05$), "b" ($p < 0.01$), and "c" ($p < 0.001$) when compared to the disease control group. Abbreviations: NC; normal control, PC; positive control, NFJ; normal rats treated with *F. johannis*, FJ; *F. johannis*; and DC; diabetic control

Biochemical analysis

The liver function tests alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) of blood samples revealed the significant improvement in the plasma samples of *F. johannis* ethanolic leaves extract treated rats. The increased values of enzymes due to induction with STZ were restored close to normal (Fig. 7A) by administration of *F. johannis* ethanolic leaves extract (125, 250, and 500 mg/kg). Lipid profile (LDL, HDL, TC, and TG) levels at 21st day of the study was reinstated close to normal (Fig. 7B, C). Atherogenic index calculated from lipid profile parameters exhibited restoration of index up to (1.41) in FJ500 group compared to DC (7.04). Treatment with ethanolic leaves extract of *F. johannis* helped in decreasing the enhanced concentration of urea in diabetic rats. However, creatinine level was not altered (Table 8). Glycosylated hemoglobin (HbA1c) was also improved in FJ500 group (5.56 ± 0.20 mg/dL), upon treatment with *F.*

Fig. 6 Effect of treatments on relative mRNA expression of functional genes. Data is presented as mean ± SD. The level of significance was estimated by one-way ANOVA followed by Fisher's LSD test and is expressed as "a" ($p < 0.05$), "b" ($p < 0.01$), and "c" ($p < 0.001$) when compared to the disease control group. Abbreviations: NC; normal control, PC; positive control, NFJ; normal rats treated with *F. johannis*, FJ; *F. johannis*; and DC; diabetic control

Table 7 Primer sequences

Gene	Sequence	Product size	Optimized temperature °C
G6P (F)	5′TGCTGGTGCTTTTGTAGGG3′	197	57
G6P (R)	5′ATGAGTTCTTGGCTGGCTTG3′		
IGF-1 (F)	5′GCAAAACATCGGAACACC3′	187	57
IGF-1 (R)	5′AATCTACCCACGCCAGGAC3′		
GAPDH (F)	5′TCATCCAAGCGTGTAAGGGT3′	239	58
GAPDH (R)	5′CCCTACTTTCTCCCCGCTTT3′		
GCK (F)	5′CCAGGAGATGCCAGAGGTTA3′	174	62
GCK (R)	5′CCCACAGTCCCAGGTAGAGA3′		
GLUT4 (F)	5′TGAGCAGGAAATGGTTGTGA3′	212	57
GLUT4 (R)	5′CCCGTGATAAGAAGTGTGTAGG3′		
PPARγ (F)	5′GCAACGGCAAATAGAAGCAG3′	224	58
PPARγ (R)	5′TCAGGAGTAGGGGAAACAGG3′		
PCNA (F)	5′TTAAACGGTTGCAGGCGTAG3′	228	59
PCNA (R)	5′GATGAGGTCCTTGAGTGCCT3′		
KI67 (F)	5′GAGGACTCGACTCGGTGG3′	225	60
KI67 (R)	5′GTCAAGTCGCACCCAAAGTC3′		
TopIIA (F)	5′ACTGGGACACCTCAGATCCA3′	176	60
TopIIA (R)	5′GCAAAGTACCTGGCAAGCA3′		
P53 (F)	5′TTTCTTTGCTGCCGTCTTCC3′	182	55
P53 (R)	5′TGACTGCTTGATGATGGCCA3′		
BAX (F)	5′GAGTGACACCCCGTTCTGA3′	161	60
BAX (R)	5′CGATGCGCTTGAGACACTC3′		

johannis ethanolic leaves extract (Fig. 7D) compared to DC group (7.36 ± 0.18 mg/dL).

