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Original Research Article

Novel insights into the antidiabetic and antioxidant effects of *Fagonia indica*: A study in alloxan-induced diabetic rats

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Abstract

Objective: *Fagonia indica* is a medicinal plant with ethnopharmacological properties. This study aimed to evaluate its phytochemical, antioxidant, and hypoglycemic effects in alloxan-induced diabetic rats.

Materials and Methods: The methanolic crude extract of *F. indica* was evaluated for *in-vitro* phytochemical activities, HPLC profiling, and antioxidant activity. *In-vivo* studies were conducted on albino rats to assess its anti-hyperglycemic effects and modulatory activity on oxidative stress markers.

Results: Phytochemical analysis revealed high phenolic (284.57±3.62 mg Quercetin Equivalent/g dry extract) and flavonoid (466.52±3.33 mg Gallic Acid Equivalent/g dry extract) contents and HPLC-based phenolic and flavonoid compounds were detected. The extracts exhibited significant antioxidant capacity, as determined by several assays, total reducing power was 18.49±1.23 mg of dry extract, and antioxidant capacity 6.96±0.51 mg of dry extract. Furthermore, extracts showed dose-dependent inhibition of radical scavenging activity *via* DPPH (81.7%), β-carotene (82.9%), and nitric oxide (37.1%) assays. Moreover, treatment with *F. indica* crude extract (FICE) significantly reduced blood glucose level by 63.8%, and normalized serum biochemical biomarkers, including alanine and aspartate transaminases, cholesterol, triglycerides, total protein, bilirubin, urea, and creatinine, and serum pancreatic biomarkers such as insulin, lipase and amylase. Moreover, in diabetic rats, FICE treatment restored antioxidant defenses in pancreas, liver, and kidney tissues by increasing superoxide dismutase, catalase, peroxidase total protein, reduced glutathione, thiobarbituric acid reactive substances levels.

Conclusion: *F. indica* possesses potential hypoglycemic and antioxidant properties, supporting its potential as a therapeutic agent for diabetes management.

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Introduction

In the 21st century, the growing human population faces escalating threats from multifactorial metabolic disorders, among which diabetes mellitus (DM) has emerged as a critical public health challenge (Zhang et al. 2024). Characterized by hyperglycemia, DM arises from impaired insulin secretion, peripheral insulin resistance or both, disrupting carbohydrates, lipid, and protein metabolism (Szablewski 2024). DM is also linked with atherosclerotic macro-vascular disease, disturbing blood vessels that supply the brain (Menafra et al. 2024). Several diabetogenic agents, which include dithizone, monosodium glutamate, high fructose load, high glucose load, and anti-insulin serum, alloxan and streptozotocin are the most widely used in diabetes studies (Gadó et al. 2024). Despite advances in conventional therapies like insulin and sulfonylureas, these treatments fail to prevent long-term complications, underscoring the need for alternative therapeutic strategies.

The WHO promotes for the plant-based therapies given their multimodal bioactive constituents, with fewer side-effects compare to synthetic drugs (Jain et al. 2024). Over, 343 plants have demonstrated hypoglycemic potential on pre-clinical studies, with many exhibiting antioxidant, anti-inflammatory, and insulin-sensitizing properties worldwide have been screened for the ability to lower blood glucose in laboratory experiments. Previous research indicates that plant extracts and their phytoconstituents impart free radical scavenger properties to cope with multiple notorious degenerative diseases in humans due to their vast reservoirs of antioxidants (Jain et al. 2024; Verma et al. 2024). *F indica*, a traditional medicinal plant, is rich in terpenoids, saponins, alkaloids, coumarins, sterols, flavonoids, amino acids and trace elements, responsible for lowering blood glucose level (Singh et al. 2023). Moreover, *F. indica* has multiple therapeutic properties, such as antimicrobial, anti-inflammatory, anticancer, hepato-protective activities, antioxidants, and antidiabetic (Ali and Khan 2021; Balkrishna et al. 2024). However, *in-vitro* and *in-vivo* antioxidant potential of *F. indica* against alloxan-persuaded oxidative stress and hyperglycemia in rats model remains underexplored.

Materials and Methods

Plant collection

The aerial parts *Fagonia indica* Burm. f. (family Zygophyllaceae) was collected during September 2023 from Mianwali, Punjab Province, Pakistan. The plant material was authenticated by Prof. Dr. Rizwana Aleem Qureshi, and voucher specimen (Accession No. HMP-461) was deposited at Herbarium of Medicinal Plants of Pakistan at Quaid-i-Azam University.

