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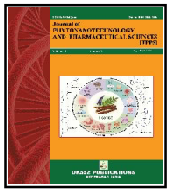

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Effect of Biochanin A and caffeic acid combination on hyperglycemia and oxidative stress in streptozotocin-induced diabetic rats

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Abstract

The present study was designed to evaluate the antihyperglycemic and antioxidant effects of biochanin A and caffeic acid, individually and in combination, in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in male Sprague Dawley rats by intraperitoneal injection of STZ (65 mg/kg), followed by nicotinamide (110 mg/kg). Oral administration of biochanin A (40 mg/kg) and caffeic acid (40 mg/kg), alone and in combination, was carried out once daily for 15 days. The diabetic control group exhibited significant hyperglycemia, body weight loss, and alterations in oxidative stress markers. Treatment with biochanin A and caffeic acid significantly reduced blood glucose levels, improved body weight, and restored antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), while decreasing malondialdehyde (MDA) levels. The combined treatment demonstrated greater efficacy than either compound alone, suggesting a possible synergistic interaction between the two phytoconstituents. These findings indicate that the combination of biochanin A and caffeic acid confers potent antihyperglycemic and antioxidant protection against STZ-induced diabetes, and may serve as a promising natural therapeutic approach for the management of diabetes and related oxidative complications.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and a major public health concern due to its increasing incidence, morbidity, and mortality. Chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels (Hashim *et al.,* 2024; Hashim *et al.,* 2023). Oxidative stress plays a pivotal role in the pathogenesis and progression of diabetes and its complications, primarily due to excessive generation of reactive oxygen species (ROS) and a decline in antioxidant defense mechanisms. Therefore, strategies that can improve glucose homeostasis and mitigate oxidative damage have gained significant interest in contemporary research (Singh *et al.,* 2024). Streptozotocin (STZ)-induced diabetes in rodents is a well-established experimental model that mimics the pathological features of human diabetes, particularly β-cell destruction and insulin deficiency. STZ exerts its diabetogenic effect by selectively damaging pancreatic β-cells through DNA alkylation and oxidative stress. The resulting hyperglycemia is accompanied by alterations in lipid metabolism, weight loss, and increased oxidative biomarkers (Shoaib *et al.,* 2020). Nicotinamide co-administration with STZ produces a partial destruction of β-cells, simulating type 2 diabetes and allowing evaluation of agents that preserve or enhance β-cell function.

In recent years, natural polyphenolic compounds have received considerable attention for their antidiabetic potential. Flavonoids and phenolic acids are known to exhibit multiple pharmacological activities, including antioxidant, anti-inflammatory, and glucose-lowering effects (Mutha *et al.,* 2021). Biochanin A, an isoflavone commonly found in red clover (*Trifolium pratense*), exhibits antioxidant, anti-inflammatory, and estrogenic properties (Chaturvedi *et al.,*2021). It has been reported to modulate glucose metabolism and protect pancreatic β-cells from oxidative injury (Anuranjana *et al.,* 2023). Similarly, caffeic acid, a hydroxycinnamic acid present in various fruits, vegetables, and coffee, demonstrates potent antioxidant and free radical-scavenging activities. It has been shown to attenuate hyperglycemia, lipid peroxidation, and oxidative stress in diabetic models (Yusuf *et al.,* 2019). Despite the individual antidiabetic effects of biochanin A and caffeic acid, limited studies have explored their potential synergistic activity in combination. Since both compounds exert complementary antioxidant mechanisms, their concurrent administration may provide enhanced protection against oxidative stress and pancreatic dysfunction associated with diabetes. Hence, the present study was designed to evaluate the antihyperglycemic and antioxidant effects of the combination of biochanin A and caffeic acid in streptozotocin-induced diabetic rats, with a view to exploring possible synergism between these phytoconstituents in mitigating hyperglycemia and oxidative stress.

2. Materials and Methods

2.1 Drugs and chemicals

The entire chemicals which were used are of analytical grade.

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2.2 Preparation of 0.1 M citrate buffer

A 0.1 M citric acid solution was prepared by dissolving 192 mg of citric acid in 10 ml of distilled water, and a 0.1 M trisodium citrate solution was prepared by dissolving 294 mg of trisodium citrate in 10 ml of distilled water. The two solutions were then mixed in a 4.5:5.5 ratio (citric acid: trisodium citrate), and the pH was adjusted to 4.5 using citric acid.