## Histopathological investigations

Significant histology changes presented in diabetic control group were reversed by *F. johannis* ethanolic leaves extract justifying its protective effect in pancreas, liver, and kidney sections (Fig. 8A, B, C). Control group exhibited no change in structure of pancreas. Inflamed acinar cells were observed after administration of STZ (Fig. 9A). On 21st day of the study, the administration of *F. johannis* ethanolic leaves extract resulted in repair of pancreatic tissue architecture exhibiting a decrease in inflammation and restoration of acinar cells. Hepatic inflammation, sinusoidal space dilation, and hydropic ballooning hepatocytes were not present in normal rats. Hepatic inflammation, sinusoidal space dilation, and ballooning hepatocytes were seen in diseased rats. Hydropic swelling and ballooning in hepatocytes was greater in the diseased rats when compared to other groups. The treatment with *F. johannis* ethanolic leaves extract exhibited a decreasing trend in above mentioned parameters (Fig. 9B). Similarly, no change in histological structure of renal tissues was observed in control rats. STZ administration resulted in altered structure of renal tissues manifested as fibrosis, dilation of renal tubule, and space in glomerular capsule (Fig. 9C). The treatment with *F. johannis* ethanolic leaves extract resulted in restoration of renal tissue with significant reductions in the scores of fibrosis, dilation of renal tubule, and space of the glomerular capsule.

Discussion

Diabetes Mellitus is an endocrinal metabolic disease, defined as persistent hyperglycemia resulting by an absolute deficiency of insulin secretion or resistance to the hormone (Rossi 2018). Present study was conducted to assess the antidiabetic and antioxidant action of *Ficus johannis* Boiss. (Moraceae).

HPLC analysis confirmed the presence of kaempferol, gallic acid, myricetin, and quercetin in *F. johannis* ethanolic leaves extract. Previously phenolic compounds have been found to increase glucose uptake, glycogen synthesis, improved glucose balance, and lipid profiles leading to decrease in complications associated with different diseases like diabetes mellitus, obesity, and cardiovascular disorders (Vinayagam et al. 2016). Different studies have suggested
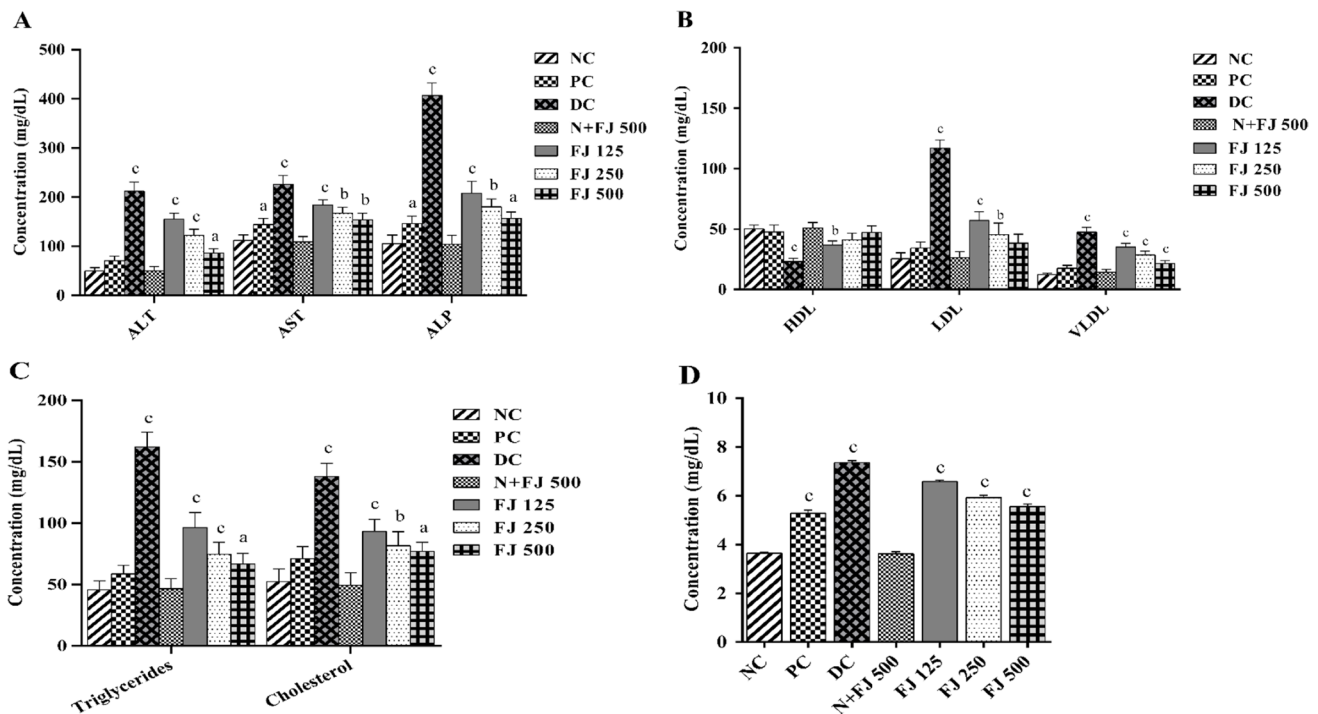
Fig. 7 Effect of treatment on **A** liver function tests, **B** and **C** lipid profile, and **D** HbA1C. Data is presented as mean±SD. The level of significance was estimated by one-way ANOVA followed by Fisher's LSD test and represented as "a" $p < 0.05$, "b" $p < 0.01$, and "c" $p < 0.001$ compared to the normal control group. Abbreviations: NC; normal control, PC; positive control, NFJ; normal rats treated with *F. johannis*, FJ; *F. johannis*, DC; diabetic control, ALT; alanine aminotransferase, AST; Aspartate transaminase, ALP; alkaline phosphatase, HDL; high-density lipoproteins, LDL; low-density lipoprotein, VLDL; very low-density lipoprotein

