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Research Article

EXPLORING THE ANTIDIABETIC, LIPID-LOWERING, AND TISSUE REPAIR BENEFITS OF MULTI-COMPONENT MICROSPHERES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

ANUSHREE CHAUHAN[1], SURBHI GUPTA[2]*, PRABHAT SINGH[3], GANESH PRASAD MISHRA[3,4]*

[1]Department of Pharmaceutics, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. [2]Department of Pharmacology, School of Pharmacy, Bharat Institute of Technology, Meerut, Uttar Pradesh, India. [3]Department of Pharmacology, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. [4]Department of Pharmaceutical Chemistry, Dr Prafulla Chandra Ray Subharti College of Pharmacy, Bijnor, Uttar Pradesh, India.
***Corresponding author: Surbhi Gupta; Email: surbhi02gupta@gmail.com**

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ABSTRACT

Objective: Dapagliflozin, linagliptin, and metformin are extensively utilized either alone or in combination to manage insulin-resistant diabetes. However, the precise administration and effectiveness of their triple combination through a microsphere formulation is yet to be thoroughly explored. This study sets out to explore the antidiabetic and hypolipidemic effects, as well as the histopathological impact of microspheres drug delivery system in streptozotocin (STZ)-administered diabetic rats.

Methods: Following an overnight fast, both healthy and diabetic rats were separated into five sets each containing six rats, receiving daily oral treatments for 21 days. Group 1 (control) was administered saline (0.9% NaCl), Group 2 (STZ) received STZ (65 mg/kg through an intraperitoneal injection.), Group 3 (STZ and metformin) received metformin (150 mg/kg), Group 4 (STZ and empagliflozin + linagliptin + metformin) received a tablet combination of these drugs, and Group 5 (STZ and dapagliflozin+linagliptin+metformin) was treated with dapagliflozin (5 mg/kg), linagliptin (5 mg/kg), and metformin microspheres (150 mg/kg). Plasma glucose concentration was monitored weekly, and lipid functions were assessed after 21 days. Histopathological examinations of the pancreas and kidneys were also conducted.

Results: The microsphere combination of dapagliflozin, linagliptin, and metformin significantly improved blood glucose levels (109.10±4.13 mg/dL), renal and hepatic functions, and showed beneficial effects on lipid parameters, particularly high-density lipoprotein levels (32.17±3.4 mg/dL), which could lower risk of cardiovascular diseases. Histopathological analysis revealed positive effects on the pancreas and kidneys. A $p<0.05$ was considered to indicate statistical significance.

Conclusion: The microsphere formulation of the triple drug combination demonstrates synergistic antidiabetic properties, enhances body weight, improves lipid profiles, and supports renal and hepatic functions. In addition, it protects against tissue damage to the pancreas and kidneys in STZ-mediated experimental diabetes.

Keywords: Dapagliflozin, Linagliptin, Metformin, Microspheres, Type II diabetes, Lipid profile.

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INTRODUCTION

Diabetes mellitus (DM) occurs when there is an inadequate secretion of insulin. It is classified as a metabolic disorder, often leading to long-term damage to various tissues and organs [1]. This condition can result in harm to the kidneys, vascular system, or nervous system. In diabetes, blood sugar levels remain elevated for extended periods due to a combination of metabolic dysfunctions. In addition, DM is marked by impaired metabolism of proteins, carbohydrates, and fats as a result of insulin deficiency, which in turn leads to persistent hyperglycemia [2,3]. Oral administration remains a preferred method of drug delivery due to its established benefits. However, it faces challenges owing to the physiology of the gastrointestinal (GI) system, which limits the effectiveness of conventional drug formulations. These methods struggle, especially for drugs primarily absorbed in the distal section of the GI tract, as they cannot effectively navigate gastric emptying or ensure proper delivery to the intestines or colon for extended-release [4]. To overcome these limitations, experts have developed advanced pharmaceutical systems that regulate drug release for prolonged effects, some of which are already available on the market. The failure of traditional systems to achieve optimal gastric absorption has led to the creation of oral systems designed for controlled drug release in the intestine. These systems allow prolonged drug contact with the absorbing membrane, improving bioavailability. Various strategies, such as bio-adhesive mucoadhesive systems, colon-targeted microparticles, liposomes, nanoparticles, and intestinal-targeted microspheres, have been explored to enhance drug effectiveness through sustained and controlled delivery [5,6].

