RESEARCH ARTICLE

Hypoglycemic and Antioxidant Activity of *Ficus arnottiana* Leaves: Insights into Diabetes Management and Oxidative Stress Mitigation

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Received: 22th March, 2024; Revised: 21th July, 2024; Accepted: 03rd August, 2024; Available Online: 31st August, 2024

ABSTRACT

Objective: To investigate the hypoglycaemic properties of a methanolic extract from *Ficus arnottiana* leaves in rodent models.

Results: The findings revealed that *F. arnottiana* leaves key active components (psoralen), notably coumarins, attributing to its prowess in combating hyperglycemia. In addition, the study observed that methanolic extract displayed the highest activity, specifically at a dosage of 500 mg/kg. This was in comparison to the standard reference, glimepiride, which exhibited activity at a dosage of 5 mg/kg. This finding suggests a potentially enhanced or superior effect associated with the methanolic extract in the context of the study, Furthermore,the finding of the result showed that the application of methanolic extract at a concentration of 500 mg/kg resulted in a noteworthy mitigation of oxidative stress and increased blood glucose levels. This was demonstrated by a significant decrease in oxidative stress markers, fasting blood glucose levels, OGT, and tissue damage in the experimental model, the control group was compared.

Conclusion: Methanolic extract of *F. arnottiana* exhibits efficacious hypoglycemic activity alongside ensured safety"

Keywords: Diabetes, Ficanone, *Ficus arnottiana*, antioxidants, free radicals.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.53

How to cite this article: Authors. Article Title. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1438-1443.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Diabetes, a labyrinthine metabolic puzzle, encompasses a spectrum of ailments characterized by heightened glucose levels in the bloodstream stemming from glitches in insulin secretion, insulin sensitivity, or both. "Its manifestations are diverse, with type 1 diabetes mellitus representing roughly 10% of confirmed cases, whereas the lion's share of diagnoses aligns with type 2 diabetes, colloquially known as NIDDM. In type 2 diabetes, individuals experience a gradual deterioration in insulin sensitivity, initially characterized by insulin resistance, which is the diminished ability of cells to respond effectively to insulin signals. Subsequently, there's a decline in the compensatory capacity of pancreatic beta cells to produce adequate insulin levels to counterbalance insulin resistance, ultimately leading to hyperglycemia and the clinical manifestations of diabetes mellitus.[1-3] In a formal context, it can be articulated that hyperglycemia demonstrates a significant association with an increased generation of free radical species and heightened levels of oxidative stress. This linkage underscores the intricate interplay between elevated blood glucose levels and the exacerbation of oxidative stress, thereby highlighting the profound impact of hyperglycemia on cellular homeostasis and overall physiological equilibriuma.[4] Prolonged high blood sugar levels cause an excess of reactive oxygen species (ROS) and free radicals, overpowering the body's antioxidant defenses. This imbalance leads to oxidative stress, damaging cellular components like proteins, lipids, and DNA. Additionally, oxidative stress contributes to endothelial dysfunction, inflammation, and disrupted cellular signaling pathways, all involved in diabetic complication development.[5] Oxidative stress worsens various diseases, such as neurodegenerative conditions and diabetes mellitus. Tissue damage mediated by lipid peroxides is involved in both types of diabetes, marked by increased TBARS and weakened antioxidant defenses, advancing the development of late complications.[6] Insulin plays a pivotal role in the treatment of diabetes but can lead to severe hypoglycemia. Nowadays, oral hypoglycemic drugs are widely used, yet they carry various side effects.

Ficus arnottiana, a member of the Moraceae family, is a botanical specimen characterized by its smooth, hairless (glabrous) appearance, presenting as either a tree or a shrub. Referred to colloquially as Paras Pipal, this species is widespread across the Indian subcontinent, flourishing particularly in regions characterized by rocky terrain and elevations reaching up to 1,350 meters above sea level. Its presence is notably prominent in rocky hillsides, where it establishes itself as an integral component of the local

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ecosystem.[7] *F. arnottiana* leaves, known as Paras Pipal, have diverse medicinal applications such as fertility control, anti-inflammatory, digestive aid, skin conditions, antioxidant properties, respiratory health and menstrual disorders.[8] The plant's leaves have within β-amyrin, bergapten, β-sisterol, friedelin, lupeol, psoralen, quercetin-3-galactoside, rutin, taraxosterol, along with lupeol.[9] Hence, in this current investigation, we embark on assessing the hypoglycemic potential of methanolic extracts derived from *F. arnottiana*, with the aim of pinpointing the specific constituent responsible for the plant's antidiabetic properties. Furthermore, our inquiry extends to examining the antidiabetic and antioxidant impacts of the active compound, psoralen, extracted from the leaves of *F. arnottiana*, in both normal and diabetic rat models".