Table 8 Effect of treatments on renal function tests

Parameters	Renal function tests (mg/dL)						
	NC	PC	DC	NFJ 500	FJ 125	FJ 250	FJ 500
Urea	42.6±4.62	55.4±6.23	103±9.53[c]	43.2±6.76	79.4±6.47[c]	64±7.97[b]	60.2±7.12[a]
Creatinine	0.5±0.07	0.48±0.08	0.46±0.05	0.46±0.05	0.46±0.05	0.44±0.05	0.48±0.08

The level of significant difference was estimated by one-way ANOVA followed by Fisher's LSD test. Data is presented as mean±standard deviation; [a] represents $p < 0.05$, [b] represents $p < 0.01$, and [c] represents $p < 0.001$ when compared to normal control group. Abbreviations: *NC*, normal control; *PC*, positive control; *NFJ*, normal rats treated with *F johannis*; *FJ*, *F johannis*; *DC*, diabetic control

that phenolic compounds decrease the chances of degenerative disorders, by providing protection against oxidative stress and imparting their role as antioxidants. The high phenolic and flavonoid contents in *F. johannis* ethanolic leaves extract elaborate its role as an antioxidant (Rice-Evans et al. 1997; Saibabu et al. 2015; Zulfqar et al. 2020).

α-amylase inhibitory potential was also observed. Phenolics such as quercetin, and gallic acid can bind themselves with proteins to inhibit glucoside hydrolases (Malik et al. 2022). FJ500 group exhibited significant inhibition of hyperglycemia (21.49%) in normal rats, an effect comparable to the one observed by metformin. Reduction in Area under curve (AUC) reflected a change in absorption process of glucose and it is attributed to the inhibited sodium glucose

transporter (SGLT 1) expressions. The results corroborate the previous findings (Amuri et al. 2017).

