INTERNATIONAL JOURNAL OF
PHARMACEUTICAL AND HEALTHCARE INNOVATION
journal homepage: www.ijphi.com

OPEN ACCESS

Research Article

Antidiabetic Activity of Polyherbal Formulation in Alloxan-Induced Diabetic Rats

Preeti Shanbhag[1]*, Navaneeth Krishna Bhat[1], Akshaya Umesh Bhandarkar[2]

[1,] PG Scholar, Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Mangalore- 574143.

[2]Associate Professor, Department of Pharmaceutics, Vidya Siri College of Pharmacy, Chikkakannalli, Bengaluru- 560035.

Article Info

Article history:

Manuscript ID:

IJPHI0404062024
Received:04-August -2024
Revised:04-September-2024
Accepted:06-October2024
Available online: October 2024

Keywords:
Polyherbal formulation, FACC-2, FACC-4, FACC-6, Antidiabetic activity, Alloxan-induced diabetes

Corresponding Author:
preetishanbhag24@gmail.com

Abstract

Background and Objective*: Diabetes mellitus is a prevalent chronic metabolic disorder necessitating effective therapeutic approaches. Traditional medicinal practices offer a promising avenue for novel antidiabetic agents, with polyherbal formulations garnering attention for their synergistic potential. This study aimed to evaluate the antidiabetic efficacy and safety of a polyherbal formulation (FACC) comprising Fenugreek, Ajwain, Cumin, and Chickpea in alloxan-induced diabetic rats.*

Methods*: Plant materials were meticulously collected, authenticated, and subjected to extraction. Three different strengths of FACC were prepared for dose optimization. Acute toxicity studies were conducted following OECD guidelines. Diabetes was induced in Wistar albino rats using alloxan, and FACC was administered orally for three weeks. Blood glucose levels and serum biochemical parameters were assessed, and histopathological examination of pancreatic and hepatic tissues was performed. Results: Acute toxicity studies revealed no adverse effects of FACC even at high doses. FACC-treated rats exhibited significant reductions in blood glucose levels in a dose-dependent manner compared to diabetic controls. Systemic improvements in metabolic and liver function markers were observed in FACC-treated rats. Histopathological examination confirmed the preservation of tissue integrity in pancreatic and hepatic tissues.*

Conclusion: *The polyherbal FACC demonstrated promising antidiabetic effects with a favorable safety profile in experimental animal models. These findings suggest that FACC could be a potential therapeutic option for diabetes management, warranting further investigation for clinical translation.*

Introduction

Diabetes mellitus, a prevalent metabolic disorder globally, prompts the exploration of complementary strategies alongside conventional treatments. Traditional medicinal practices, deeply rooted in cultural heritage, present a promising avenue for novel antidiabetic agents[1-4]. Polyherbal formulations, blending various plant-based ingredients synergistically, have emerged as a focal point in this quest. Fenugreek, known for its medicinal properties, contains bioactive compounds like trigonelline and saponins that aid in reducing blood glucose levels by enhancing insulin sensitivity [5-9]. Ajwain, rich in thymol, exhibits antioxidant properties and potential for improving insulin sensitivity. Cumin, with constituents like cuminaldehyde, shows antidiabetic effects by enhancing insulin secretion. Chickpeas, beyond their nutritional value, offer polyphenols and soluble fiber that aid in glycemic control [10-15]

The study investigates the synergistic potential of traditional ingredients in a polyherbal blend for diabetes management. By combining historical wisdom with scientific scrutiny, it aims to explore the efficacy and safety of natural antidiabetic agents through biochemical and clinical studies.

MATERIALS AND METHODS

Plant material

The initial phase of this research involved meticulous collection and authentication of the plant materials. The seeds of Fenugreek, Ajwain, Cumin and Chickpea were procured from the local market of Mangalore. To ensure their authenticity, the samples were subjected to rigorous examination and verification by the Siddaraju M. N., Assistant professor, Department of Botany at Mangalore university, Mangalore.

Chemicals:

For the experimental procedures, specific chemicals were carefully selected and procured. Alloxan, obtained from SD fine chem-limited, Mumbai, Tolbutamide, obtained from Jiyan chemicals, Gujarat, along with Ethanol and Normal Saline Solution, were utilized in the subsequent phases of the research.