MATERIALS AND METHODS

Plant Material

F. arnottiana leaves were gathered from the forests of Madhya Pradesh in April. The plant's identification and authentication were conducted by Dr. S.N Dwivedi, Janta P G College ,APS University, who holds a Master's degree and a PhD in Botany.

Drugs and Chemicals

Streptozotocin, a crucial component for our research, was generously provided as a gift sample by Sun Pharma. Meanwhile, "the standard antidiabetic medication, glimepiride, was obtained from Ranbaxy Research Laboratories located in Gurgaon, India, ensuring the highest quality for our experiments.

Plant Extracts Preparation

The leaves were picked, dried at room temperature, and then finely ground into powder after being shaded. About 450 g of this powder were then extracted using solvents of increasing polarity, beginning with methanol. Each solvent underwent 24-hour hot extraction in a Soxhlet apparatus at 60°C. After the extraction process, the solvents were evaporated using a rotary evaporator at reduced pressure until a consistent weight was reached. The extracted materials were gathered, placed in a drying chamber, and saved for future examination.

Preliminary Phytochemical Study

A fraction of the residue obtained from the methanolic extract underwent phytochemical analysis to ascertain the presence of various compounds within the leaf extracts, including alkaloids, sterols, carbohydrates, tannins, phenols, and others.[10,11]

Determination of Oxidative Stress and Blood Glucose Level

Animals

36 Wistar rats male, weighing approx 150 to 200 g, were obtained from the Animal House of the institution. They were kept under standard 12-hr light/12-hr dark conditions with free access to water and food. The rats were randomly divided into six groups: control (non-diabetic and untreated) or experimental (low, medium, and high dose). All animal experiments adhered to the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals. (CPCSEA). A one-week acclimatization period was provided for the animals before the experiment.[12]

Acute oral toxicity study

Testing on mice for acute oral toxicity was carried out following the guidelines of OECD Test Guideline No. 425, set forth by the Organization for Economic Cooperation and Development (OECD).This guideline provides standardized procedures for evaluating the potential adverse effects of substances administered orally.[13]

Experimentalmethods

Diabetes inducing process

The diabetic model used in this study was the streptozotocin-alloxan (STZ-A) model. This model involves inducing diabetes by administering streptozotocin (STZ) and alloxan (A), chemical agents known to selectively destroy pancreatic cells, resulting in insulin deficiency and hyperglycemia.[14] Male Wistar rats were induced with DM using intraperitoneal injections of neonatal alloxan and streptozotocin (STZ). Initially, 110 mg/kg of alloxan was administered in 0.9% normal saline, followed by 60 mg/kg of STZ in 0.1 M Citrate buffer with a pH of 4.5 after a 15-minute interval. To prepare the citrate buffer, trisodium citrate was dissolved in a citric acid solution to achieve a pH of 4.5, and ice was used to keep the temperature low. Citrate buffer was administered intraperitoneally to normal control rats.

On the fourth day after diabetes induction, rats with FBG levels above 126 mg/dl or OGTT readings in the first hour OG1 or second hour OG2 that exceeded 200 mg/dl were classified as diabetic rats and enrolled in the study.[14]

Therapy schedule

The Wistar rats were organized into six groups, with each group comprising 6 rats. Treatment commenced on the 4th day subsequent to the diabetes induction procedure, designated as Day 1 for treatment initiation, and extended for duration of 30 days. Throughout this period, the animals received treatment for the specified timeframe. The treatment regimen for each group unfolded as follows: Normal control (CON) treatment with normal saline per oral, Diabetic group (DB),diabetic groups were treated with standard drug glimepiride(STN+DB) (5 mg/kg, per oral), three test groups were treated with *F.Arnotianna* extract of (100 mg/kg, p.o, 250 mg/kg, per oral and 500 mg/kg, p.o)".[14]

Group 1, the control group (CON), the rats were orally feeded with normal saline (10 mL/kg) for 30 consecutive days.