STZ damages pancreatic β-cells, resulting in hypo-insulinemia and hyperglycemia (Graham et al. 2011). STZ is a nitrosourea alkylating agent which is selectively taken into the pancreatic β-cells due to resemblance with structure of glucose (Papich 2007). Dose-dependent hypoglycemic effect was seen by the treatment with *F. johannis* ethanolic leaves extract. Decrease in glucose levels may be attributed to phenolic and flavonoid compounds present in *F. johannis* ethanolic leaves extract. Quercetin exhibits protective effects in experimental diabetes as it decreases the oxidative stress and preserves the integrity of pancreatic β-cells (Yelumalai et al. 2019).
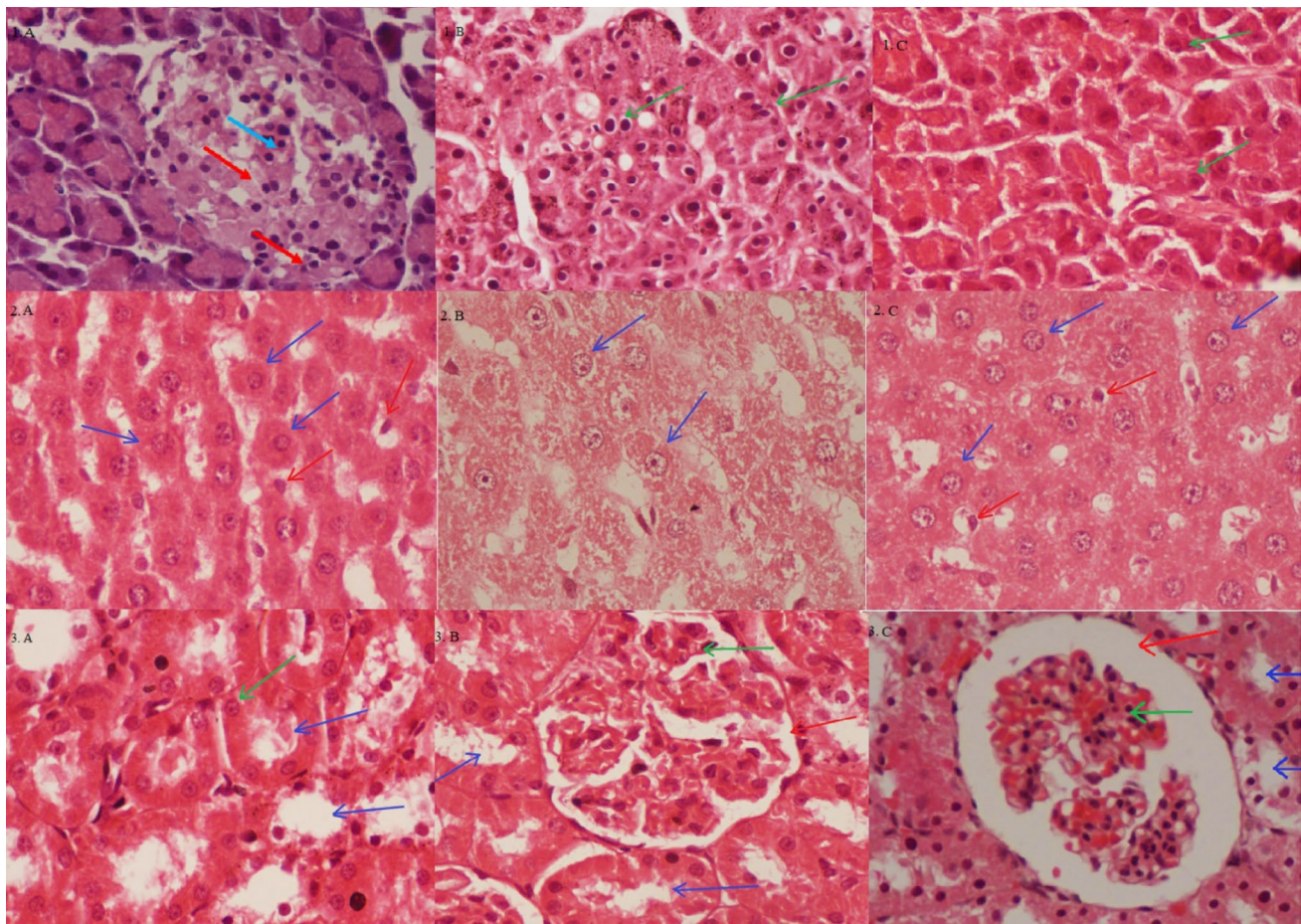
A: Normal control, **B**: Diabetic control, **C**: *F.johannis* 500mg/kg treated