Preparation of plant crude extract

The aerial plant parts were washed, shade-dried, and ground into fine power and soaked in methanol-chloroform (1:1) of 4 L, for 72 hr at 25ºC, then filtrated and concentrated using a rotary evaporator, described in supplementary data.

In-vitro studies

Phytochemical analysis

Total phenolic content (TPC) was quantified using Folin-Ciocalteu's phenol reagent with minor modifications (Khan et al. 2021). The total flavonoid content (TFC) was determined *via* the aluminium chloride calorimetric method (Shehab et al. 2020). HPLC analysis of *F. indica* crude extract (FICE) using an Agilent 1200 HPLC system with a diode array detector. The detail protocol is described in supplementary data.

Antioxidant activity

Total Reducing Power (TRP) assay was assessed following a modified Oyaizu method (Olofinsan et al. 2022). Total antioxidant capacity (TAC) was evaluated

using the phosphomolybdenum assay with adaptations (Hameed et al. 2021), DPPH radical scavenging activity was measured according to an adjusted protocol (Atiq ur et al. 2021). Nitric Oxide Scavenging assay was evaluated using a modified method (Ali and Khan 2021). Further, antioxidant activity was tested *via* β-carotene bleaching following optimized procedures (Zafar et al. 2023), described in supplementary data.

In-vivo evaluation

Animal and housing

Healthy adult Sprague Dawley albino male rats (age: 8-9 weeks, and weight 200-250 g) were housed under controlled conditions (12 hr light/dark cycle) at Quaid-i-Azam University, Islamabad, following NIH and ARRIVE (n = 6/group).

Diabetes induction in rats

After 24 hr fasting, rats were intraperitoneal (i.p.) injected with a single dose of alloxan monohydrate agent (120 mg/kg in 10% DMSO). Post 72 hr of injection, the blood glucose levels ≥250 mg/dl or confirm diabetes.

Glucose tolerance test

Groups incudes normal control (NC; 10% DMSO), diabetic control (DC), diabetic control treated with alloxan + Glibenclamide-10 mg/kg per body mass (DC+Glib), and diabetic control treated with alloxan + *F. indica* crude extract (FICE) 400 mg/kg per body mass. Blood glucose and body weight were monitored 0, 1, 2, 24, 48 and 72 hr. In the experiment, the body weight of rats was measured and recorded using a digital balance at the start (day 0), on days 03, 07, and 11, and before slaughter.

Dissection and sample collection

Rates were anesthetized with isoflurane and sacrificed, and blood was collected *via* cardiac puncture. Centrifuged (10,000 rpm at 4°C, 15 min) and organs (split for enzymatic assays and histopathology) were stored at -80°C, described in supplementary data.

Tissue and serum analysis

Organ (100 mg) were homogenized in 1 ml of potassium phosphate buffer containing EDTA (100 mM; pH 7.4) and centrifuged at 10,000 rpm at 4°C for 30 min. The tissues of liver and kidney were assessed for total protein estimation, α-amylase activity (Mollania and Sahabi 2022), lipase activity (Rehman 2025) detailed procedure is described in the supplementary file. AMP one space diagnostic kit was used to measured cholesterol, triglyceride (TG), total protein, alanine and aspartate aminotransferase (ALT/AST), total bilirubin, urea, creatinine (Abdallah et al. 2023) levels in serum.

Enzyme activity of tissue samples

The enzymatic activity of the samples were determined by Catalase (CAT) (Selim et al. 2021), Peroxidase (POD) (Almilaibary et al. 2022), Superoxide dismutase (SOD) (Abdallah et al. 2023), reduced glutathione (GSH) (Younas et al. 2022), and thiobarbituric acid reactive substances (TBARS) (Leon and Borges 2020) detailed procedure is described in the supplementary data.

Statistical analysis

Values were represented as means ± standard deviation of triplicate analyses. Graph Pad Prism version 10 software was used for statistical analysis and determination of IC_{50}. $p < 0.05$ was considered significant using ANOVA followed by Tukey's multiple comparison test.

Results

Phytochemical analysis and HPLC-based profiling of *F. indica*

The TPC of FICE was 510.31±1.39 GAE/g dry extract (Table 1) using the standard curve (R^2=0.91) of Gallic acid, indicating significant phenolic compounds,

which contribute to its antioxidant potential. The TFC of FICE was 284.89±2.74 QE/g dry extract using standard curve (R^2 = 0.91) (Table 1). Next, we investigated HPLC-based profiling of *F. indica* methanolic extract, which exhibited significant quantities of phytochemicals, including quercetin (554.78 µg/g of dry weight), myricetin (502.82 µg/g of dry weight), and coumaric acid (469.44 µg/g of dry weight) (Table 1; Figure 1).