2.3 Preparation of STZ solution

Solution of STZ (65 mg/kg b.w., i.p.) was prepared by dissolving it in an ice-cold citrate buffer (pH 4.5, 0.1 M).

2.4 Animals

Adult male Sprague - Dawley rats weighing 120-200 g were procured from the Central Drug Research Institute (CDRI), Lucknow, and housed in the Animal House Facility, Faculty of Pharmacy, Integral University, Lucknow. The animals were kept in polypropylene cages (five rats per cage) under controlled environmental conditions of 12 h light/dark cycle, temperature 23 ± 2°C, and relative humidity 50 ± 15%. All rats had free access to standard pellet diet and water ad libitum. Animals were allowed to acclimatize for seven days prior to experimentation. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Integral University, Lucknow (Approval No.: IU/IAEC/20/23).

2.5 Experimental design

2.5.1 Diabetes induction

Rats were deprived of food but not water overnight prior to the induction of diabetes. Streptozotocin (STZ) was freshly dissolved in ice-cold citrate buffer (0.1 M, pH 4.5) and administered intraperitoneally (i.p.) at a dose of 65 mg/kg body weight within 5 min of preparation. This was followed, after 15 min, by an intraperitoneal injection of nicotinamide (NIC; 110 mg/kg) to partially protect pancreatic β-cells and induce a type 2 diabetes-like state. To prevent mortality due to acute hypoglycemia, rats were provided with 5% glucose solution for 24 h following STZ administration (Table 1).

After 72 h, blood glucose levels were determined using blood collected from the tail vein and analyzed with a glucometer (Accu-Chek, Roche Diagnostics, Germany). Animals exhibiting fasting plasma glucose levels above 250 mg/dl were considered diabetic and selected for further experimentation.

Table 1: Treatment schedule

Groups	Treatment	Dosage, route of administration, and duration
Group I (NC)	Vehicle (1% CMC)	10 ml/kg, p.o. once a day for 15 days
Group II (DC)	STZ + Nicotinamide	65 mg/kg, i.p. STZ (single dose) +110 mg/kg, i.p. NIC after 15 min
Group III (Met 250 mg)	Diabetic rat + Metformin	250 mg/kg, p.o. once a day for 15 days
Group IV (BCA 40 mg)	Diabetic rat + Caffeic acid	40 mg/kg, p.o. once a day for 15 days
Group V (CA 40 mg)	Diabetic rat + Biochanin-A	40 mg/kg, p.o. once a day for 15 days
Group VI (BCA 40 mg + CA 40 mg)	Diabetic rat + Biochanin-A + Caffeic acid	40 mg/kg + 40 mg/kg, p.o. once a day for 15 days

(N = Number, NC = Normal control, DC = Diabetic control, STD = Standard, i.p. = intraperitoneal, STZ = Streptozotocin, p.o. = per oral).

Diabetic rats were then divided into six groups, each comprising five animals, to evaluate the antihyperglycemic effects of biochanin A (40 mg/kg) and caffeic acid (40 mg/kg), administered orally, either alone or in combination, for a period of 14 days. The initial and final body weights of all animals were recorded. At the end of the treatment period, animals were anesthetized with thiopentone sodium, and blood samples were collected *via* retro-orbital puncture for the estimation of blood glucose and other biochemical parameters.

2.6 Blood glucose estimation

At the initial and final day of the treatment blood sugar measurements were taken by using the tail's vein blood (by ACCUCHEK- ACTIVE kit made by Roche, Germany).

2.7 Assessment of oxidative biomarkers in pancreas

2.7.1 Preparation of pancreas supernatant

After the final dose of treatment, the animals were sacrificed under anesthesia. The pancreas was carefully excised, thoroughly washed with cold 0.9% saline, weighed, and subsequently homogenized in ten volumes (10% w/v) of ice-cold potassium phosphate buffer (0.1 M, pH 7.4) using a Teflon-glass homogenizer. The resulting homogenate was centrifuged at 1000 × g for 3 min at 4°C, and the supernatant was collected for subsequent biochemical estimations.