Preparation of plant extract

The extraction process for each plant followed a standardized methodology[16]. Fenugreek, Ajwain, Cumin and Chickpea seeds were individually collected, air-dried, and finely powdered. Subsequently, 1500 grams of each powdered seed were subjected to extraction using a Soxhlet apparatus, employing 70% ethanol as the solvent. This meticulous extraction process aimed to capture and concentrate the bioactive compounds present in each plant, laying the foundation for the subsequent stages of the study.

Preparation of Polyherbal Formulation:

Development of Poly Herbal Formulation: The Poly Herbal Formulation was prepared in three different strengths, 200 mg/kg B. Wt, p.o/day (FACC-2), 400 mg/kg B.Wt, p.o/day (FACC-4), 600 mg/kg B.Wt, p.o/day (FACC-6) for dose optimization and to find out the most effective and safer dose. The following formulations were prepared:

Table1: Formulation 1 (FACC-2)

Ingredients	Quantity (mg)
Fenugreek	13.6
Ajwain	32.3
Curcumin	53.64
Chickpea	100.46

Table 2: Formulation 2 (FACC-4)

Ingredients	Quantity (mg)
Fenugreek	27.2
Ajwain	64.6
Curcumin	107.28
Chickpea	200.92

Table 3: Formulation 3 (FACC-6)

Ingredients	Quantity (mg)
Fenugreek	40.8
Ajwain	96.9
Curcumin	160.92
Chickpea	301.38

Animals

The study followed CCSEA guidelines, approved by the Institutional Animals Ethics Committee, Srinivas College of Pharmacy. Wistar albino rats, 180-200g, were obtained from Geniron Biolabs Pvt. Ltd. Rats were housed in polypropylene cages at Srinivas College of Pharmacy, Mangalore, under standard conditions with a 12-hour light-dark cycle and ad libitum access to food and water. The animal house maintained a temperature of 25±20°C with relative humidity at 50±15%. Throughout the experiments, ethical norms were strictly observed [17].

Acute Toxicity studies

The acute oral toxicity study followed guidelines outlined by the Organization for Economic Cooperation and Development (OECD) and draft guidelines 420 received from the CCSEA, Ministry of Social Justice and Empowerment, Government of India. Six female albino rats were employed, and a single dose of 3000 mg/kg of the Poly Herbal Formulation (FACC) was administered via gavage. Continuous monitoring of each animal took place within the initial 30 minutes post-dosing, at intervals over the first 24 hours, and subsequently daily for a period of 14 days.

Induction of Diabetes: The experimental procedure involved inducing diabetes in albino rats through an intraperitoneal injection of Alloxan at 120 mg/kg body weight after an overnight fasting period. Alloxan selectively damages the insulin-producing β-cells in the pancreas, leading to diabetes. To prevent fatal hypoglycemia, rats were given a 20% glucose solution for 6 hours post-injection, followed by access to water and a 5% glucose solution for the next 24 hours. Blood glucose levels exceeding 200 mg/dl, measured with the ACCU-CHEK ACTIVE Glucometer, confirmed diabetes. Rats with elevated glucose levels were maintained in a diabetic state for 72 hours to ensure diabetes development. This rigorous protocol ensured the establishment of a stable diabetic condition in the experimental animals [14].

Study design

The Poly Herbal Formulations (FACC) were suspended in 2% acacia solution and the given by oral route using a catheter. Tolbutamide 100mg/kg was used as a standard drug.[15 -16]

Animals were divided into six groups of six each.

Group-1: Healthy normal animals received only the water served as Normal control.

Group-2: Untreated, alloxan-induced diabetic animals served as a Diabetic control group and also received water.

Group-3: Standard group was treated with Tolbutamide 100 mg/kg B.Wt., p.o.

Group-4: Diabetic control animals treated with FACC-2 p.o.

Group-5: Diabetic control animals treated with FACC-4 p.o.

Group-6: Diabetic control animals treated with FACC-6 p.o.