Table 1. Quantitative and HPLC-based profiling of phytochemicals in *Fagonia indica* crude extract.

Quantitative analysis *F. indica*		
Total phenolic content		**Total flavonoid content**
510.31±1.39 GAE/g dry extract		284.89±2.74 QE/g dry extract
HPLC analysis of *F. indica*		
Wavelength	**Compound**	**µg/g of dry weight**
257	Rutin	441.66
279	Gallic acid	432.29
279	Coumaric acid	469.4
325	Ferulic Acid	91.84
368	Myricetin	502.82
368	Quercetin	554.78

Represented as means ± SD

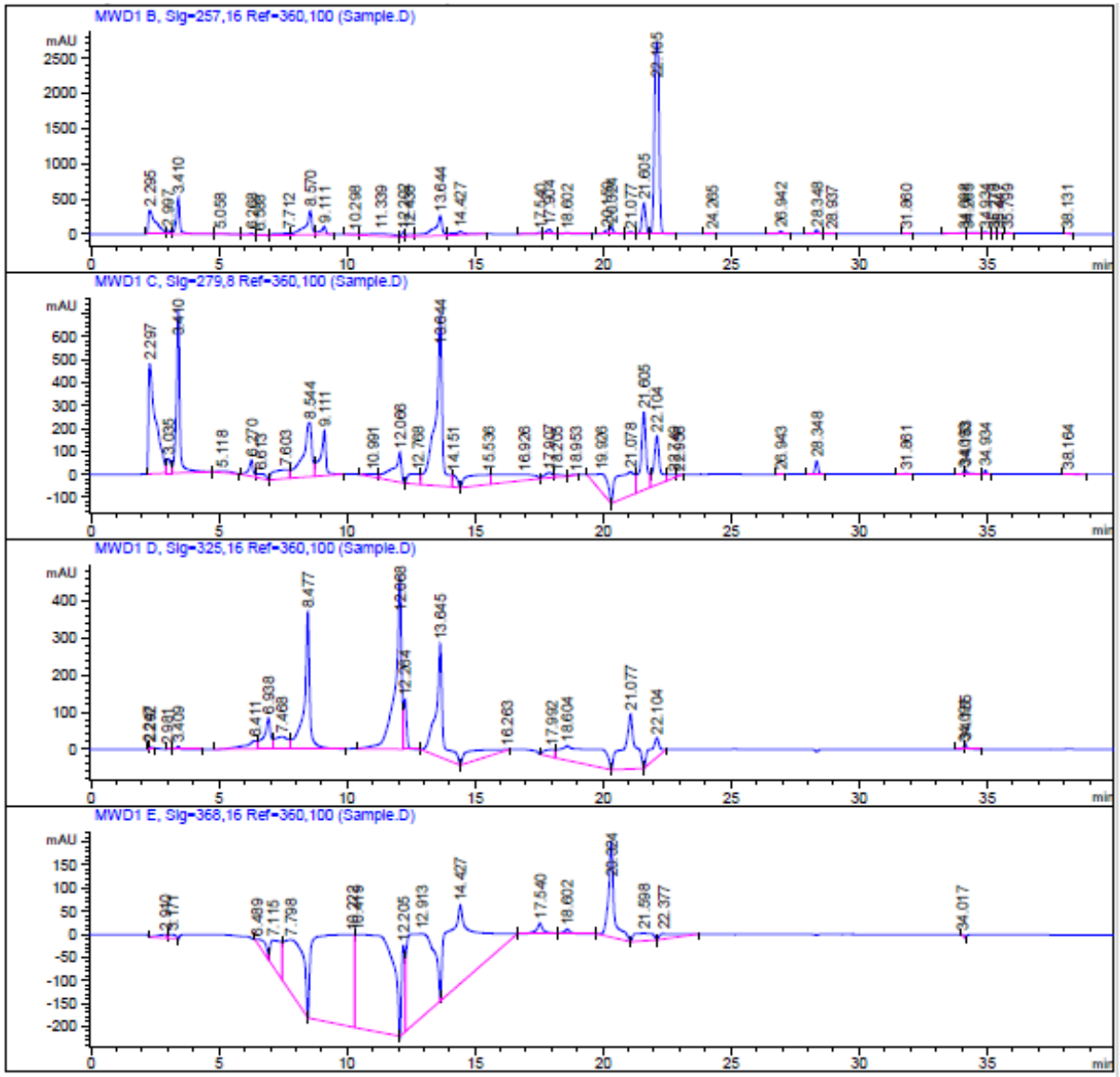

Figure 1. HPLC Chromatogram of phytochemicals

Antioxidant potential of *F. indica*

The results showed that highest activity of FICE was exhibited by the DPPH radical scavenging activity (81.7% inhibition, IC_{50} 5.5±0.3 mg/ml), reflecting hydrogen-donating ability. Additionally, significant β-carotene (82.9% inhibition, IC_{50} 1.44±0.4 mg/ml), indicating potent lipid peroxidation inhibition. However, nitric oxide scavenging exhibited moderate effect (37.1% inhibition, IC_{50} 122.5±6.8 mg/ml), suggesting selective radical quenching (Table 2). Moreover, total reducing power of FICE was 18.49±1.23 mg/g of dry extract, and total antioxidant capacity was 6.96±0.51 mg/g of dry extract, confirming its potent free radical scavenging ability (Table 2).

Table 2. Assessment of *in-vitro* antioxidant potential of *Fagonia indica* crude extract using various antioxidant assays

Assays	Percentage inhibition				IC_{50} mg/ml
	40 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	
DPPH	81.7±1.5**	75.8±1.4**	68.1±1.1**	44.5±1.8**	5.5±0.3
β-Carotene	82.9±3.1**	77.6±1.4**	68.2±3.9***	65.4±2.6**	1.44±0.4
Nitric oxide	37.1±1.5**	31.2±1.1**	24.4±2.5**	18.4±1.9**	122.5±6.8
Ascorbic acid	97.4±1.0	90.2±1.1	88.1±1.3	83.5±1.4	0.45±0.5
	mg/g of dry extract				
Total reducing power	18.49±1.23				
Total antioxidant capacity	6.96±0.51				

Data are expressed as means ± SD. Statistical significance compared to ascorbic acid was determined by one-way ANOVA with *p<0.05, **p<0.01 and ***p<0.001 significant.

Glucose tolerance test