Group2, Negative control group or diabetic group (DB), the rats were subcutaneously injected with "STZ (60 mg/kg) + NA (110 mg/kg) on the first four day to induce diabetes and then orally fed with normal saline (10 mL/kg) for 30 consecutive days.

Group3, the treatment group, the rats were orally given glimepiride (5 mg/kg) (STN+DB) for 30 consecutive days.

Groups 4, the treatment group, the rats were orally feeded with

F. arnotianna extract (100 mg/kg) (DB+FAE 100 mg/kg) for 30 consecutive days.

Groups 5, the treatment group, the rats were orally feeded with *F. arnotianna* extract (250 mg/kg) (DB+FAE 250 mg/kg) for 30 consecutive days.

Groups 6, the treatment group, the rats were orally feeded with *F. arnotianna* extract (500 mg/kg) (DB+FAE 500 mg/kg) for 30 consecutive days.

Estimation of FBG and OGT

Before giving STZ and A to the rats, all of them underwent measurement of fasting blood glucose levels to ensure they were below 126 mg/dl. Oral glucose tolerance tests (OGTT) were conducted on day 1 (4th day post-induction) and Day 30 of the treatment. For the OGTT, the rats fasted overnight for 12 hours and then received a glucose solution orally (2 g/kg body weight). Fasting blood glucose levels (FBG) were measured at 0 minutes before administering glucose. Additional readings (OG1 and OG2) were taken at 60 and 120 minutes after glucose administration. Blood samples for FBG, OG1, and OG2 were collected from the rat tail tip using an Accu-Check Glucometer. On Day 30, after ether anesthesia, blood was drawn via cardiac puncture from overnight-fasted rats into red-colored procoagulant vacutainers. The blood was subsequently centrifuged at 3000 rpm for 7 minutes to obtain serum.[14-16]

Analysis of oxidative stress parameter

To assess oxidative stress parameters, pancreatic tissue underwent homogenization in an ice-cold phosphate-buffered saline medium with 3 mM EDTA using a homogenizer. Subsequently, centrifugation at 7000 rpm for 10 minutes at 4°C was conducted to remove cell debris, utilizing a refrigerated centrifuge to clarify supernatants. Protein estimation, following the method outlined by Lowry et al., was then performed to determine protein concentration in the supernatant. The prepared supernatants were subjected to analysis for malondialdehyde (MDA), nitrite, superoxide dismutase (SOD), and catalase (CAT) activity. All results were normalized to the total protein content, expressed as per milligram of protein.[17-20]

Histopathological investigation of pancreatic, liver and kidney tissues

Pancreatic, hepatic, and kidney specimens from all experimental groups were excised and preserved in 10% neutral buffered formalin for fixation. Subsequent tissue processing involved washing the specimens with PBS, followed by dehydration in alcohol (ethanol) concentrations ranging from 70 to 100%. After dehydration, tissues were cleared with xylene, embedded in paraffin to form tissue blocks, and sectioned at a 5 µm thickness for histopathological examination. The tissue sections obtained were stained using hematoxylin and eosin to facilitate histopathological assessment.[21]

Statistical investigation

Statistical analysis involved employing one-way ANOVA followed by Tukey's post hoc test to identify significant differences among the study variables across various treatment groups. SPSS version 21.0 software was utilized for rigorous data evaluation.[13]

RESULT

Effect of *F. arnotianna* Extract on FBG and OGT at Day1stand Day 30th

On Day 1 of treatment, which was 4 days after induction, blood glucose levels were significantly higher in the diabetes-induced groups (DB, STN+DB, DB+FAE 100 mg/kg, DB+FAE 250 mg/kg, DB+FAE 500 mg/kg) compared to the Control group ($p < 0.0001$). All rats subjected to diabetes induction through STZ-NA treatment met the inclusion criteria (fasting blood glucose >126 mg/dl or OG1 reading >200 mg/dl), confirming successful induction of diabetes in all animals.