Fig. 8 **1A** The examination of the submitted sample of pancreas exhibited no pathological changes, Islets of Langerhans were normal in size. Insulin producing beta cells were normal. **1B** Degenerative changes were observed. The acinar cells were undergoing Hydropic degeneration. Lumen of blood vessels had fat lobules. **1C** No inflammatory cells were present. Acinar cells were normal. **2A** The examination of the submitted sample of liver exhibited no pathological changes. Most of the hepatocytes were normal. **2B** Most of the hepatocytes were undergoing coagulative necrosis and nucleus are not seen. Severe degenerative changes were observed. The hepatocytes have more eosinophilic and granular cytoplasm. **2C** Most hepatocytes were normal. Few hepatocytes had undergone necrotic changes. **3A** Renal tubules were normal. Glomerulus and Bowman's capsule were normal. No pathological changes were observed in the kidney Section **3B** The epithelial cells of renal tubules were undergoing severe coagulative necrosis. **3C** The renal tubules were almost normal. The glomerulus and Bowman's capsule were normal

Irrespective of the high feed and water consumption in the untreated rats, the body weight decreased compared to *F. johannis* ethanolic leaves extract treated groups. The improved consumption of food and water in the *F. johannis*-treated rats may have resulted in enhanced metabolism. The results are comparable with previous observations which have also reported weight loss in diabetic rats. This evidently indicates that *F. johannis* ethanolic leaves extract amplified glucose metabolism and has preventive effects on degradation of structural proteins which increased body weights in the rats (Soni et al. 2018).

Levels of liver biomarkers (ALT, AST, and ALP) were restored upon treatment with *F. johannis* ethanolic leaves extract in a dose-dependent fashion. The effect can be attributed to the amelioration of hepatic phosphatases and transaminases related with decreased oxidation (Malik et al. 2022). Decrease in ALT upon administration of *F. johannis* ethanolic leaves extract could be attributed to improved insulin activity which results due to decrease of transcription of ALT associated gluconeogenesis (Farhangi et al. 2016). Elevated urea levels were restored near to normal after treatment with *F. johannis* ethanolic leaves extract. However, no effect was observed on creatinine level in diabetic rats(Chandran et al. 2016).