The glucose tolerance test revealed different glycemic responses across (Figure 2A), NC group showed stable glucose levels (101–110 mg/dl); DC group exhibited progressive hyperglycemia (102-481 mg/dl by day 15). DC+Glib showed significant reduction from 400 mg/dl on day 5 to 104 mg/dl on the day 15 (78.4% decrease). The FICE group initially exhibited elevated glucose levels, peaking at 390 mg/dl on the day 5 to reduction ($p<0.05$) 174 mg/dl on day 15 (63.8% decrease).

Change in body weights

Body weight analysis revealed different patterns among treatment groups: NC group rats maintained stable weights (238-252 g), indicating physiological consistency and homeostasis. DC groups rats showed progressive body weight loss (221-168 g) over 15 days, demonstrating disease progression. The FICE group demonstrated significant preservation (245-234 g), DC+Glib group showed (225-219 g) by day 15, reflecting better preservation of the parameter compared to the NC group (Figure 2B).

Effect of *F. indica* on serum for biochemical profile

Notably, administration of FICE significantly reduced both cholesterol and triglyceride levels compared to DC, bringing them closer to normal values (Table 3). Results showed that serum liver markers (ALT, AST, bilirubin, and total proteins), diabetic rats group elevations were restored with the treatment of FICE rats' group (Table 3). The present study revealed that DC rats exhibited significantly elevated serum levels of kidney biomarkers, including urea and creatinine, in comparison to NC animals (Table 3). Treatment with FICE resulted in a statistically significant reduction in these renal biomarkers, suggesting a potential reno-protective effect (Table 3).

Serum analysis revealed significant pancreatic dysfunction in the diabetic rats. Compared to NC, DC rats exhibited elevated insulin, lipase and amylase levels, indicating exocrine pancreatic stress. Although insulin levels were remained statically unchanged. Treatment with FICE significantly reduced serum lipase and amylase levels while restoring insulin levels closer to the normal range (Table 3).