Metformin is an antihyperglycemic medication that helps improve glucose tolerance in Type II diabetes. When taken orally, its bioavailability is reported to be 50–60%, and it has a physiological elimination half-life of 1.5–1.6 h, with the proximal small intestine being the main site of absorption [7,8]. Dapagliflozin is a potent oral hypoglycemic agent that works as a selective, reversible inhibitor of the human sodium-glucose cotransporter 2 protein. Linagliptin is a dipeptidyl peptidase-4 inhibitor used to treat Type II diabetes. In addition, dapagliflozin has shown beneficial impacts on cardiovascular and renal outcomes, making it a valuable option in the management of Type II diabetes [9,10]. It is classified as a class-III drug characterized by high solubility and low permeability [11]. This research was undertaken to explore the antidiabetic, lipid-lowering, and tissue repair benefits of dapagliflozin–linagliptin and metformin [12].

METHODS

Drugs and chemicals

Dapagliflozin, linagliptin, and metformin were obtained from Sun Pharma (Mumbai, India), a renowned global leader in pharmaceutical innovation. The excipients, including HPMC K 100, ethyl cellulose, sodium alginate, Tween 80, and streptozotocin (STZ), were sourced from CDH Laboratory Reagents, New Delhi, a trusted supplier of laboratory essentials.

Blood glucose measurements were assessed with the Accu Chek glucose meter from Roche Diabetes Care Pvt. Ltd., India. All additional chemicals and reagents employed in the formulation belonged to the highest pharma grade, ensuring optimal purity along with performance throughout the preparation process.

Microspheres preparation

The microspheres of metformin were prepared using the emulsification (o/w) solvent evaporation method, incorporating HPMC K 100 as the polymer, along with ethanol, dichloromethane, and Tween 80 as the surfactant. For dapagliflozin microspheres, HPMC K 100, sodium alginate, and ethyl cellulose were used as polymers, also utilizing the solvent evaporation method. Linagliptin microspheres were formulated with HPMC K 100, ethyl cellulose, polyvinyl alcohol, and Tween 80, employing the emulsification (o/w) solvent evaporation method. The formulation processes for all three microsphere types were optimized using a Box-Behnken design (Design Expert Software), where polymer concentration, concentration of surfactant along, and stirring rate were considered independent parameters, and size of the particle, percentage yield, and drug entrapment efficiency as dependent parameters [13-17].

In vivo evaluation of the dapagliflozin, linagliptin, and metformin encapsulated microsphere

Experimental animal and ethical approval

An animal-based experimentation study was conducted using male Wistar rats with body weight ranging from 180 to 220 g (n=6), which were handled and cared for in accordance with the standards set by the committee for the control and supervision of experiments on animals. The rats were acclimatized to regulated conditions (temperature: 25±5°C, relative humidity: 55±10%) and also a regular day–night cycle. During the experiment, the animals resided in polypropylene-based cages, provided with a normal pellet feeding regimen, with unlimited access to water, as shown in Fig. 1. All procedures followed the ethical guidelines approved by the Animal Ethics Committee of the Department of Pharmaceutical Technology, Subharti University, India (Registration number: 1204/PO/Re/S/08/CPCSEA) [18].

Diabetes induction

A single intraperitoneal dose of STZ (65 mg/kg) was administered to induce diabetes in the experimental rats [19]. Before injection, the rats underwent fasting for 12 h to enhance the effectiveness of the STZ. Following the administration of STZ, blood glucose levels were monitored after 48 h using a blood glucose meter. Diabetes was confirmed in rats whose blood glucose levels surpassed a set threshold (e.g., 250.00 mg/dL), and these rats were included in the study. Plasma glucose concentration was assessed weekly throughout the study (for a duration of 3 weeks) utilizing glucose measurement test strips and an Accu-Chek blood glucose meter (India). Plasma glucose readings and body weight were monitored on days 0, 7, 14, and 21 following the oral dosing of the microspheres [20].

Experimental design

Post-overnight fasting, both healthy and diabetic rats were assigned to five groups, consisting of six rats in every group, and given oral treatment once a day for a 3-week duration. All experimental operations were conducted as per the study protocol outlined below [21,22].