Blood samples were obtained through the retro-orbital plexus puncture method, and electronic glucometer (ACCU-CHEK ACTIVE" Glucometer from Roche Diagnostics GmbH, Germany) was utilized for blood glucose level assessments. Sampling occurred at weekly intervals throughout the three-week study period, specifically on days 0, 7, 14, and 21. On the 21st day, blood collection took place through cardiac puncture under mild ether anesthesia from overnight fasted rats, and fasting blood sugar was estimated. Subsequently, serum was separated for a comprehensive analysis, encompassing serum cholesterol, triglycerides, HDL, LDL, creatinine, urea, alkaline phosphatase (ALP), bilirubin, serum glutamate oxalate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT).

Statistical Analysis

The statistical analysis of experimental data was conducted using Graph Pad Prism software, version 10.2.0. The analysis involved employing analysis of variance (ANOVA) followed by Dunnet's test for post hoc comparisons. Significance levels were defined as $p < 0.001$, $p < 0.01$, and $p < 0.05$.

Histopathology of isolated liver and pancreas:

Tiny portions of liver and pancreas tissues were carefully gathered and preserved in 10% formalin to ensure proper fixation. For histopathological investigations, these tissues underwent fixation in Bouin's fixative (without acetic acid). Tissue sections, precisely 6 microns thick, were stained with haematoxylin and eosin (H and E) to facilitate histological examination. The resulting photomicrographs from these histological studies are visually depicted in figures 3 and 4, providing a detailed insight into the cellular structures and morphological changes within the tissues.

RESULT

The Acute Toxicity studies on FACC-treated rats didn't show any noticeable behavioral changes when administered orally. There were no instances of mortality even when a high dose of 3g/kg body weight was given orally, which is higher than the effective antihyperglycemic dose, during the observation period. The rats were closely monitored for 24 hours for any signs of mortality, and for up to 14 days for any delayed toxic effects on their behavioral activitie

Antidiabetic activity

Table 4 - Effect of 3-week treatment with the standard drug and FACC on blood glucose level after alloxan-induced diabetic rats.

S.N.	GROUP	0-DAY	7-DAY	14-DAY	21-DAY
1.	**Normal control**	77.32 ± 2.582	78.45 ± 1.860	79.89 ± 1.34	77.88 ± 0.976
2.	**Diabetic control**	317.3 ± 4.168	354.87 ± 6.12	368.2 ± 9.38	371.33 ± 9.898
3.	**Standard**	359.2 ± 4.790*#	245.12 ± 20.78*#	198.76 ± 12.79*#	137.89 ± 13.58*#
4.	**Diabetic control +FACC-2**	372.3 ± 7.342*#	328.12 ± 7.672*	273.78 ± 4.887*#	256.11 ± 9.689*#

5.	**Diabetic control +FACC-4**	369.7 ± 7.763*#	269.12 ± 9.52*#	196.48 ± 16.34*#	162.89 ± 13.756*#
6.	**Diabetic control +FACC-6**	353.2 ± 6.186*#	256.12 ± 10.89*#	185.98 ± 18.12*#	156.12 ± 13.89*#

The values presented in the table represent the Mean ± SEM of animals. * P<0.01 (Tukey test) significant when treated with Normal control # P<0.01 (Tukey test) significant when treated with Diabetic control.