The expressions of GLUT4, GCK, and IGF-1 were improved whereas expressions of G6P were declined
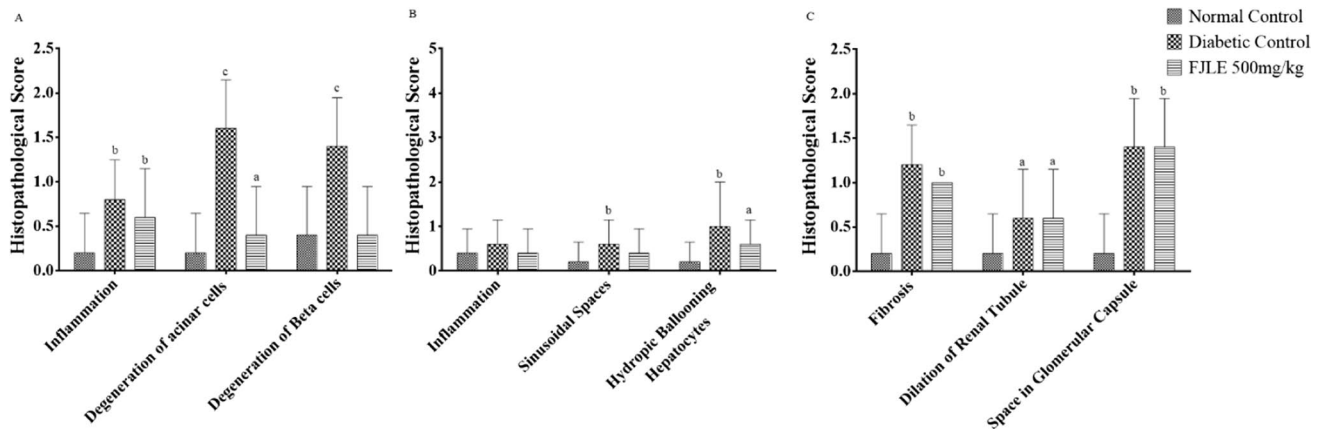
Fig. 9 Effect of *F.johannis* leaf extract on histological scores of **A** pancreas, **B** liver, and **C** kidney. Data is presented as mean ± SD. The level of significance was estimated by one-way ANOVA followed by Fisher's LSD test and is expressed as "a" ($p < 0.05$), "b" ($p < 0.01$), and "c" ($p < 0.001$) when compared to the normal control group. Abbreviations: FJLE; *F. johannis* leaves extract

upon treatment with *F. johannis* ethanolic leaves extract. GLUT4 is responsible for glucose homeostasis however, GLUT4 translocation is disturbed by insulin deficiency during diabetes. As a result, it remains inside the cells, non-functional, causing an elevation of blood glucose level (Balakrishnan et al. 2019). Previously, *Ficus carica* was found to improve glucose utilization by restoring GLUT4 translocation to plasma membrane. *F. johannis* ethanolic leaves extract up-regulated the gene expression of GLUT4 in diabetic rats correlating with the previous study (Irudayaraj et al. 2016). Gallic acid has the ability to initiate adipogenesis and fat accumulation by activating PPAR-γ and C/EBPα. It also has the ability to translocate GLUT-4 leading to an increase uptake of glucose. Myricetin also exhibits anti-hyperglycemic properties, mediated by the regulation of glucose transport through GLUT2. Myricetin administration was also linked with increased insulin sensitivity and inhibition of pancreatic β-cell apoptosis (Lalitha et al. 2020). The increased activities of G6P in diabetic rats are attributed to the increased synthesis of the enzymes involved in glucose production during diabetes by liver. Kaempferol ameliorates glucose intolerance, fasting hyperglycemia, and insulin resistance. These effects are linked with the ability of kaempferol to inhibit G6P activity and hepatic gluconeogenesis. *F. johannis* treatment significantly decreased blood glucose levels and G6P gene expression leading to decrease gluconeogenesis probably by reducing the activities of G6P, thereby reducing the endogenous production of glucose (Hemmati et al. 2017). *F. johannis* ethanolic leaves extract improved the gene expression of GCK improving carbohydrate metabolism in comparison to diabetic rats. It can be suggested that *F. johannis* exhibits protective effects against oxidative stress in rats and its hypoglycemic effect

could be attributed to increase in insulin level, resulting in glycolysis, gluconeogenesis pathways regulation (Pari and Chandramohan 2017). Kaempferol increases the GCK activity and improves hepatic glucose metabolism. These findings suggest, kaempferol as an effective antidiabetic natural compound which regulates hepatic gluconeogenesis and improves insulin sensitivity (Alkhalidy et al. 2018). It is suggested that *F. johannis* ethanolic leaves extract have the potential to be used for the treatment of type II DM acting on multiple targets attributed to the presence of kaempferol and quercetin (Al-Nour et al. 2019). Treatment with *F. johannis* ethanolic leaves extract, significantly decreased blood glucose levels by up regulation of IGF-1 gene expression probably by improving lipogenesis and insulin sensitivity and overcoming insulin resistance (Kang et al. 2016).