Baseline readings for OG1 and OG2 were significantly elevated in the diabetes-induced groups compared to the Control group ($p < 0.0001$).

By day 30 after treatment, fasting blood glucose (FBG) levels notably increased in the DB group compared to both the Control group and other treatment groups ($p < 0.001$). Furthermore, FBG levels in the DB+FAE 500 mg/kg group were significantly higher than those in the DB+FAE 250 mg/kg and DB+FAE 100 mg/kg treated groups".

Likewise, OG1 and OG2 measurements in the DB group showed significant increases compared to the other treatment groups post-treatment. Specifically, the OG1 measurement in the DB+FAE 500 mg/kg group was notably higher than that in the "DB+FAE 250 mg/kg group, while the OG2 measurement in the DB+FAE 500 mg/kg group was significantly higher than those in the DB+FAE 250 mg/kg and DB+FAE 100 mg/kg treatment groups.

CON: Normal Control; DB: Diabetic; treated;STN+DB: Metformin treated;DB+FAE 100 mg/kg, DB+FAE 250 mg/kg and DB+FAE 500 mg/kg :*F. arnotianna* extract 100, 250 and 500 mg/kg treated group.

Effect of *F. arnotianna* Extract on oxidative stress among various treatment groups

Malondialdehyde (MDA) serves as a reliable biomarker of lipid peroxidation, reflecting the oxidative degradation of lipids within cellular membranes.[22] The negative control group exhibited markedly elevated levels of MDA compared to the control. However, administration of *F. arnottiana* extract (at doses of 100, 250, and 500 mg/kg) significantly decreased MDA levels in comparison to the negative control group (DB). Furthermore, the pancreatic tissue of the negative control group (DB) displayed a significant increase in Nitric Oxide levels compared to the control group. Conversely", treatment with *F. arnottiana* Extract (at all doses) resulted in a notable reduction in Nitric Oxide levels.

The vital endogenous antioxidants, including superoxide dismutase, catalase, and glutathione, play a pivotal role in protecting tissues against oxidative stress and reactive oxygen species. In this study, a noteworthy decrease was observed

Table 1: FBG level, OGT test (first and second hour) (mg/dL)

Treatment Group	*Day 1 reading (baseline readings)*			*Day 30 readings (after treatment)*		
	FBG	*OG-1*	*OG-2*	*FBG*	*OG-1*	*OG-2*
	Mean ± SEM	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*	*Mean ± SEM*
CON	83.73	97.08	86.66	83.33	96	90.83
DB****	124.05	395.78	336.66	394.5	469	417.5
STN+DB$^{\#\#\#\#}$	127.25	420.83	302	143.16	327.66	202.5
DB+FAE 100 mg/kg^{aaaa}	130.83	388.83	327.66	81	239.66	109.5
DB+FAE 250 mg/kg^{bbbb}	130.5	369.5	336.33	80.3	138.33	92.5
DB+FAE 500 mg/kg^{cccc}	127.79	439.66	359.16	195.66	286	278.5

p ****< 0.0001 compared to CON; *p* $^{\#\#\#\#}$< 0.0001 compared to DB; *p* $^{\mathbf{aaaa}}$< 0.0001, *p* $^{\mathbf{bbbb}}$< 0.0001, *p* $^{\mathbf{cccc}}$< 0.0001 compared to DB

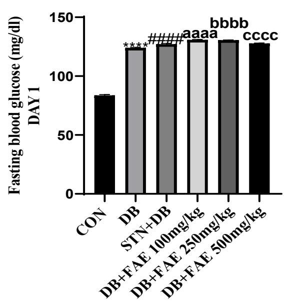

Figure 1: Fasting blood glucose level at Day 1where p****< 0.0001 compared to CON; p$^{\#\#\#\#}$ < 0.0001 compared to DB; p^{aaaa}< 0.0001,p^{bbbb}< 0.0001, p^{cccc}< 0.0001 compared to DB

