

Prophylactic Antidiabetic Effect of Turmeric (*Curcuma longa* L.) in a High-Fat High-Sugar Diet and Alloxan-Induced Type 2 Diabetic Rat Model – Biochemical and Histopathological Evaluation

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ABSTRACT

Introduction

Diabetes mellitus represents a rapidly expanding global health challenge, characterised by insulin resistance and progressive pancreatic β -cell dysfunction driven by metabolic and oxidative stress. The present study evaluated the prophylactic efficacy of turmeric co-administered with black pepper (TBP) in a combined high-fat high-sugar (HFHS) diet and alloxan-induced diabetic rat model.

Methods

Forty-eight male Sprague-Dawley rats were randomised into six groups: three controls; [normal (NC), control (DC), and metformin (MC)] groups, and three prophylactic TBP [high-dose extract (PTBP-H), low-dose extract (PTBP-L), and whole powder without extraction (PTBP-W)] groups. Diabetes was induced by HFHS diet and alloxan. Prophylactic TBP treatment was given throughout the study, whereas metformin was initiated only after induction. Body weight and fasting blood glucose (FBG) were monitored weekly. Fasting serum insulin (FSI), glycated haemoglobin (HbA1c), oral glucose tolerance (OGTT), and pancreatic histopathology were assessed. Data were analysed by one-way ANOVA followed by Tukey's post-hoc test.

Results

Prophylactic TBP significantly improved glycaemic control compared with the DC group ($p < 0.001$). High-dose extract (PTBP-H) demonstrated the strongest effect, reducing FBG to 114.67 ± 9.22 mg/dL and HbA1c to $4.45 \pm 0.45\%$, while maintaining higher FSI levels ($p < 0.001$). All PTBP formulations markedly improved oral glucose tolerance and reduced the area under the curve. PTBP treatment significantly reduced pancreatic islet necrosis, inflammation, and atrophy, with the high-dose group exhibiting the best preservation of islet architecture, comparable to DC group.

Conclusion

Prophylactic administration of turmeric in combination with black pepper preserved pancreatic islet architecture and function, and hence improved glycaemic parameters, indicating its potential to delay the progression of diabetes and its complications

Keywords

Alloxan; diabetes; diabetes prophylaxis; diabetes treatment; high-fat diet; pancreatic histopathology; piperine; turmeric

INTRODUCTION

Diabetes mellitus has become a major global public health burden, with a current prevalence of approximately 11.1% attributable largely to sedentary lifestyles and the widespread adoption of high-fat, high-sugar (HFHS) dietary patterns.¹ Type 2 diabetes constitutes the great majority of cases and is characterised primarily by insulin resistance, which over time is accompanied by progressive pancreatic β -cell dysfunction.² Sustained hyperglycaemia leads to structural and functional damage across multiple organ systems, with pancreatic islet dysfunction occupying a central role in disease progression.³ Preclinical models that combine HFHS diet-induced metabolic stress with selective β -cell injury via alloxan closely replicate key features of human type 2 diabetes and are widely employed in preventive intervention research.^{4,5}

Curcuma longa (turmeric) and its principal bioactive constituents, the curcuminoids, have demonstrated antioxidant, anti-inflammatory, and glucose-lowering properties across experimental and clinical investigations.^{6,7} Mechanistically, curcumin modulates oxidative stress pathways, inflammatory signalling cascades, and cellular survival mechanisms implicated in β -cell preservation.⁸ Preclinical evidence further supports its protective effects on pancreatic structure and function.^{9,10} Despite these promising findings, evidence regarding the prophylactic efficacy of turmeric against diet-induced type 2 diabetes and its associated pancreatic histopathological alterations remains limited, warranting further preclinical investigation. The present study therefore evaluated the prophylactic antidiabetic potential of turmeric in an HFHS diet and alloxan-induced diabetic rat model through biochemical and histopathological assessments.

METHODS

This experimental study was conducted from December 2023 to May 2024 in the laboratories of the Karnali Academy of Health Science (KAHS), Jumla, Nepal, specifically in the Department of Clinical Anatomy & Cell Biology, Department of Clinical Pharmacology & Therapeutics, and Department of Clinical Pathology, in collaboration with National Ayurveda Research & Training Center

(NARTC), Kathmandu, Nepal and University of Cyberjaya (UoC), Selangor, Malaysia.