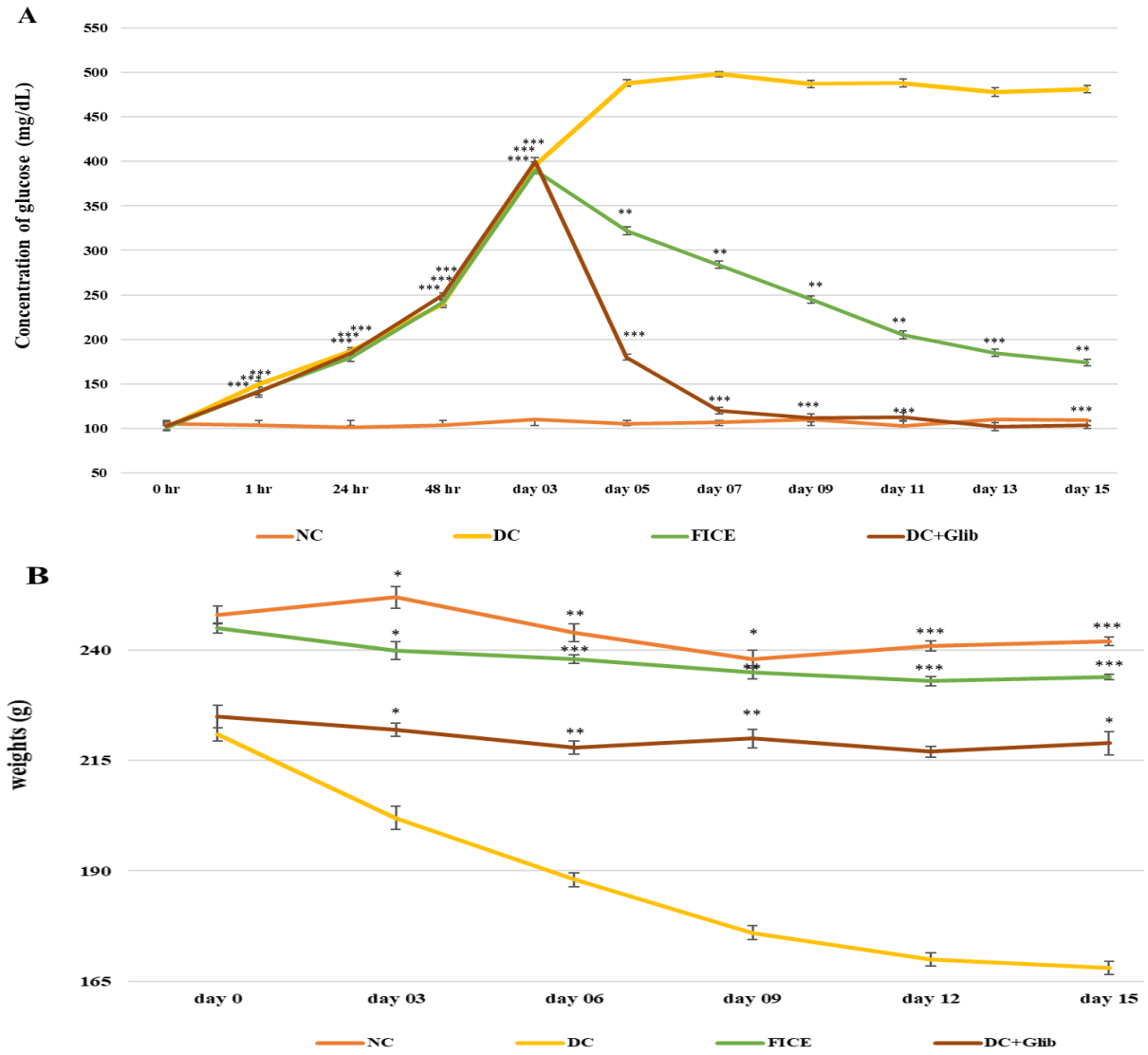

Figure 2. Effect of *F. indica* crude extract and glibenclamide in various studied groups. (A) blood glucose levels (mg/dl), and (B) body weights (grams). Normal control (NC), diabetic control (DC), diabetic control group, treated with 400 mg/kg *F. indica* crude extract (FICE), diabetic control group, treated with 10 mg/kg of glibenclamide (DC+Glib). Values are presented as means ± SD. Statistical significance compared to DC is donated as *p<0.05, **p<0.01 and ***p<0.001.

Table 3. Effect of *Fagonia indica* crude extract on serum biochemical profile of diabetic rats.

Items	Groups			
	NC	DC	FICE	DC+Glib
Cholesterol (mg/dl)	124.8±4.6***	175.6±1.4	53.6±3.8***	49.7±3.4***
Triglycerides (mg/dl)	150.5±3.6**	200.3±1.5	112.4±2.5***	100.4±2.3***
Total proteins (g/dl)	6.1±0.55**	7.7±0.46	6.1±0.60**	5.2±0.25***
ALT (U/L)	34.3±1.79***	76.5±2.88	38.3±2.90***	28.1±3.87***
AST (U/L)	110.4±1.63***	174.4±2.6	140.4±1.50***	109.1±1.80***
Bilirubin (mg/dl)	1.2±0.12*	1.3±0.1	1.1±0.04*	1.0±0.03*
Urea (mg/dl)	35±1.59***	121.0±4.80	140.4±1.39**	109.1±1.80***
Creatinine (mg/dl)	0.3±0.01***	1.5±0.25	0.9±0.12***	0.8±0.16***
Insulin (U/L)	3.78±0.03**	3.9±0.06	3.3±0.05***	3.3±0.03***
Lipase (U/L)	128.3±2.52***	193.3±4.16	155.0±3.00***	131.7±3.06***
Amylase (U/L)	775.7±3.05***	811.7±3.51	735.7±2.08***	678.3±4.73***

Normal control (NC), diabetic control (DC), diabetic control group, treated with 400 mg/kg *F. indica* crude extract (FICE), diabetic control group, treated with 10 mg/kg of glibenclamide (DC+Glib). Data are expressed as means±SD. Statistical significance compared to DC was determined by one-way ANOVA with *p<0.05, **p<0.01 and ***p<0.001 significant.