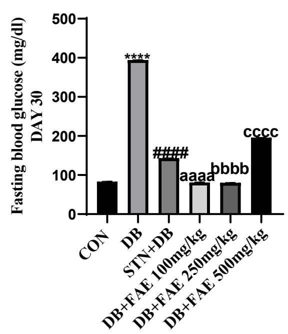

Figure 2: Fasting blood glucose level level at Day 30 where p****< 0.0001 compared to CON; p$^{\#\#\#\#}$ < 0.0001 compared to DB; p^{aaaa}< 0.0001, p^{bbbb}< 0.0001, p^{cccc}< 0.0001 compared to DB

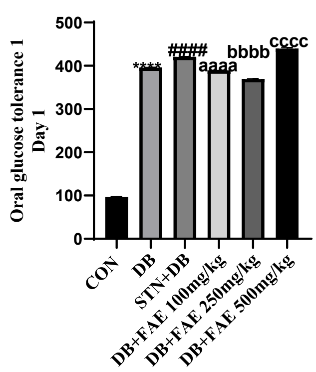

Figure 3: Oral glucose tolerance level 1 at Day 1 where p****< 0.0001 compared to CON; p$^{\#\#\#\#}$ < 0.0001 compared to DB; p^{aaaa}< 0.0001, p^{bbbb}< 0.0001, p^{cccc}< 0.0001 compared to DB

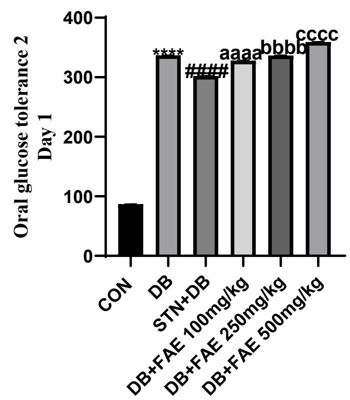

Figure 4: Oral glucose tolerance level 2 at Day 1 where p****< 0.0001 compared to CON; p$^{\#\#\#\#}$ < 0.0001 compared to DB; p^{aaaa}< 0.0001, p^{bbbb}< 0.0001, p^{cccc}< 0.0001 compared to DB

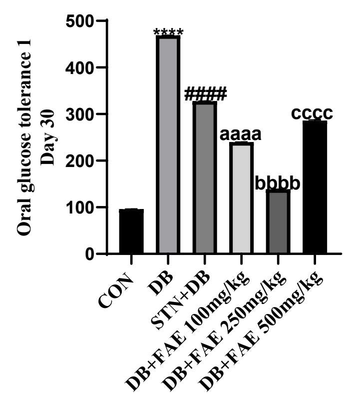

Figure 5: Oral glucose tolerance level 1 at Day 30 where p****< 0.0001 compared to CON; p$^{\#\#\#\#}$ < 0.0001 compared to DB; p^{aaaa}< 0.0001, p^{bbbb}< 0.0001, p^{cccc}< 0.0001 compared to DB

in the levels of SOD, CAT, and GSH in the negative control group (DB) compared to the control group. However, treatment with *F. arnottiana* Extract (at doses of 100, 250, and 500 mg/kg) markedly enhanced these antioxidant levels, significantly surpassing those of the negative control group (DB).

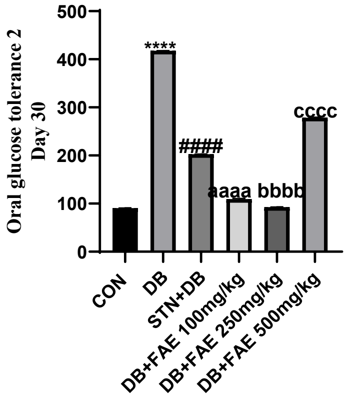

Figure 6: Oral glucose tolerance level 2 at Day 30 where $p^{****} < 0.0001$ compared to CON; $p^{\#\#\#\#} < 0.0001$ compared to DB; $p^{aaaa} < 0.0001$, $p^{bbbb} < 0.0001$, $p^{cccc} < 0.0001$ compared to DB