- Group 1 (Control): The rats received daily saline treatment (0.9% of NaCl) and served as the control group animals
- Group 2 (STZ): The rats of this group received STZ (65 mg/kg;ip) and served as a negative control
- Group 3 (STZ+metformin): In this group, the STZ diabetic rats received the reference drug metformin orally at a dose of 150 mg/kg body weight
- Group 4 (STZ+empagliflozin+linagliptin and metformin): STZ diabetic rats in this group were administered empagliflozin+linagliptin and metformin tablet in suspension form to compare with the microsphere formulation
- Group 5 (STZ+Dapagliflozin+linagliptin and metformin): This group received a combination treatment of dapagliflozin (5 mg/kg body weight), linagliptin (5 mg/kg body weight), and metformin microspheres (150 mg/kg body weight) administered orally, to explore the synergistic effects of the combination therapy on glucose regulation.

Blood sampling and analysis of biochemical parameters

Blood samples (ranging from 0.5 to 0.6 mL) were drawn through the tail vein using cold heparinized collection tubes. Plasma glucose levels were assessed every week on 0, 7, 14, and 21 days using an Accu-Chek glucometer, following daily oral administration of the formulation. On day 21, after the final glucose measurement, a cardiac puncture was performed to collect whole blood under mild ether sedation. Serum cholesterol, diglycerol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very LDL (VLDL) were analyzed in both healthy and STZ-treated diabetic rats. Liver function tests included the measurement of serum alanine transaminase (ALT) and aspartate transaminase (AST), whereas kidney function tests involved assessing urea and creatinine levels [23].

Histopathology of pancreas and kidneys

At the conclusion of the study, three rats from every group were randomly chosen for euthanasia. Pancreatic and renal tissues were carefully dissected, with any excess tissue removed. The tissues were then washed with normal saline, weighed, and sectioned into small fragments. These samples were preserved in 10% buffered formalin, dehydrated using ethanol and xylene, and then embedded in paraffin. Thin slices (5 µm) were prepared, stained with hematoxylin and eosin (H and E), and analyzed using a light microscope (Eclipse TS100, Nikon, Japan) [24].

Fig. 1: Rats placed in polypropylene cages with unrestricted access to water

Statistical assessment

The results are presented as the average±standard error of the mean. A Student's t-test was used to compare two groups, whereas a one-way analysis of variance followed by Dunnett's post-test was used for comparisons among multiple groups. A p-value below 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Microsphere preparation

The optimized microsphere formulation of the three drugs is compiled in Table 1.

Antidiabetic effect on rats

Blood glucose levels in rats typically range from 50 to 135 mg/dL, and above 250 mg/dL were considered diabetic [25]. A single STZ injection (65 mg/kg) destroys insulin-producing β-cells, leading to insulin deficiency and severe hyperglycemia. This causes a rise in blood glucose, elevated cholesterol, and increased liver enzymes. After 21 days of daily oral administration of a microsphere's formulation (160 mg/kg), plasma glucose levels exhibited a marked reduction ($p<0.05$), and insulin levels were improved. The microsphere formulation appears to reduce elevated glucose levels and may stimulate insulin secretion, suggesting its potential as an antidiabetic treatment, as shown in Table 2. The F-value obtained for this data was found to be approximately 38.74.

Table 1: Characterization details of dapagliflozin, linagliptin, and metformin microspheres

Microsphere formulation	Particle size (μm)	Percentage yield (%)	Entrapment efficiency (%)
Dapagliflozin microspheres	278.69±0.36	81.78±0.04	82.21±0.14
Linagliptin microspheres	165.54±0.12	84.24±1.47	86.15±0.15
Metformin microspheres	430.48±0.28	89.54±0.36	92.61±1.14

Impact on rat body weight

The study revealed that the weight of the normal control rats steadily increased over time. On administration of a single dose of STZ (65 mg/kg), diabetic control rats cause a decrease in body weight throughout the experiment, as shown in Table 3. There was an improvement in body weight with the marketed product, but the treatment with the microsphere formulation (160 mg/kg) led to a notable enhancement in the body weight of diabetic rats, with no further reduction observed. The F-value calculated for the data was found to be 8.81.