Antioxidant regulatory effects of *F. indica* on tissues samples

Catalase activity was significantly elevated in DC rats compared to NC rats. This elevation may represent a compensatory response to alloxan-induce oxidative stress. Treatment with FICE significantly attenuated CAT activity across all tissues (pancreas: 13.53±0.35, liver: 15.2±0.91 and kidney: 12.8±0.31 U/min tissue), normalizing levels comparable to those observed in glibenclamide treatment (Table 4). Similarly, peroxidase activity was markedly increases in DC tissue sample of pancreas, liver, and kidney than in NC tissues. Moreover, treatment with FICE significantly decreased POD activity in pancreas: 5.87 ± 0.59, liver: 5.30 ± 0.10, and kidney: 4.37 ± 0.21 U/min tissue, mirroring the effects of glibenclamide (Table 4). Likewise, superoxide dismutase activity was significantly higher in DC rats' group than in NC group. Whereas, administration of FICE showed significant reduction in the SOD levels in pancreas: 7.67 ± 0.47, liver: 4.06 ± 0.23, and kidney: 8.63 ± 0.51 U/min tissue, which demonstrating a regulatory effect similar to glibenclamide (Table 4).

Next, we investigated the total protein content in pancreas, liver, and kidney tissue homogenates. Results indicated that levels were significantly elevated in DC rats compare to NC rats' group. However, substantial reduction was observed after treatment with FICE (pancreas: 14.7 ± 0.50 µg/mg; liver: 14.2 ± 0.31 µg/mg; kidney: 11.0 ± 0.50 µg/mg) compared to DC rats indicating a restorative effect of the extract on tissue protein homeostasis (Table 4). Subsequent, we assessed lipid peroxidation using TBARS assay, which revealed a significant elevation in pancreas, liver, and kidney tissues of DC rats (42.2 ± 0.84, 58.2 ± 0.65, and 60.3 ± 0.35 nM/min/mg tissue, respectively) compared to NC rats, indicating pronounced oxidative damage under diabetic conditions. However, treatment with FICE significantly reduced TBARS levels in the pancreas (40.0±0.28 nM/min/mg), liver (55.9±0.87 nM/min/mg), and kidney (59.2±0.70 nM/min/mg), demonstrating its potent antioxidative effects (Table 4). Our results demonstrate that GSH levels were significantly elevated in the pancreas, liver, and kidney tissues of DC rats compared to NC rats, suggesting compensatory response to oxidative stress. Though, treatment with FICE significant reduced GSH levels (pancreas: 8.13±0.25; liver: 8.63±0.59; kidney: 4.37±0.21 mM/g), restoring them to normal values, further confirming it antioxidative efficacy (Table 4).

Table 4. Effect of *Fagonia indica* crude extract on antioxidant enzymes in pancreas, liver, and kidney tissues of diabetic rats

	Tissue Samples											
	Pancreas				**Liver**				**Kidney**			
	Groups				**Groups**				**Groups**			
Enzymes	NC	DC	FICE	DC+Glib	NC	DC	FICE	DC+Glib	NC	DC	FICE	DC+Glib
CAT (U/min)	14.13±0.40***	15.03±0.47	13.53±0.35***	12.37±0.15***	12.9±0.35***	16.8±0.70	15.2±0.91*	12.7±0.50***	13.9±0.35**	15.6±0.51	12.8±0.31***	14.5±0.35**
POD (U/min)	5.47±0.25**	7.17±0.35	5.87±0.59**	5.40±0.46*	3.40±0.20***	7.53±0.35	5.30±0.10***	4.63±0.25***	4.10±0.17**	5.53±0.35	4.37±0.21**	2.90±0.40***
SOD (U/min)	6.67±0.15***	8.23±0.31	7.67±0.47***	7.03±0.48***	4.36±0.21***	5.53±0.35	4.06±0.23***	2.87±0.35***	4.07±0.42**	9.03±0.61	8.63±0.51***	4.53±0.31**
Total protein estimation (µg/mg)	11.4±0.15***	16.7±0.10	14.7±0.50***	10.6±0.25***	14.5±0.31**	15.6±0.25	14.2±0.31***	10.9±0.72***	10.8±0.35***	12.3±0.36	11.0±0.50**	10.5±0.31***
TBARS (nM/min/mg)	30.3±0.26***	42.2±0.84	40.0±0.28**	32.6±0.25***	35.2±0.40***	58.2±0.65	55.9±0.87**	37.8±0.45***	29.2±0.30***	60.3±0.35	59.2±0.70*	33.1±0.56***
GSH (mM/g)	5.93±0.51**	9.83±0.35	8.13±0.25***	8.23±0.31***	3.73±0.42***	11.0±0.61	8.63±0.59**	4.63±0.25***	4.10±0.17**	5.53±0.35	4.37±0.21**	2.91±0.40***