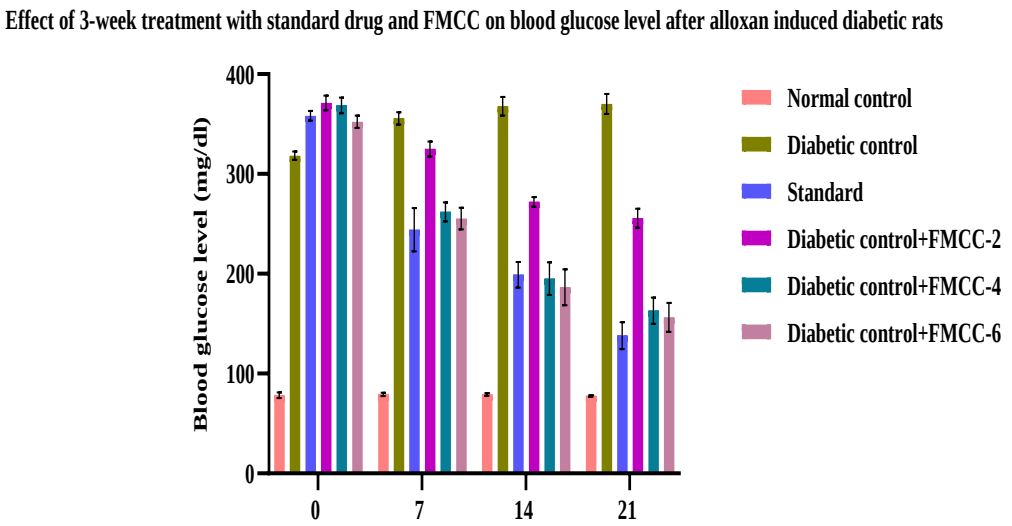

Fig. 1: Effect of 3-week treatment with standard drug and FACC on blood glucose level after alloxan induced diabetic rats

Table 5 - Effect of FACC on serum profile in alloxan induced diabetic albino rats after 21 days treatment

S.N.	Serum profile	Normal control	Diabetic control	Standard	FACC-2 treated	FACC-4 treated	FACC-6 treated
1	**Cholesterol**	74.22±6.395	147.35±9.352	87.75±4.812#	102±2.357#	95.21±7.89#	94.93±16.68#
2	**Triglycerides**	41.12±3.123	92.46±4.345	52.88±3.885#	74.42±5.186*	56.78±5.235#	81.48±8.729*
3	**HDL**	25.88±0.132	13.367±1.12	21.89±0.981*#	17.45±0.8513*	20.72±0.8652*#	21.14±0.677*#
4	**LDL**	29.45±3.678	94.80±5.89	41.89±3.989#	53.75±6.356#	40.88±4.502#	47.50±5.575#
5	**Urea**	74.25±4.678	132.78±13.67	69.78±4.678#	80.96±5.822#	72.22±4.415#	76.15±7.598#
6	**Creatinine**	0.525±0.026	1.56±0.167	0.663±0.212#	0.7475±0.2016*#	0.6675±0.0292#	0.667±0.0396#
7	**Albumin**	3.854±0.032	1.775±0.137	3.193±0.0423#	2.625±0.2562*	3.30±0.124#	2.425±0.449*
8	**SGPT**	56.75±5.963	149.8±4.090	68±2.972#	97.50±5.951#	69.50±4.664#	105.8±28.19*

9	**SGOT**	52.15±4.614	139±14.08	82.13±6.514$^{\#}$	97.85±8.556*#	84.35±4.870#	96.25±9.88*#
10	**ALP**	79.50±6.193	145.8±5.528	94.38±2.348$^{\#}$	106.5±3.304*#	96.19±2.618#	97.30±2.489#
11	**Bilirubin**	0.2125±0.013	0.4475±0.0482	0.2450±0.0236$^{\#}$	0.3275±0.01315	0.265±0.0188#	0.235±0.016#

NC= Normal control, DC= Diabetic control, STD= Standard. The values presented in the table represent the Mean ± SEM of animals. * P<0.01 (Tukey test) significant when treated with Normal control # P<0.01 (Tukey test) significant when treated with Diabetic control.