Influence on lipid markers

Hyperglycemic rats typically exhibited common lipid abnormalities like hypertriglyceridemia and hypercholesterolemia when compared with the normal group. The administration of microsphere formulation (160 mg/kg) led to a significant reduction in cholesterol and triglyceride levels, while HDL, LDL, and VLDL cholesterol levels were significantly improved after 21 days of treatment, as shown in Table 4. Remarkably, microsphere formulation significantly ($p<0.05$) reduced these lipid imbalances, possibly through decreased cholesterol synthesis and fatty acid production.

Effect on liver activity

The liver function results of the treatment are shown in Table 5. In STZ-treated diabetic rats, the levels of liver enzyme markers ALT and AST were remarkably elevated, indicating liver stress. However, after 21 days of treatment with the microsphere formulation, a notable ($p<0.01$) reduction in the elevated liver enzyme levels was recorded, suggesting an improvement in liver function.

Influence on kidney activity

The microsphere formulation significantly enhanced kidney function in STZ-induced diabetic rats over a 21-day period. Elevated

Table 2: Influence of microsphere formulation on plasma glucose concentration in STZ stimulated hyperglycemia in rats

Groups/treatment	1st day (mg/ld.)	7th day (mg/dL)	14th day (mg/dL)	21st day (mg/dL)
I: Control (0.9% NaCl)	78.47±2.21	80.42±1.03	77.47±2.11	81.11±2.61
II. Negative control (65 mg/kg STZ)	230.15±6.80	285.0±8.73	310.50±4.18	325.13±9.53
III. Positive control (STZ and metformin)	218.14±4.65	198.65±7.45*	184.50±3.17*	172.13±4.01*
IV. (STZ and empagliflozin+linagliptin and metformin)	180.07±3.51	158.4.65*	133.04±3.13*	115.10±5.14*
V. (STZ and dapagliflozin+linagliptin and metformin)	178.24±11.02	152.14±3.06*	129.00±2.14*	109.10±4.13*

*Responses are shown as the average±standard error of the mean (SEM), and statistical significance ($p<0.05$) was evaluated when groups III, IV, and V were compared with diabetic (negative control, i.e., group II). STZ: Streptozotocin

Table 3: Effect of microsphere formulation on weight of diabetic rats

Groups/treatment	1st day	7th day	14th day	21st day
I. Control (0.9% NaCl)	185±0.80	192±1.05	205±0.95	218±1.17
II. Negative control (STZ)	210±0.28	150±0.30	126±0.55	120±3.60
III. Positive control (STZ and Metformin)	205±1.30	170±2.40*	182±0.82*	188±1.52*
IV. (STZ and empagliflozin+linagliptin and metformin)	205±0.73	192±1.62*	202±0.55*	204±1.02*
V. (STZ and dapagliflozin+linagliptin and metformin)	209±0.18	188.±2.40*	206±2.10*	208±0.14*

*Values are expressed as the mean body weight (g)±SEM (6 animals), and statistical significance is indicated by $p<0.05$. When groups III, IV, and V were compared with negative control. STZ: Streptozotocin, SEM: Standard error of the mean

Table 4: Impact of microsphere formulation in regard to lipid parameters in STZ-administered diabetic rats after 21 days

Category/therapy	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I. Control (0.9% NaCl)	142.45±2.12	84.14±4.22	3.83±2.43	89.17±5.53
II. Negative control (STZ)	268.65±2.93	198.12±2.53	18.06±2.62	178.20±2.01
III. Positive control (STZ and metformin)	150.18±3.25*	92.47±1.42*	37.47±2.91*	94.25±3.85*
IV. (STZ and empagliflozin+linagliptin and metformin)	144.18±1.83*	86.63±3.71*	35.24±4.23*	92.14±2.19*
V. (STZ and dapagliflozin+linagliptin and metformin)	141.61±4.75*	84.20±4.83*	32.17±3.44*	90.57±1.83*

*Findings are presented as mean±SEM (6 animals per category), with $p<0.05$ indicating statistical significance. When groups III, IV, and V were compared with negative control. STZ: Streptozotocin, SEM: Standard error of the mean, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

levels of kidney health indicators, such as creatinine and urea, were observed in rats with induced diabetes. However, microsphere formulation treatment led to a notable reduction in both creatinine and urea levels, indicating an improvement in kidney function. These findings, shown in Table 6, highlight the potential of the microsphere formulation in alleviating kidney dysfunction associated with diabetes.