Expressions of KI67, PPAR-γ, PCNA, and topoisomerase II enzyme (TOP2A) were increased whereas BAX expressions were decreased upon treatment with *F. johannis* ethanolic leaves extract. The mRNA expression of KI67 was significantly increased by the antioxidant activity of *F. johannis* ethanolic leaves extract causing the preservation of pancreatic β-cell integrity and maintaining tissue homeostasis and growth (Bologna-Molina et al. 2013). The gene expression of PPAR-γ was significantly increased in *F. johannis*-treated groups in a dose-dependent fashion, thus improving glucose homeostasis by activation of GCK and GLUT2 in liver and pancreas. Improvement in gene expression levels of PPAR-γ is also linked with increased sensitization of insulin in peripheral tissues by stimulating its secretion and controlling blood glucose by increased sensitivity in β-cells of pancreas (Assaei et al. 2016). The treatment with *F. johannis* ethanolic leaves extract significantly increased the gene expression level of PCNA in diabetic rats indicating normal cell

proliferation and preservation of pancreatic β-cells (Boddu-luru et al. 2016). The mRNA expression of topoisomerase II enzyme (TOP2A) was increased significantly in STZ-NA diabetic rats upon treatment with *F. johannis* ethanolic leaves extract in comparison of the diabetic group. It exhibits proliferation and normal functioning of pancreatic β-cells due to the antioxidant activity of extract (Harkin et al. 2016). *F. johannis* reduced apoptosis and increases cell survival in pancreatic β-cells as the P53 expression was down regulated after treatment with extract indicating a decline in oxidative stress which was previously reported. P53 gene expression was increased in diabetic rats (Gharib et al. 2018). BAX expression increases in diabetes at mRNA and protein levels due to apoptosis. Previously experimental models support in vitro and in vivo over expression of BAX in apoptosis (Duchen 2004). The gene expression of BAX was decreased significantly in *F. johannis*-treated diabetic rats compared to normal control group indicating a decrease in progression of diabetes linked with apoptosis.

Type II DM is associated with hyperlipidemia. Hyper-lipidemia is associated with the hindrance of glucose which in turn leads to the lipid availability. LDL deposition on the arteries wall leads to the transportation of the plaques into the liver via HDL. Hence an increase in level of HDL and lowering of cholesterol and triglycerides can be a useful therapeutic alternative (Mooradian 2009). Treatment with *F. johannis* ethanolic leaves extract effectively improved dys-lipidemia in a dose-dependent manner by improvement in insulin sensitivity. Results are in concurrence with a study where the findings suggested that flavonoids are responsi-ble for producing hypo-lipidemia (El-Hadary and Ramadan 2019). Marked improvement observed in atherogenic index after administration of FJ500 is attributed with the reduction in the risk of infarction, diabetes mellitus, and atheroscle-rotic conditions (Zhang et al. 2016).

The histopathological examinations showed dose-depend-ent improvement in liver, kidney, and pancreas of STZ-induced diabetic rats when treated with *F. johannis* ethanolic leaves extract compared to the disease control group. The findings of the current study corroborated the earlier inves-tigation on herbal drugs suggesting their role in the ame-lioration of oxidative damage in organs and prevention of diabetic complications (Wang et al. 2017; Zafar et al. 2021).

Conclusion

Present study confirms that *F. johannis* leaves ethanolic extract possess remarkable antidiabetic activity based upon both in vitro and in vivo analysis. It does so by modulation of pancreas function and also evident from cellular morphol-ogy of pancreas in STZ induced diabetic rats. Biophenol

(Gallic acid) and flavonoids (kaempferol, myricetin, and quercetin) were quantified and considered to be responsi-ble for the in vitro and in vivo antidiabetic potential. These macromolecules present in the *F. johannis* leaves ethanolic extract has the potential to regulate the concentration of glu-cose by modulation of GLUT4, G6P, IGF-1, and GCK in T2DM rats.

Declarations

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