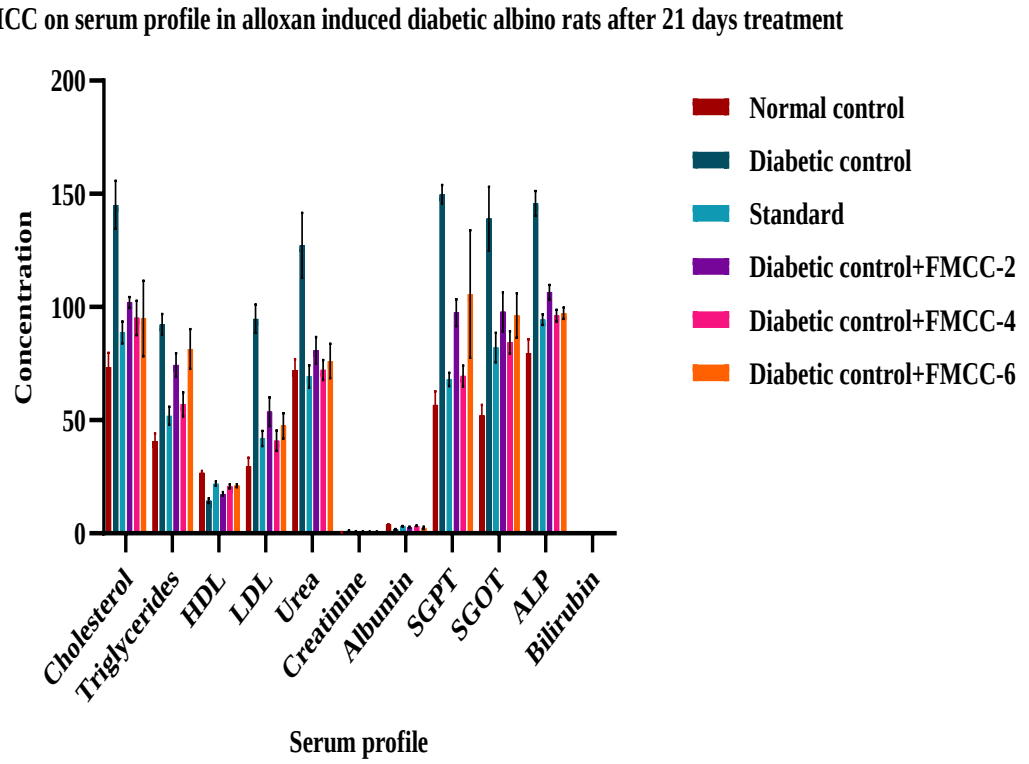

Fig 2: Effect of FACC on serum profile in alloxan induced diabetic albino rats after 21 days treatment

Histopathology

Photomicrographs (Fig.3) shows normal acini and normal cellular population in the islets of langerhans in pancreas of normal control and lesions in diabetic rats which maintained significantly after treatment by standard drug and FACC-4 up to normal.