Histology of pancreas

The histological analysis of the pancreas was performed at 10×magnification using a light microscope, as shown in Fig. 2. The figure represents H and E-stained pancreatic sections from treated rats, highlighting the cell morphology across different groups. In the untreated healthy class (Group I), islets of Langerhans exhibited a normal cellular structure. In the diabetic control group (Group II), extensive clumping of structures within the islets of Langerhans was observed, with beta cells showing signs of shrinkage, necrosis, and disorganized lobular arrangements. The islets showed signs of shrinkage, and the cellular structure was severely damaged, with a noticeable vacuolation. However, treatment with the microsphere formulation led to a restoration of the islet cellular population, bringing it closer to normal. The microsphere-treated groups (Group IV and Group V) showed significant recovery from cellular damage. In contrast, the group treated with the 21-day oral drug regimen showed proliferating islets of Langerhans and improved lobular cell organization, with the most notable regeneration seen in the microsphere-treated group, which demonstrated significant recovery of pancreatic cells.

Histology of kidneys

The kidney histology was examined under a light microscope at 10×magnification, as shown in Fig. 3. In normal animals (Group I), the typical nephron structure and cup-shaped Bowman's space were observed. Group II, treated with STZ, and known to stimulate the production of free radicals and result in tissue damage, exhibited significant organ stress. This group showed no development of endocytic vacuoles but had disorganized glomerular vessel arrangements and altered shapes in the Bowman's capsule. Group III hyperglycemic rats exhibited a slight increase in the thickness of the basement membrane in the arterioles of the glomeruli was noted; however, no other significant alterations were observed. In the treated groups (marketed product and microsphere formulation), nephrons were reconstructed, and glomerular vessel arrangements improved, with changes returning closer to normal conditions. Furthermore, a significant recovery of endocytic vacuoles was observed in both the microsphere formulation and marketed formulation (Groups IV and V). In contrast to the diabetic control group, the treated groups exhibited recovered cells and a marked improvement in kidney tissue structure.

Table 5: Impact of microsphere formulation on liver function tests in STZ-treated diabetic rats after 21 days

Category/Therapy	AST (IU/L)	ALT (IU/L)
I. Control (0.9% NaCl)	142.45±2.14	84.14±4.23
II. Negative control (STZ)	268.65±2.93	198.12±2.54
III. Positive control (STZ and metformin)	150.18±3.26*	92.47±1.25*
IV. (STZ and empagliflozin+linagliptin and metformin)	144.18±1.84*	86.63±3.77*
V. (STZ and dapagliflozin+linagliptin and metformin)	141.61±4.75*	84.20±4.83*

*Responses are shown as mean±SEM (6 animals per category), with $p<0.05$ indicating statistical significance. When groups III, IV, and V were compared with negative control. STZ: Streptozotocin, SEM: Standard error of the mean, AST: Aspartate transaminase, ALT: Alanine transaminase

Table 6: Effect of microsphere formulation on kidney function tests in STZ-treated diabetic rats after 21 days

Classes/therapy	Creatinine (mg/dL)	Urea (mg/dL)
I. Control (0.9% NaCl)	0.44±0.42	29.12±2.14
II. Negative control (STZ)	45.62±0.33	79.45±1.57
III. Positive control (STZ and metformin)	1.18±3.25*	50.60±0.83*
IV. (STZ and empagliflozin+linagliptin and metformin)	0.78±1.83*	36.63±2.74*
V. (STZ and dapagliflozin+linagliptin and metformin)	0.46±0.34*	31.20±1.58*