2.7.2 Superoxide dismutase (SOD)

The pyrogallol autoxidation method, as outlined by Marklund and Marklund (1974), was used to spectrophotometrically measure the activity of superoxide dismutase (SOD) in pancreatic homogenates. The assay relies on the enzyme's capacity to prevent pyrogallol from oxidizing at alkaline pH. One unit of SOD activity was defined as the quantity of enzyme needed to produce 50% inhibition of pyrogallol autoxidation. The change in absorbance was measured at 420 nm (Kannan *et al.,* 2022).

2.7.3 Estimation of thio barbituric acid reactive substances (TBARS)

The Ohkawa *et al.* (1979) method of measuring thiobarbituric acid reactive substances (TBARS) was used to determine the amount of lipid peroxidation in pancreatic tissue. The basis for the assay is the production of a pink chromogen when thiobarbituric acid (TBA) and malondialdehyde (MDA), a byproduct of lipid peroxidation, react in an acidic, high-temperature environment. Using a molar extinction coefficient of 1.56 × 10u M^{-1} cm^{-1}, the concentration of MDA was determined by measuring the absorbance of the supernatant at 540 nm (Kherouf *et al.,* 2021).

2.7.4 Reduced glutathione (GSH) level

The concentration of reduced glutathione (GSH) in tissue homogenates was estimated spectrophotometrically using the method of Sedlak and Lindsay (1968). The assay is based on the reduction of 5,52 - dithiobis-(2-nitrobenzoic acid) (DTNB) by sulfhydryl groups to form a yellow-colored 2-nitro-5-mercaptobenzoic acid, which is measured at 412 nm. The intensity of the color produced is directly proportional to the GSH concentration. The results were expressed as µg of GSH per mg of protein (Sakr and Rashad, 2023).

2.7.5 Catalase

Catalase (CAT) activity in tissue homogenates was determined spectrophotometrically according to the method of Aebi (1984). The assay is based on the decomposition of hydrogen peroxide (H_2O_2) by catalase, which results in a decrease in absorbance at 240 nm. The rate of change in absorbance (ÄA/min) is directly proportional to the enzyme activity. Catalase activity was expressed as nanomoles of H_2O_2 decomposed per minute per milligram of protein (Ertik *et al.,* 2023).

2.8 Statistical analysis

Data was represented as the mean ± SEM of four animals in every faction. Statistical analysis was performed by using one way ANOVA followed by Dunnett: compare all vs control (Graph Pad Instat, USA).

3. Results

3.1 Effect of biochanin-A and caffeic acid on body weight

In the diabetic control group (DC) the body weight significantly decreased ($p<0.001$) when compared with normal control group (NC). The body weight was significantly increased ($p<0.001$) with all the treated groups compared with diabetic control (DC) except standard group (Table 2).

Table 2: Effect of biochanin-A and caffeic acid on body weight

Groups	Change in body weight (g)			
	At week 0	At week 2 (g)	% Body weight variation	
			% Weight gain	% Weight loss
Group I (NC)	147.5 ± 3.23	175 ± 2.04	18.64%	-
Group II (DC)	151.25 ± 2.39	112.5 ± 7.22***	-	25.61%
Group III (Met 250 mg)	147.5 ± 3.23	122.5 ± 3.23ns	-	16.94%
Group IV (BCA 40 mg)	150 ± 2.04	175 ± 2.04###	16.66%	-
Group V (CA 40 mg)	148.75 ± 2.39	173.75 ± 2.39###	16.80%	-
Group VI (BCA 40 mg + CA 40 mg)	151.25 ± 2.39	175 ± 2.04###	15.70	-

All values were expressed as mean ± SEM. Significant difference between various groups (ANOVA) and individual comparison was done by Dunnett's t-test. ***$p<0.001$=Significant, when compared with normal control (NC), ns $p>0.05$ &###$p<0.001$= Significant when compared with diabetic control (DC).

3.2 Effect of biochanin-A and caffeic acid on blood glucose

In the diabetic control group (DC) the glucose level significantly increased ($p<0.001$) when compared with normal control group (NC). The glucose level was significantly decreased ($p<0.001$) with all the treated groups compared with diabetic control (DC) (Table 3).