Ethical Approval and Regulatory Compliance

Prior to the commencement of the study, ethical clearance was obtained from the institutional animal ethics committee of the Nepal Veterinary Council (NVC) with Ref. No. 154/2080/81 on 9th November, 2023 and from the University of Cyberjaya Animal Care and Use Committee (CACUC) with reference number CACUC/1/2023/4 on 24 November, 2023.

The study protocol was strictly executed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Care was taken to adhere to the principles of 3R (Replacement, Reduction and Refinement) principle ensuring that the number of animals was kept to the minimum necessary and that any potential distress was alleviated through proper housing, daily monitoring, and timely use of analgesia where indicated. The study was reported in accordance with the ARRIVE guidelines 2.0 (Animal Research: Reporting of In Vivo Experiments) to ensure transparency and reproducibility of the in vivo procedures (NC3Rs.org.uk). A complete record of approvals, consent forms, and compliance documentation was maintained separately and remains available for audit or institutional review.

Experimental Animals

Forty-eight healthy male Sprague-Dawley rats (160–190g) were procured and acclimatised for seven days under standard laboratory conditions ($23 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity, 12-h light/dark cycle). Rats were housed in polypropylene cages with unrestricted access to standard chow and water. All procedures adhered to national and international animal welfare guidelines and were reported according to the guide ARRIVE standards.

Plant Material and Extraction

Turmeric rhizomes were collected from the herbal garden of NARTC, Kirtipur, and black pepper fruits were obtained from a certified supplier. Both plant materials were authenticated at the National Herbarium and Plant Laboratory, Nepal, and voucher specimens were deposited with reference numbers KATH168001 and KATH168002.

Following cleaning and shade-drying, samples were pulverised and stored under controlled conditions.

Hydroalcoholic extracts of turmeric (*Curcuma longa* L.) and black pepper (*Piper nigrum* L.) were prepared by double successive Soxhlet extraction using 70% ethanol (ethanol: water, 70:30 v/v). Fresh rhizomes and fruits were cleaned, shade-dried, and ground to a fine powder (particle size ≤ 0.42 mm). Each powdered material was extracted individually at a solid-to-solvent ratio of 1:10 (w/v) for 6 h at approximately 60 °C. The extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator at 40 °C, and freeze-dried to obtain dry powders. The dried extracts were stored in amber glass vials at 4 °C, and fresh suspensions were prepared in distilled water immediately before each oral administration.

Experimental Groups and Drug Administration

Rats were randomly allocated to six groups of eight animals each: normal control (NC); diabetic control (DC); metformin control (MC); prophylactic turmeric-black pepper whole powder without extraction (PTBP-W); prophylactic high-dose extract (PTBP-H); and prophylactic low-dose extract (PTBP-L). Prophylactic PTBP treatments were administered daily by oral gavage from week 1 through week 9 of the experimental period.

Induction of Diabetes

Diabetes was induced using a combined HFHS diet and the diabetogenic agent alloxan. All groups except NC received an HFHS diet supplemented with 30% fructose solution for four weeks to establish insulin resistance. During the fifth week, multiple low-to-medium doses of alloxan (MLM-ALX) were administered intraperitoneally at 35, 45, and 55 mg/kg body weight on alternate days. Rats with FBG exceeding 250 mg/dL after induction were classified as diabetic; confirmation was further supported by HbA1c > 5% and reduced FSI values. From week 6 onwards, MC rats received metformin at 200 mg/kg body weight daily until week 9, while DC rats remained untreated and were maintained on a normal diet alongside other groups.

Dosing Rationale

PTBP-W received 800 mg/kg turmeric and 80 mg/kg black pepper whole powder (without

extraction). PTBP-H and PTBP-L groups received high-dose (500+50 mg/kg) and low-dose (250+25 mg/kg) hydroalcoholic TBP extracts, respectively, suspended in distilled water and administered orally once daily. Doses were selected based on published literature on antidiabetic effects of *Curcuma longa* extracts in alloxan-induced diabetic rat models and similar HFHS diet studies, with minor modifications to ensure safety and avoid toxicity.^{6,7,9} Turmeric was co-administered with black pepper primarily to enhance the bioavailability of curcumin through the action of piperine, a well-established bioenhancer that inhibits hepatic and intestinal glucuronidation, thereby increasing curcumin absorption and systemic bioavailability substantially.^{9,10} This combination is standard practice in both traditional Ayurvedic formulations and contemporary phytopharmacological research to overcome curcumin's inherently poor oral bioavailability.