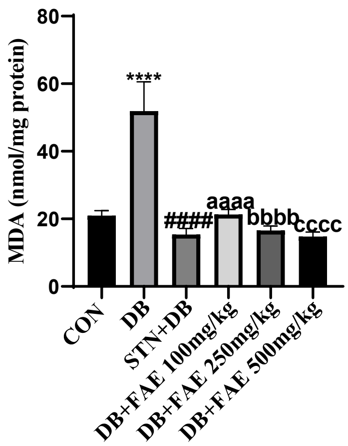

Figure 7: MDA level after 30 day treatment where $p^{****} < 0.0001$ compared to CON; $p^{\#\#\#\#} < 0.0001$ compared to DB; $p^{\mathbf{aaaa}} < 0.0001$,$p^{\mathbf{bbbb}} < 0.0001$, $p^{\mathbf{cccc}} < 0.0001$ compared to DB

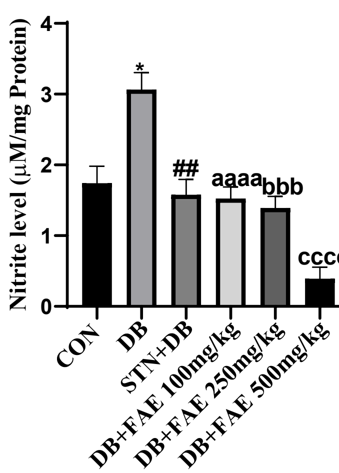

Figure 8: Nitrite level after 30 day treatment where $p^{*} < 0.1$ compared to CON; $p^{\#\#} < 0.01$ compared to DB; $p^{\mathbf{aaaa}} < 0.0001$,$p^{\mathbf{bbb}} < 0.001$, $p^{\mathbf{cccc}} < 0.0001$ compared to DB

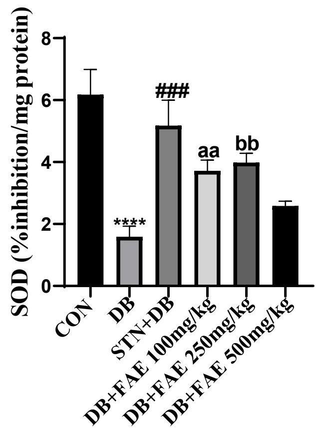

Figure 9: SOD level after 30 day treatment where $p^{****} < 0.0001$ compared to CON; $p^{\#\#\#} < 0.001$ compared to DB; $p^{\mathbf{aa}} < 0.01$,$p^{\mathbf{bb}} < 0.01$compared to DB

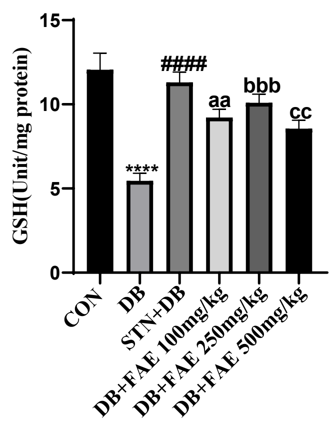

Figure 10: GSH level after 30 day treatment where $p^{****} < 0.0001$ compared to CON; $p^{\#\#\#\#} < 0.001$ compared to DB; $p^{\mathbf{aa}} < 0.01$,$p^{\mathbf{bbb}} < 0.001$, $p^{\mathbf{cc}} < 0.01$ compared to DB

Effect of *F. Arnotianna* Extract on pancreatic tissue among various treatment groups

To assess the damage at the cellular level, we performed histopathology of the pancreatic tissue by H&E staining. "Diabetic group (DB) animals showed shrunken and distorted islet of Langerhans displaying cells with vacuolated cytoplasm, while the *F. arnotianna* extract (500 mg/kg) treatment showed improvement in the shrunken and distorted islets of Langerhans cells.

Figure 10. (A-D): Pancreas sections stained with hematoxylin and eosin revealed distinct histological features:(A): The control group (CON) rats exhibited a normal histological view of the pancreas.(B): In the pancreas section of a rat from the DB group, the islet of Langerhans appeared shrunken and distorted, with cells exhibiting vacuolated cytoplasm and large darkly stained nuclei (arrows).(C): Pancreas sections from the *F. arnottiana* extract treated group (DB+FAE 250 mg/kg) rats displayed shrunken and distorted islets of Langerhans (arrows), along with cells showing degenerative and necrotic

changes (Arrowhead).(D): Conversely, pancreas sections from rats treated with *F. arnottiana* extract (DB+FAE 500 mg/kg) exhibited a normal islet of Langerhans with numerous β-cells.