3.3 Effect of biochanin-A and caffeic acid on SOD

The superoxide dismutase (SOD) activity in pancreatic tissue was significantly decreased ($p<0.001$) in the diabetic control (DC) group compared with the normal control (NC) group, indicating enhanced oxidative stress due to diabetes induction. Treatment with biochanin A (40 mg/kg), caffeic acid (40 mg/kg), and their combination markedly increased SOD activity ($p<0.001$) relative to the diabetic control. Among all treated groups, the combination of biochanin A and caffeic acid produced the highest elevation in SOD levels (51.18 ± 0.61 U/mg of protein), approaching near-normal values, suggesting a synergistic antioxidant effect (Table 4).

Table 3: Effect of biochanin-A and caffeic acid on blood glucose.

Groups	Blood glucose concentration (mg/dl)	
	Initial blood glucose level	Final blood glucose level
Group I (NC)	87.75 ± 8.37	111.5 ± 1.936
Group II (DC)	279.25 ± 18.45	405.5 ± 11.079***
Group III (Met 250 mg)	366.5 ± 5.752	133.75 ± 4.385###
Group IV (BCA 40 mg)	375.5 ± 24.888	182.5 ± 4.093###
Group V (CA 40 mg)	369.75 ± 18.103	172.25 ± 4.535###
Group VI (BCA 40 mg + CA 40 mg)	393.75 ± 6.524	151.5 ± 3.428###

All values were expressed as mean ± SEM. Significant difference between various groups (ANOVA) and individual comparison was done by Dunnett's t-test. ***$p<0.001$=Significant, when compared with normal control (NC), ###$p<0.001$=Significant when compared with diabetic control (DC).

3.4 Effect of biochanin A and caffeic acid on catalase activity

A significant reduction ($p<0.001$) in catalase (CAT) activity was observed in the diabetic control group compared with the normal control, confirming impaired enzymatic antioxidant defense in diabetic rats. Administration of biochanin A, caffeic acid, and metformin (250 mg/kg) significantly restored CAT activity ($p<0.001$), while the combined treatment of both compounds exhibited the most pronounced increase (6.66 ± 0.27 U/mg of protein) relative to diabetic rats. This suggests that the dual administration of these phytoconstituents provides enhanced protection against hydrogen peroxide-induced oxidative damage in pancreatic tissue (Table 4).

3.5 Effect of biochanin A and caffeic acid on reduced glutathione (GSH) levels

Diabetic rats showed a marked decline ($p<0.001$) in reduced glutathione (GSH) levels (18.43 ± 0.89 µg/mg of protein) when compared to the normal control group (65.0 ± 0.91 µg/mg of protein), reflecting depletion of endogenous antioxidant reserves. Treatment with biochanin A and caffeic acid, either alone or in combination, significantly ($p<0.001$) increased GSH concentrations. The combination group demonstrated the greatest elevation (47.95 ± 0.61 µg/mg of protein), surpassing the effects of individual treatments and the standard metformin group, indicating a synergistic restoration of the glutathione-dependent antioxidant system (Table 4).

3.6 Effect of biochanin A and caffeic acid on malondialdehyde (MDA) levels

The malondialdehyde (MDA) content, a marker of lipid peroxidation, was significantly elevated ($p<0.001$) in the diabetic control rats (6.72 ± 0.45 nM/mg protein) compared to the normal control group (1.92 ± 0.083 nM/mg protein). Treatment with biochanin A, caffeic acid, and metformin markedly reduced MDA levels, indicating attenuation of lipid peroxidation. The combination of biochanin A and caffeic acid produced the most pronounced reduction (3.67 ± 0.20 nM/mg protein; $p<0.001$) relative to diabetic control, suggesting strong free radical scavenging potential and improved oxidative stability of cellular membranes (Table 4).