Biochemical Measurements

FBG was measured using a glucometer (Accu-Chek, Roche Diagnostics). FSI was quantified with a commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kit (Rat Ins1/Insulin ELISA Kit, Cat. No. RAB0904, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), performed according to the manufacturer's instructions; the kit has a reported sensitivity of 5 μ IU/mL and a detection range of 4.69–300 μ IU/mL. HbA1c was measured using a rat-specific enzymatic assay kit (Rat Haemoglobin A1c (HbA1c) Assay Kit, Cat. No. 80300, Crystal Chem Inc., Downers Grove, IL, USA) following the manufacturer's protocol with 5 μ L of EDTA-treated whole blood per well. Absorbance was measured at 450 nm using a microplate reader, and sample concentrations were derived from a standard curve generated using the reference standards provided with the kit.

Monitoring and Tissue Collection

Body weight and FBG were monitored weekly throughout the study. FSI and HbA1c were measured at baseline (week 0), post-induction (week 5), and at the end of the experiment (week 9). At study termination, rats were euthanised under deep anaesthesia induced by intramuscular administration of ketamine (80 mg/kg) and xylazine (10 mg/kg), followed by cervical dislocation. Terminal blood

samples were collected from the right atrium, after which pancreatic tissues were harvested, fixed in 10% neutral buffered formalin, processed, and embedded in paraffin. Tissue sections were stained with haematoxylin and eosin (H&E) according to standard histopathological protocols. Morphological changes in the exocrine (acinar) and endocrine (islet) pancreas, including inflammation, necrosis, and atrophy, were evaluated using a semi-quantitative four-point scoring system (0–3) as detailed in *Supplementary Table 1*. All slides were independently scored by two blinded observers.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0. Normality of data distribution was evaluated using the Shapiro–Wilk test. For normally distributed data, inter-group comparisons were conducted by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. When the normality assumption was violated, the non-parametric Kruskal–Wallis test was applied, followed by Dunn's post-hoc test for multiple comparisons. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

This study evaluated the prophylactic antidiabetic effects of TBP in diabetic rat model induced by HFHS diet combined with

alloxan. Body weight, FBG, FSI, HbA1c, OGTT, and histopathological changes in pancreatic tissue were assessed using a semi-quantitative scoring.

Baseline Physical and Biochemical Characteristics

At baseline, all experimental groups were comparable with respect to body weight, FBG, FSI, and HbA1c. Mean body weight ranged from 166.9 ± 4.2 g to 175.8 ± 11.2 g across groups, and FBG values were within the normoglycaemic range (77.9 ± 5.1 to 88.3 ± 9.4 mg/dL). FSI (17.45 ± 2.29 to 18.75 ± 2.86 μ U/mL) and HbA1c ($3.7 \pm 0.4\%$ to $4.3 \pm 0.9\%$) levels were similarly within normal ranges, confirming adequate group homogeneity before intervention.

Effect of Prophylactic TBP on Body Weight

NC rats demonstrated continuous weight gain throughout the study, from 172.50 ± 6.48 g at baseline to 249.75 ± 3.85 g at week 9. DC rats, by contrast, experienced progressive weight loss after alloxan exposure, declining from 168.25 ± 5.65 g to 159.0 ± 7.07 g by week 9. Rats in the PTBP-W group gained steadily from 171.50 ± 5.10 g at baseline to 211.88 ± 6.29 g at week 5 and 235.67 ± 4.68 g at week 9. PTBP-L rats similarly increased from 175.0 ± 11.20 g to 212.63 ± 13.30 g at week 5 and reached 235.71 ± 13.87 g by study end (Figure 1). All treated groups-maintained body weight trajectories approaching those of the NC group, contrasting sharply with the catabolic decline observed in untreated DC group. (Figure 1)