Effect of *F. arnotianna e*xtract onliver tissue among various treatment groups

To assess the damage at the cellular level, we performed histopathology of the liver tissue by H&E staining. Diabetic group (DB) animals showed coagulative necrosis in hepatocytes, while the *F. arnotianna* extract (500 mg/kg) treatment showed almost normal morphology.

Figure 11. Liver sections stained with hematoxylin and eosin displayed distinctive histological features:(A): The control group (CON) rats exhibited a normal histological view of the liver.(B): Liver tissue from rats in the diabetic group (DB) displayed vacuolar hydropic degeneration (Arrow) and coagulative necrosis in hepatocytes (arrowheads).(C): Liver sections from animals treated with *F. arnottiana* extract (DB+FAE 250 mg/kg) showed dissociation in hepatic cordons (arrow).(D): Liver sections from animals treated with *F. arnottiana* extract (DB+FAE 500 mg/kg) exhibited an almost normal morphology.

Effect of *F. Arnotianna* Extract on kidney tissue among various treatment groups

To assess the damage at the cellular level, we performed histopathology of the kidney tissue by H&E staining. In the diabetic group (DB), animals exhibited notable pathological changes, including necrosis and vacuolar degeneration in tubular epithelial cells, along with inflammatory cell infiltration and dilatation of tubules. Conversely, treatment with *F. arnottiana* extract at a dosage of 500 mg/kg resulted in a significant improvement in renal morphology, displaying an almost normal appearance.

Figure 12. (A-D): Kidney sections stained with hematoxylin and eosin revealed distinct histological features:(A): The control group (CON) rats exhibited a normal histological view of the kidney.(B): Kidney sections from rats in the DB group displayed necrosis (thin arrows) and vacuolar degeneration (thick arrows) in tubular epithelial cells, inflammatory cell infiltration (arrowheads), and dilatation of tubules (star).(C): Kidney sections from rats treated with *F. arnottiana* extract at a dosage of 250 mg/kg (DB+FAE 250 mg/kg) showed hydropic degeneration in tubular epithelial cells (arrows), dilatation of tubules (star), and deterioration of the glomerular structure. (D): Conversely, kidney sections from rats treated with *F. arnottiana* Extract at a dosage of 500 mg/kg (DB+FAE 500 mg/kg) exhibited nearly normal histological features comparable to the control group.

SUMMARY

Research into the hypoglycemic and antioxidant properties of *F. arnottiana* leaves has centered on exploring the role of psoralen, a coumarin compound found in these leaves. The study investigated psoralen's effects in both normal and streptozotocin-induced diabetic rats. Methanol extracts of *F. arnottiana* leaves were orally administered at a dose of 500 mg/kg for 30 days. This treatment resulted in a significant reduction in fasting blood sugar (FBS) levels in diabetic rats. According to OECD-425 guidelines, acute oral toxicity studies indicated no mortality up to the highest tested dose of methanol extract (2000 mg/kg, p.o.). Phytochemical analysis identified psoralen as a furano coumarin compound present in *F. arnottiana* leaves. When administered orally at 500 mg/kg to diabetic rats, psoralen caused a substantial (p <0.0001) decrease in fasting blood glucose levels. Moreover, Psoralen treatment led to a notable reduction in lipid peroxidation and significant improvement in antioxidant enzyme levels, such as reduced glutathione, superoxide dismutase, and catalase in diabetic rats. Histopathological examination of the pancreas, kidney, and liver tissues further confirmed the beneficial effects of Psoralen treatment, showing improvement in β-cell condition in the pancreas, suggesting protective effects against diabetes-induced damage.

Overall, the findings of the study provide compelling evidence for the antioxidant potential and antidiabetic activity of psoralen. These results offer a scientific rationale for the traditional use of *F. arnottiana* leaves as an antidiabetic agent. Further research and clinical studies are warranted to explore the therapeutic potential of psoralen and its application in the management of diabetes and related complications."