*Data are represented as mean±SEM. SEM: Standard error of the mean

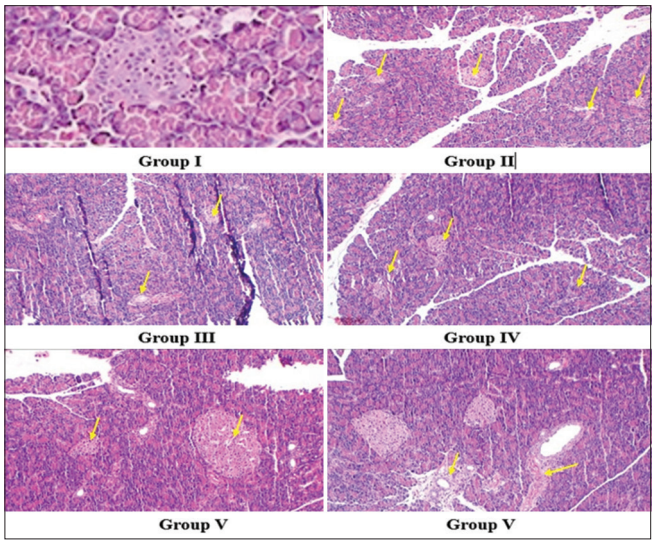

Fig. 2: Hematoxylin and eosin-stained pancreas section of treated rats show the cellular morphology of group I (control, 0.9% NaCl), group II (negative control, 65 mg/kg STZ), group III (positive control, STZ and metformin) group IV (STZ and empagliflozin+linagliptin and metformin marketed product tablet), and group V (STZ and dapagliflozin+linagliptin and metformin microspheres). STZ: Streptozotocin

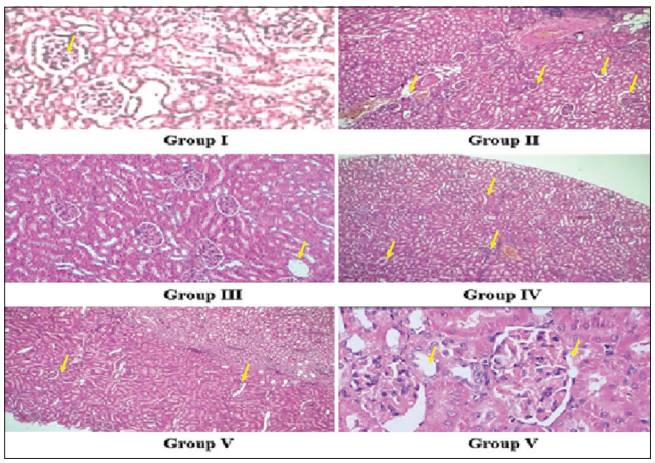

Fig. 3: Hematoxylin and eosin-stained kidney sections of treated rats show the cellular morphology of group I (control, 0.9% NaCl), group II (negative control, 65 mg/kg STZ), group III (positive control, STZ, and metformin) group IV (STZ and empagliflozin+linagliptin, and metformin marketed product tablet), and group V (STZ and dapagliflozin+linagliptin, and metformin microspheres). STZ: Streptozotocin

CONCLUSION

The study demonstrated that dapagliflozin-linagliptin-metformin microspheres were both safe and effective in repairing damage caused by STZ-stimulated diabetes in rats. The treatment normalized blood glucose levels, improved body weight, and enhanced liver and kidney functions while also reducing hyperlipidemia. Histopathological analysis revealed repair of pancreatic and kidney damage. The combination of dapagliflozin, linagliptin, and metformin improved glucose uptake, inhibited hepatic glucose production, and regulated gluconeogenesis. The microsphere formulation provided sustained release in the intestine, improving drug delivery and achieving more effective blood glucose reduction than free-form metformin while requiring lower doses and less treatment time. The treatment controlled blood glucose, increased body weight, and improved biochemical markers such as ALT, AST, creatinine, and urea levels. It also positively impacted lipid profiles, lowering cholesterol and reducing cardiovascular risks. Overall, the microsphere formulation shows promising antidiabetic potential, though further studies are needed to investigate its release behavior and long-term effects.

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AUTHOR CONTRIBUTIONS

The conceptualization and supervision of the study was carried out by Dr. Prabhat Singh. The original draft of the manuscript was written by Anushree Chauhan, and review and editing were done by Dr. Surbhi Gupta and Dr. Ganesh Prasad Mishra. Project administration was managed by Dr. Prabhat Singh and Dr. Ganesh Prasad Mishra. All authors have gone through and given their approval for the final manuscript.

CONFLICTS OF INTEREST

This research has no personal conflicts. There are no hidden agendas and no personal or financial stakes influencing our work – our findings are entirely unbiased and impartial.

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