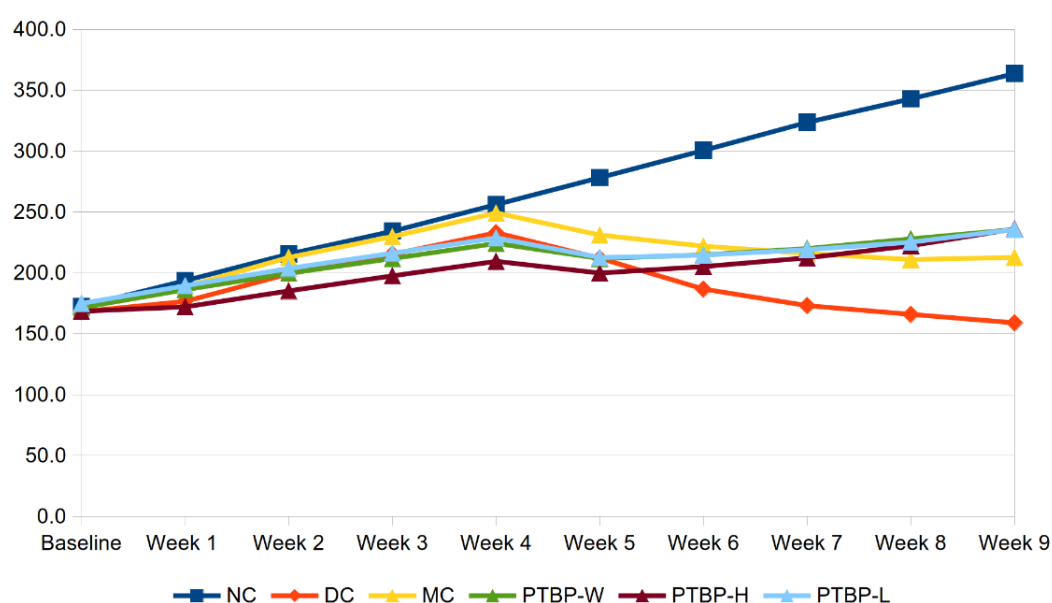


Figure 1. Effect of prophylactic TBP on weekly change in body weight (gain/loss) in different experimental groups from baseline (week 0) until the end of the experiment (EoE) at week 9.

Effect of Prophylactic TBP on Fasting Blood Glucose (FBG)

At baseline, FBG levels were comparable across all groups (range: 77.88–88.25mg/dL), with no significant inter-group differences ($F=1.672$, $p=0.123$). Following diabetes induction at week 5, the DC group developed severe hyperglycaemia (341.63 ± 109.07 mg/dL). Prophylactic TBP significantly attenuated FBG rise in all PTBP groups (PTBP-W: 273.88 ± 42.68 mg/dL; PTBP-H: 250.50 ± 20.98 mg/dL; PTBP-L: 274.25 ± 25.78 mg/dL) compared with DC ($p<0.001$), though levels remained elevated above NC values. At the end of the experiment, the DC group showed persistent marked hyperglycaemia (364.20 ± 22.08 mg/dL). All PTBP-treated groups demonstrated substantial FBG reduction, with PTBP-H (114.67 ± 9.22 mg/dL) and PTBP-W (122.33 ± 7.20 mg/dL) approaching MC group values. Differences across all groups at this

time point were highly significant ($F=400.192$, $p<0.001$) (Table 1).

Effect of Prophylactic TBP on HbA1c

Baseline HbA1c values ranged from 3.75% to 4.11% across groups, with no significant differences ($F=0.612$, $p=0.765$). After induction at week 5, HbA1c rose markedly in the DC group ($6.68 \pm 1.24\%$, $p < 0.001$), while PTBP formulations substantially limited this increase. By the end of the experiment, the DC group exhibited severe long-term glycaemic deterioration (HbA1c $9.44 \pm 0.71\%$), whereas all PTBP-treated groups-maintained values between 4.45% and 5.70%, statistically comparable to NC and MC groups. PTBP-H recorded the lowest value ($4.45 \pm 0.45\%$), closely approaching NC levels, followed by PTBP-W ($4.65 \pm 0.91\%$). PTBP-L showed a moderate but significant reduction relative to DC (5.70 ± 1.07 vs $9.44 \pm 0.71\%$, $p<0.001$). Overall

Table 1. Effect of prophylactic TBP on glycemic profile among different experimental groups