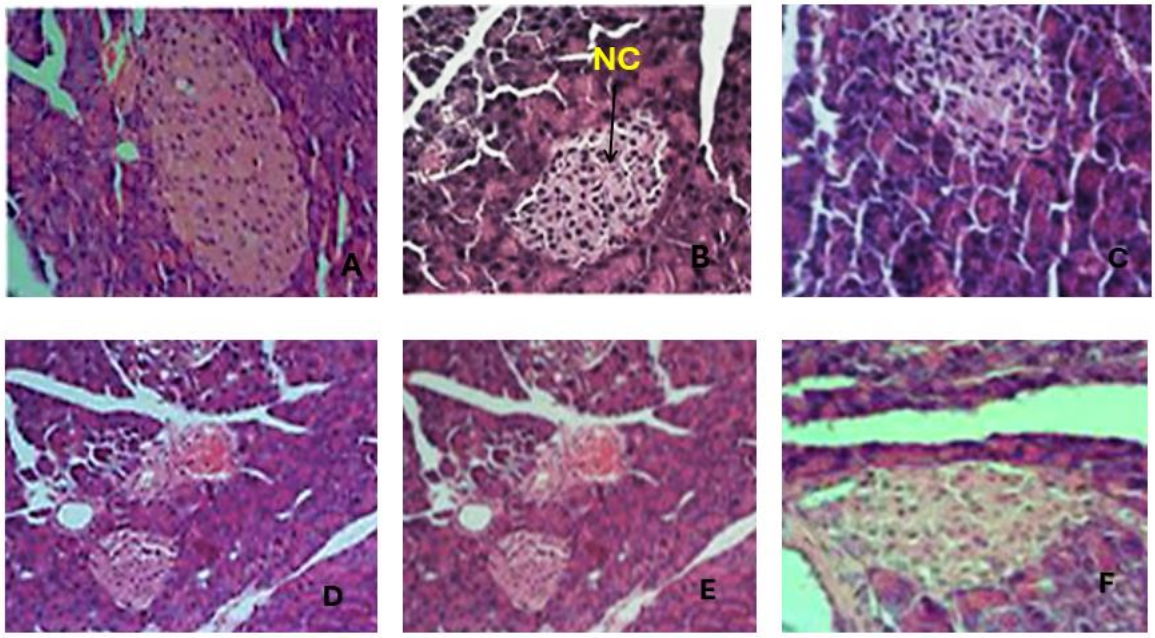

Fig. 3: Photomicrograph of rat pancreas stained by haematoxylin and eosin of normal control (A) diabetic control (B) standard (tolbutamide) treated (C) FACC-2 treated (D) FACC-4 treated (E) FACC-6 treated (F)

Photomicrographs (Fig.4) shows normal hepatocytes and lesions in diabetic rats which maintained significantly after treatment by standard drug and FACC-4.

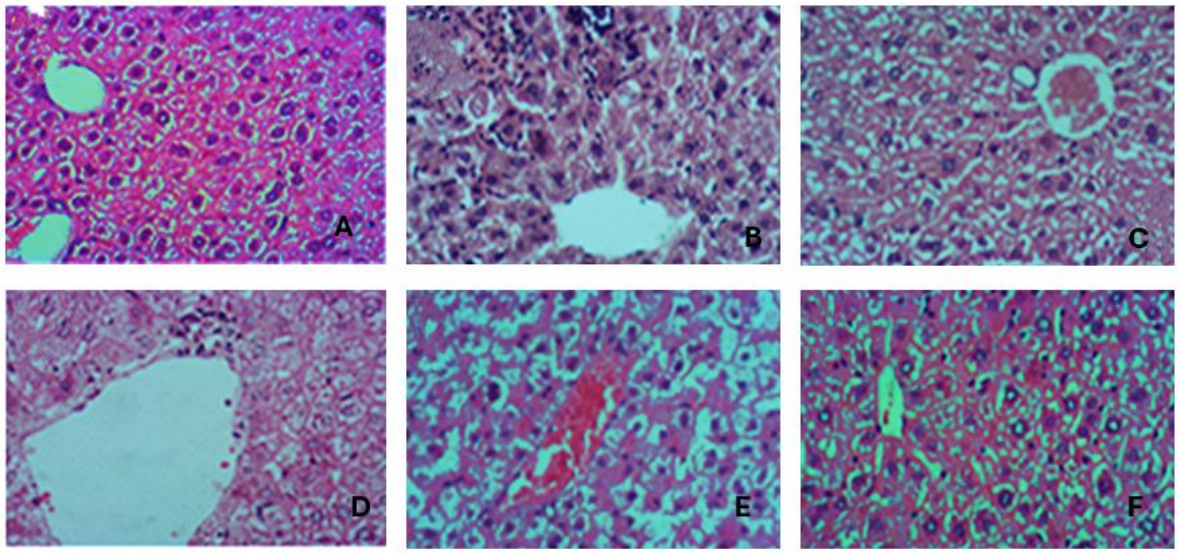

Fig. 4: Photomicrograph of rat liver stained by haematoxylin and eosin of normal control (A) diabetic control (B) standard (tolbutamide) treated (C) FACC-2 treated (D) FACC-4 treated (E) FACC-6 treated (F)

DISCUSSION

The utilization of medicinal plants as a source of bioactive agents for treating various ailments, including diabetes, is gaining widespread acceptance globally[19]. The ethnobotanical perspective offers valuable insights into the development of novel drugs to combat human diseases, particularly diabetes [20]. In both urban and rural settings, the popularity of safe, effective, and affordable indigenous remedies is on the rise, especially in countries like India.

Alloxan-induced diabetes serves as a common animal model for studying diabetes in experimental settings [21,22]. Alloxan exerts its diabetogenic effects by rapidly targeting insulin-secreting beta cells in the pancreas, leading to their destruction through the generation of reactive oxygen species [23,24]. Despite this cytotoxic action, some beta cells may survive, as evidenced by the observed antihyperglycemic activity of oral hypoglycemic agents in alloxan-induced diabetes [25-27].