Normal control (NC), diabetic control (DC), diabetic control group, treated with 400 mg/kg *F. indica* crude extract (FICE), diabetic control group, treated with 10 mg/kg of glibenclamide (DC+Glib). Data are expressed as means±SD. Statistical significance compared to DC was determined by one-way ANOVA with *p<0.05, **p<0.01 and ***p<0.001 significant.

Discussion

The present study demonstrates the potent phytochemical and antioxidant properties of *F. indica*, highlighting its therapeutic potential in mitigating diabetes associated complications. HPLC analysis revealed the presence of bioactive compounds such as quercetin, and myricetin, which are well known for their multifaceted antidiabetic mechanisms. Quercetin exhibits clinical efficacy in glycemic control, reducing HbA1c by ~4% at 500 mg/day over 12 weeks in type 2 diabetics (Mantadaki et al. 2024), while preclinical studies demonstrates it preserves β-cells function, improves glucose tolerance and oxidative stress in diabetic animal model (Li et al. 2020). Myricetin inhabits α-glucosidase/α-amylase and enhances acting as insulin secretion *via* GLP-1 agonism. These compounds, along with phenolics and flavonoids show significant antioxidant and anti-inflammatory activity, potentially mitigating diabetes-associated oxidative stress and complications (Nabil-Adam et al. 2023; Niisato and Marunaka 2023). Antioxidant potential of FICE indicating strong electron-donating and free radical-neutralizing capabilities. The antioxidant activity of phytochemicals is attributed to their hydroxyl groups, which form stable complex with reactive species (Banc et al. 2023; Tak and Kumar 2020). FICE exhibited a gradual glucose-lowering effect in the glucose tolerance test, suggesting insulinotropic or insulin-sensitizing mechanism, akin to glibenclamide but with slower kinetics (Dikkala et al. 2023). The optimal hypoglycemic activity at 400 mg/kg aligns with previous study (Dikkala et al. 2023), and the observed weight stabilization suggest improved metabolic control, potentially through insulin-mediated pathways (Singh et al. 2024). Furthermore, diabetogenicity in rats by alloxan endorsed upgraded serum lipid profile due to the cascade of free radicals generation (Singdam et al. 2022). FICE significantly reduced cholesterol and triglyceride levels in the serum analysis. Additionally, standard mechanisms of fatty acid synthesis, biliary secretions, uptake of bilirubin and its conjugation and excretion get disturbed owing to injured liver parenchyma (Thakur et al. 2024). FICE treatment reduced elevated serum ALT and AST, indicating hepatoprotective effects. This aligns with the extracts ability to mitigate alloxan-induced oxidative stress and restore liver function in diabetic rats (Adwani et al. 2024). FICE treatment significantly reduced renal biomarkers (urea and creatinine). These regulations are reflective of impaired renal function commonly associated with diabetic nephropathy, driven by disturbances in protein metabolism and nitrogen balance. The rise in urea and creatinine levels indicates increased protein catabolism and reduced renal clearance capacity (Ávila et al. 2025). Serum analysis exhibited elevated insulin, lipase and amylase levels, indicating exocrine pancreatic stress. FICE normalized serum lipase and amylase levels, suggesting a protective effect on both endocrine and exocrine pancreatic function. These findings of pancreatic enzyme levels supports its therapeutic potential in alleviating diabetic-related pancreatic complications, consistent with previous studies on plant-derived hydrolase modulators (Egbuna et al. 2021).