Table 4: Effect of biochanin-A and caffeic acid on SOD, CAT, GSH and MDA levels

Groups/Treatment	SOD level (U/mg of protein)	Concentration of catalase (U/mg of protein)	Concentration of GSH (µg/mg of protein)	Concentration of MDA (nM/mg protein)
Group I (NC)	59.64 ± 0.61	7.72 ± 0.56	65.0 ± 0.91	1.92 ± 0.083
Group II (DC)	31.93 ± 0.66***	2.65± 5.43***	18.43 ± 0.89***	6.72 ± 0.45***
Group III (Met 250 mg)	47.77 ± 0.72$^{\#\#\#}$	5.43 ± 0.47$^{\#\#\#}$	43.8 ± 0.65$^{\#\#\#}$	3.47 ± 0.17$^{\#\#}$
Group IV (BCA 40 mg)	46.35 ± 1.01$^{\#\#\#}$	6.06 ± 0.30$^{\#\#\#}$	38.03 ± 0.75$^{\#\#\#}$	4.40 ± 0.24$^{\#}$
Group V (CA 40 mg)	46.93 ± 0.83$^{\#\#\#}$	5.75 ± 0.10$^{\#\#\#}$	37.85 ± 1.7$^{\#\#\#}$	4.25 ± 0.92$^{\#}$
Group VI (BCA 40 mg + CA 40 mg)	51.18 ± 0.61$^{\#\#\#}$	6.66 ± 0.27$^{\#\#\#}$	47.95 ± 0.61$^{\#\#\#}$	3.67 ± 0.20$^{\#\#\#}$

All values were expressed as mean ± SEM. Significant difference between various groups (ANOVA) and individual comparison was done by Dunnett's t-test. $^{***}p<0.001$=Significant, when compared with normal control (NC), $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$=Significant whencompared with diabetic control (DC).

4. Discussion

The findings of the present study demonstrated that oral administration of biochanin A and caffeic acid, either alone or in combination, exerted significant antihyperglycemic and antioxidant effects in streptozotocin (STZ)-induced diabetic rats. The combination therapy displayed superior efficacy compared to either compound alone, suggesting a synergistic interaction in mitigating hyperglycemia, oxidative stress, and diabetes-associated metabolic alterations.

STZ is widely known for its selective cytotoxicity to pancreatic β-cells, resulting in insulin deficiency and hyperglycemia (Zhu, 2022). Consistent with this, the diabetic control group exhibited a marked elevation in fasting blood glucose levels (405.5 ± 11.08 mg/dl) compared with the normal control group (111.5 ± 1.93 mg/dl), confirming successful induction of diabetes. Treatment with biochanin A (182.5 ± 4.09 mg/dl) and caffeic acid (172.25 ± 4.53 mg/dl) significantly ($p<0.001$) lowered blood glucose concentrations, while their combination produced the most pronounced effect (151.5 ± 3.42 mg/dl), approaching near-normal levels. These findings highlight the ability of the phytoconstituents to improve glucose utilization and preserve β-cell function, likely through their antioxidant-mediated protection against STZ-induced oxidative injury (Rais *et al.,* 2022).

Body weight loss is a hallmark of diabetes, primarily due to excessive catabolism of proteins and lipids resulting from insulin deficiency (Beloucif *et al.,* 2022). In the present study, diabetic control rats exhibited a 25.6% reduction in body weight, whereas treatment with biochanin A and caffeic acid significantly restored body weight toward normal values (16-17% gain). The combined treatment group (15.7% gain) demonstrated a comparable effect to metformin (250 mg/kg), suggesting an improvement in overall metabolic efficiency and insulin sensitivity.

Oxidative stress is a major pathological contributor to the onset and progression of diabetes and its complications (Chen *et al.,* 2025). In the current study, diabetic rats exhibited a significant ($p<0.001$) reduction in key antioxidant enzyme activities superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) alongside a substantial increase in malondialdehyde (MDA) levels, indicating heightened lipid peroxidation and oxidative damage in pancreatic tissue. Specifically, SOD, CAT, and GSH levels in diabetic control animals were 31.93 ± 0.66 U/mg, 2.65 ± 5.43 U/mg, and 18.43 ± 0.89 µg/mg, respectively, compared to 59.64 ± 0.61 U/mg, 7.72 ± 0.56 U/mg, and 65.0 ± 0.91 µg/mg in normal controls. The elevated MDA value (6.72 ± 0.45 nM/mg) further confirmed oxidative deterioration of cell membranes. Following treatment, biochanin A and caffeic acid significantly enhanced the antioxidant defense system. In the

combination group, SOD, CAT, and GSH levels increased to 51.18 ± 0.61 U/mg, 6.66 ± 0.27 U/mg, and 47.95 ± 0.61 µg/mg, respectively, while MDA levels decreased to 3.67 ± 0.20 nM/mg. These results indicate potent free-radical scavenging activity and restoration of the antioxidant enzymatic system. The findings are consistent with previous reports that biochanin A exerts antioxidant and cytoprotective effects by modulating oxidative enzymes and reducing ROS-mediated lipid peroxidation (Anuranjana *et al.,* 2023), while caffeic acid acts as a chain-breaking antioxidant and enhances endogenous antioxidant enzyme expression (Yusuf *et al.,* 2019).