The acute toxicity study on polyherbal FACC in rats showed no adverse behavioral effects even at high doses, with no mortality recorded. In assessing antidiabetic activity, FACC significantly reduced blood glucose levels in diabetic rats over 3 weeks, demonstrating dose-dependent improvement.

The antidiabetic activity assessment, the study examined the effect of polyherbal FACC on blood glucose levels in alloxan-induced diabetic rats over a 3-week treatment period. The data presented in Table 4 illustrate a significant reduction in blood glucose levels in rats treated with polyherbal FACC compared to diabetic control rats. Notably, rats treated with polyherbal FACC exhibited dose-dependent improvements in blood glucose levels, with considerable reductions observed at 21 days post-treatment. These results underscore the potential of polyherbal FACC as an effective antidiabetic agent, offering promise for the management of diabetes mellitus.

Furthermore, the impact of polyherbal FACC treatment on various serum parameters associated with diabetes was investigated. Table 5 provides insights into the favorable alterations in serum cholesterol, triglycerides, HDL, LDL, urea, creatinine, albumin, SGPT, SGOT, ALP, and bilirubin levels in polyherbal FACC-treated rats compared to diabetic control rats. These systemic improvements in metabolic and liver function further support the antidiabetic potential of polyherbal FACC.

Histopathological examination revealed that polyherbal FACC preserved normal cellular structure in the pancreas and liver of diabetic rats, highlighting its protective effects. Overall, these findings suggest that polyherbal FACC has the potential to be an effective antidiabetic agent, offering hope for diabetes management. Overall, the findings suggest that polyherbal FACC exhibits promising antidiabetic activity with a favorable safety profile in experimental animal models, warranting further research to explore its mechanisms of action and translational potential in diabetes management.

CONCLUSION

The polyherbal FACC demonstrates promising antidiabetic effects with a favorable safety profile, as evidenced by the lack of acute toxicity and significant reductions in blood glucose levels in alloxan-induced diabetic rats. Moreover, FACC treatment leads to systemic improvements in metabolic and liver function, supported by favorable alterations in serum parameters. Histopathological examination further confirms the protective effects of FACC on pancreatic and hepatic tissues. These findings underscore the potential of polyherbal FACC as a safe and effective therapeutic option for diabetes management, warranting further investigation.

Conflict of interest: None.
Funding: None.

Ethical statement:

The study was carried out as per CPCSEA norms after obtaining approval from the Institutional Animal Ethical Committee. Ref no: SCP/IAEC/F165/P117/2023 dated 13/08/2023.