FICE demonstrated robust antioxidative activity by modulating key enzymes. It restored catalase activity, enhancing the neutralization of reactive oxygen species, including superoxide and hydroxyl radicals (Morsy et al. 2010). These findings are in concordance with previous studies reporting catalase dysregulation in diabetic tissues and demonstrated the corrective potential of antioxidant-rich plant extracts. FICE shows potential in mitigating oxidative stress through restoration of enzymatic redox balance (Almilaibary et al. 2022). Additionally, its ability to normalize peroxidase activity suggests efficacy on in

reducing peroxidative stress and enhances antioxidant homeostasis. This is consistent with the previous studies demonstrating the capacity of plant-based compounds to mitigate alloxan-induced oxidative stress by enhancing enzymatic antioxidant defense mechanisms in vital organs (Rusli et al. 2024). Furthermore, FICE enhances superoxide dismutase activity, supporting its role in maintaining redox equilibrium, which is align with previous studies highlighting the antioxidant properties of medicinal plant extracts (Mihailović et al. 2021).

The study revealed significantly elevated total protein levels in pancreas, liver, and kidney tissues of diabetic rats, reflecting metabolic stress and pathological protein accumulation due to chronic hyperglycemia and oxidative damage (Almilaibary et al. 2022). Our results indicate that treatment with FICE effectively restored tissue protein homeostasis, as evidenced by the normalization of total protein levels in pancreas, liver, and kidney tissues. This regulatory effect likely contributes to the mitigation of diabetes-related metabolic dysfunction. Furthermore, FICE treatment significantly reduced TBARS levels in the pancreas, liver, and kidney tissues demonstrating potent anti-lipid peroxidation activity. As TBARS serves as a well-established marker of lipid peroxidation, these findings strongly suggest that FICE contains bioactive compounds with ROS-scavenging and membrane-stabilizing properties (Akbari et al. 2022). This protective effect against oxidative membrane damage is particularly relevant in diabetes, where chronic hyperglycemia exacerbates lipid peroxidation cascades. Additionally, FICE treatment normalized GSH levels in the pancreas, liver, and kidney tissues, indicating an adaptive response to oxidative stress. The restoration of GSH reflects improved redox homeostasis, given a GSH's critical role in neutralizing ROS and maintaining cellular redox regulation (Bhattacharya 2015). Collectively, these findings suggest that FICE's therapeutic potential extends beyond basic antioxidant activity to the restoration of the entire redox regulatory system, making it a promising candidate for mitigating the complex oxidative pathophysiology of diabetes and its complications

This study comprehensively evaluated the pharmacological potential of FICE, demonstrating its significant *in-vitro* and *in-vivo* antioxidant and hypoglycemic activities. In antidiabetic rats, the FICE exhibited remarkable efficiency in mitigating oxidative stress and hyperglycemia as evidenced by its ability to normalize key biomarkers (CAT, POS, SOD, GSH, and TBARS) and restore pancreatic function. The findings position *F. indica* as a promising natural source for developing novel therapeutic against diabetes and oxidative stress-related complications. Its bioactive constituents appear to modulates enzymatic antioxidant defenses and glucose hemostasis, aligning with traditional uses of medicinal plants in metabolic disorder management. This research underscores *F. indica* potential as a complementary therapy, bringing ethnopharmacological knowledge with evidence-based drug discovery. Further studies are warranted to isolate and characterize its active compounds and elucidate their precise mechanism of action.

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Conflicts of interest

The authors declare no conflicts of interest

Ethical Considerations

The study design was accepted by the Institutional Animal Ethics Committee of University of Gujrat (IRB No 320), and all provisions were carried to reduce animal sufferings.

Code of Ethics

IRB No 320

Authors' Contributions

Conceptualization, H.I. and M.Z.B.; methodology, H.I, M.Z.B, B.A., T.R. and H.A; formal analysis, A.A., and A.R; resources Z.H.; writing—original draft preparation, S.I.H., B.A and R.G.; writing—review and editing, H.I., and M.Z.B. All authors have read and agreed to the published version of the manuscript.

Abbreviation

DM: Diabetes mellitus, WHO: World health organization, BGL: Blood glucose level, TFC: Total flavonoid content, TRP: Total reducing power assay, TCA: Trichloroacetic acid, AST: Aspartate aminotransferase, ALT :Alanine aminotransferase, SGPT: Serum glutamic pyruvic transaminase, SGPT: Oxaloacetic transaminases, SGOT: Serum glutamic-oxaloacetic transaminase EDTA: Ethylene diamine tetra acetic acid, CAT: Catalase, POD: Peroxidase, SOD: Superoxide dismutase, GSH: Reduced glutathione, TBARS: Thiobarbituric acid reactive substances, TG: Triglyceride, FICE: *Fagonia indica* crude extract, DC+Glib: Diabetic control group with 10 mg/kg of standard drug glibenclamide, NC: Normal control, DC: Diabetic control, H_2O_2: Hydrogen peroxidase

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