The superior efficacy of the combined therapy compared to single-agent treatments can be attributed to the complementary antioxidant mechanisms of both compounds. Biochanin A, as a flavonoid, donates hydrogen atoms to neutralize free radicals and stabilize ROS, whereas caffeic acid, a hydroxycinnamic acid derivative, interrupts lipid peroxidation chain reactions and enhances the regeneration of endogenous antioxidants. Together, these mechanisms reinforce redox homeostasis in pancreatic tissues and preserve β-cell viability.The observed restoration of antioxidant biomarkers is particularly relevant because excessive ROS generation in diabetes not only causes β-cell apoptosis but also contributes to insulin resistance and vascular dysfunction. The current findings are in line with the work of Kannan *et al.* (2022), who reported improved antioxidant enzyme activity in diabetic models treated with polyphenolic compounds, and with Ertik *et al.* (2023), who demonstrated that melatonin ameliorates pancreatic oxidative stress by elevating SOD and CAT activities. Moreover, polyphenols are known to activate the nuclear factor erythroid 2 related factor 2 (Nrf2) pathway, enhancing cellular antioxidant defenses and reducing oxidative damage (Krawczyk *et al.,* 2023). Collectively, the experimental data suggest that biochanin A and caffeic acid exert synergistic antihyperglycemic and antioxidant effects in diabetic rats by improving glucose regulation, restoring enzymatic antioxidant balance, and protecting pancreatic tissue from oxidative damage. The combination therapy produced biochemical results comparable to metformin, indicating its potential as an adjunct natural therapeutic strategy for diabetes management. However, further investigations incorporating molecular assays, and clinical studies are warranted to confirm the underlying mechanisms and translational relevance of these findings.

5. Conclusion

The present investigation clearly demonstrates that the combination of biochanin A and caffeic acid exerts a significant antihyperglycemic and antioxidant effect in streptozotocin-induced diabetic rats. Oral administration of both compounds for 15 days not only reduced elevated blood glucose levels but also improved body weight and restored the activities of key antioxidant enzymes such as superoxide dismutase, catalase, and glutathione, while simultaneously lowering malondialdehyde levels. The combined treatment showed superior efficacy compared with either compound alone, indicating a possible synergistic interaction in combating oxidative stress and metabolic imbalance associated with diabetes.These findings suggest that biochanin A and caffeic acid together may offer a promising natural therapeutic strategy for the prevention and management of diabetes mellitus and its oxidative complications. Nevertheless, further studies involving molecular pathway analysis, and clinical validation are essential to confirm their safety, mechanism of action, and translational potential in humans.

Availability of data and material

All data are provided within the manuscript.

Authorship contribution statement

Mohammad Adnan: Contributed to conceptualization, methodology design, data curation, and writing the original draft of the manuscript. **Badruddeen:** Contributed to supervision, conceptualization, validation, and critical review and editing of the manuscript. **Muhammad Arif:** Contributed to data analysis, interpretation, and validation of experimental results. **Juber Akhtar:** Contributed to resources, formal analysis, and manuscript proofreading. **Anas Islam:** Contributed to investigation, methodology execution, and software handling. **Mohammad Irfan Khan:** Contributed to experimental work, visualization, and data presentation. **Mohammad Ahmad:** Contributed to literature review, data organization, and editing of the final draft. **Gazala Noor:** Contributed to project administration, validation, and final manuscript approval.

Consent for publication

All authors gave their full consent for publication and submission to this journal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Ethics approval

Ethical clearance was obtained from Institutional animal Ethical Committee (IAEC) (Approval No: IU/IAEC/20/23) Integral University, Lucknow.

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