REFERENCES

1. Karunamoorthi K, Jegajeevanram K, Vijayalakshmi J, Mengistie E. Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. J Evid Based Complementary Altern Med. 2013;18(1):67-74.
2. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biol. 2020;9(9):252.
3. Lankatillake C, Huynh T, Dias DA. Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. Plant Methods. 2019;15(1):105.
4. Srinivasa UM, Naidu MM. Fenugreek (*Trigonella foenum-graecum* L.) seed: promising source of nutraceutical. Stud Nat Prod Chem. 2021;71:141-84.
5. Verma PP, Nayyer MA, Singh S, Siddiqui S, Kumar D, Agri B, et al. Exploring genetic and morphological diversity of fenugreek (*Trigonella foenum-graecum* L.) in Uttarakhand: A Review. J Ayurvedic Herbal Med. 2023;9(3):133-7.
6. Bhat R, Himasvi MM, Varshini N, Shabaraya AR. Emerging Frontiers and Future Directions in Nanomedicine: A Comprehensive Review. Int J Curr Res Physiol Pharmacol. 2024;11:25-32.
7. Bahmani M, Shirzad H, Mirhosseini M, Mesripour A, Rafieian-Kopaei M. A review on ethnobotanical and therapeutic uses of fenugreek (*Trigonella foenum-graceum* L). J Evid Based Complement Altern Med. 2016;21(1):53-62.
8. Maqbool M, Chaudhary K, Chalotra R, Chauhan S, Dahiya RS. Phyto-pharmacology of Most Common Indian Culinary Spices and their Potential in Developing New Pharmaceutical Therapies. Curr Tradit Med. 2024;10(2):117-40.
9. Bader GN, Rashid R, Ali T, Hajam TA, Kareem O, Mir SA, et al. Medicinal Plants and their Contribution in Socio-Economic Upliftment of the Household in Gurez Valley (J&K). In Edible Plants in Health and Diseases: Volume 1: Cultural, Practical and Economic Value. Singapore: Springer Nature Singapore; 2022. p. 107-136.
10. Singh N, Yadav SS, Kumar S, Narashiman B. A review on traditional uses, phytochemistry, pharmacology, and clinical research of dietary spice *Cuminum cyminum* L. Phytother Res. 2021;35(9):5007-30.
11. Sarkar C, Jamaddar S, Islam T, Mondal M, Islam MT, Mubarak MS. Therapeutic perspectives of the black cumin component thymoquinone: A review. Food Funct. 2021;12(14):6167-213.
12. Saad B, Zaid H, Shanak S, Kadan S, Saad B, Zaid H, Shanak S, et al. Hypoglycemic and Anti-obesity Polyherbal Mixtures. In: Anti-diabetes and Anti-obesity Medicinal Plants and Phytochemicals: Safety, Efficacy, and Action Mechanisms. 2017. p. 217-51.
13. Zandoná GP, Siebeneichler TJ, Hoffmann JF, Ferreira CD, De Oliveira M. Bioactive Profile and Antioxidant Properties of Chickpea and Cowpea: Part I. In: Chickpea and Cowpea. CRC Press; 2023. p. 137-172.
14. Shanbhag P, Satish S, Bhat R, Shabaraya AR. A need of the hour: pharmacovigilance in healthcare setting–nothing less than safety. Int J Curr Res Physiol Pharmacol. 2023;19:19-24.
15. Shanbhag P, Bhat R, Mestha SV, Nagesh S, Nayak DRK. Investigation of Anti-anxiety Activity of Hydroalcoholic Extract of *Plectranthus scutellarioides* Leaves in Experimental Animal Models. Int J Pharm Sci Rev Res. 2022;76(1):115-8.
16. Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and

clinical implications. Diabetol Metab Syndr. 2017;9:1-2.

17. Moura A, Lopes L, Matos LC, Machado J, Criado MB. Converging Paths: A Comprehensive Review of the Synergistic Approach between Complementary Medicines and Western Medicine in Addressing COVID-19 in 2020. BioMed. 2023;3(2):282-308.
18. Bhat R, Shanbhag P, Shabaraya AR. Diuretic Activity of Ethanolic Extract of *Bauhinia tomentosa* Linn Roots. Int J Pharm Phytopharmacol Res. 2023;13(2):25-9.
19. Mestha SV, Nagesh S, Shanbhag P, Bhat R. Evaluation of anti-depressant activity of methanolic extract of *Averrhoa bilimbi* using various animal models. World J Curr Med Pharm Res. 2022;4(5):118-21.
20. Bhat R, Varshini H, Shabaraya R. Pharmacogenomic Approaches in Alzheimer's Disease: A Comprehensive Review. Int J Pharm Phytopharmacol Res (eIJPPR). 2023;13(4):7-13.
21. Shanmugam KR, Shanmugam B, Subbaiah GV, Ravi S, Reddy KS. Medicinal plants and bioactive compounds for diabetes management: important advances in drug discovery. Curr Pharm Des. 2021;27(6):763-74.
22. Bhat R, Shanbhag P. Evaluation of anticonvulsant activity of *Bixa Orellana Linn*. Seed. J Pharmacovigil Drug Res. 2023;4(3):34-9.
23. Bhat R, Shanbhag P. Emerging Trends in Biomaterials for Cancer Immunotherapy and Genome Editing: A Comprehensive Review. Indian J Pharm Drug Stud. 2024;3(2):1-9.
24. Durie M. Understanding health and illness: research at the interface between science and indigenous knowledge. Int J Epidemiol. 2004;33(5):1138-43.
25. Halberstein RA. Medicinal plants: historical and cross-cultural usage patterns. Ann Epidemiol. 2005;15(9):686-99.