Experimental groups at w0, w5 & w9	Glycemic Profile (Mean $\pm$ SD)		
	FBG (mg/dL)	FSI (μ IU/mL)	HbA1c (%)
Baseline at week 0			
NC (n=8)	81.75 $\pm$ 6.50	18.48 $\pm$ 3.21	4.11 $\pm$ 0.49
DC (n=8)	88.25 $\pm$ 9.44	18.30 $\pm$ 2.88	4.05 $\pm$ 0.55
MC (n=8)	77.88 $\pm$ 5.06	18.16 $\pm$ 2.70	3.75 $\pm$ 0.43
PTBP-W (n=8)	85.25 $\pm$ 9.22	18.75 $\pm$ 2.86	3.90 $\pm$ 0.53
PTBP-H (n=8)	80.38 $\pm$ 8.82	18.00 $\pm$ 3.34	4.08 $\pm$ 0.74
PTBP-L (n=8)	85.88 $\pm$ 7.94	17.45 $\pm$ 2.29	3.88 $\pm$ 0.71
F (8,63)*	1.672	0.475	0.612
p-value*	0.123	0.869	0.765
Post-induction at week 5			
NC (n=8)	84.75 $\pm$ 5.83 ^c	19.35 $\pm$ 1.61 ^d	4.18 $\pm$ 0.57 ^d
DC (n=6)	316.63 $\pm$ 72.22 ^a	31.90 $\pm$ 4.06 ^a	6.68 $\pm$ 1.24 ^a
MC (n=8)	406.25 $\pm$ 11.84 ^a	30.61 $\pm$ 6.29 ^{ab}	5.45 $\pm$ 0.79 ^{bc}
PTBP-W (n=6)	273.88 $\pm$ 42.68 ^b	24.03 $\pm$ 2.56 ^{cd}	4.54 $\pm$ 0.79 ^{cd}
PTBP-H (n=6)	250.50 $\pm$ 20.98 ^b	23.86 $\pm$ 1.95 ^{cd}	4.90 $\pm$ 0.44 ^{cd}
PTBP-L (n=7)	274.25 $\pm$ 25.78 ^b	29.70 $\pm$ 4.18 ^{ab}	4.91 $\pm$ 0.36 ^{cd}
F (6,43)*	37.388	13.372	10.745
p-value*	<0.001	<0.001	<0.001
End of the experiment (week 9)			
NC (n=8)	84.75 $\pm$ 5.70 ^a	20.01 $\pm$ 1.91 ^a	4.24 $\pm$ 0.38 ^a
DC (n=6)	363.83 $\pm$ 16.94 ^c	9.27 $\pm$ 2.15 ^c	9.40 $\pm$ 0.64 ^c
MC (n=8)	110.63 $\pm$ 7.71 ^b	16.85 $\pm$ 3.02 ^{ab}	4.81 $\pm$ 0.68 ^a
PTBP-W (n=6)	122.33 $\pm$ 7.20 ^b	17.47 $\pm$ 3.49 ^{ab}	4.65 $\pm$ 0.90 ^a
PTBP-H (n=6)	114.67 $\pm$ 9.22 ^b	19.27 $\pm$ 3.79 ^a	4.45 $\pm$ 0.45 ^a
PTBP-L (n=7)	131.57 $\pm$ 7.70 ^b	16.13 $\pm$ 3.27 ^{ab}	5.70 $\pm$ 1.07 ^{ab}
F (6,43)*	533.54	10.04	23.69
p-value*	<0.001	<0.001	<0.001

*Values are expressed as Mean $\pm$ SD. *Statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey's HSD post-hoc test for multiple comparisons.

^{a,b,c,d}Values in the same column with different superscript letters are significantly different (Tukey's HSD test, $p < 0.05$).

Note: NC = Normal Control; DC = Diabetic Control; MC = Metformin Control; PTBP = Prophylactic Turmeric Black-Pepper formulations (H = High dose, L = Low dose, and W = Whole powder without extraction).

inter-group differences at study end were highly significant ($F=20.851$, $p<0.001$) (Table 1).

Effect of Prophylactic TBP on Fasting Serum Insulin

Baseline FSI levels did not differ significantly among groups ($F=0.475$, $p=0.869$; range: 17.45–18.75 $\mu\text{IU/mL}$). At week 5, post-induction, diabetic groups developed hyperinsulinaemia, with FSI levels significantly elevated compared with NC ($p<0.001$). By the end of the experiment, DC rats exhibited markedly depleted insulin levels ($8.78\pm2.00\mu\text{IU/mL}$), indicative of β -cell exhaustion. All PTBP-treated groups maintained significantly higher FSI (16.13 – $19.27\mu\text{IU/mL}$), comparable to MC and NC groups. PTBP-H achieved the highest insulin recovery ($19.27\pm3.79\mu\text{IU/mL}$), followed by PTBP-W ($17.47\pm3.49\mu\text{IU/mL}$) and PTBP-L ($16.13\pm3.26\mu\text{IU/mL}$). One-way ANOVA confirmed highly significant inter-group differences at study end ($F=9.905$, $p<0.001$) (Table 1).

Effect of Prophylactic TBP on Oral Glucose Tolerance and AUC

At the end of the experiment, oral glucose tolerance was severely impaired in DC rats, with persistently elevated glucose concentrations at all time points and the diet and alloxan-induced glucose intolerance (Table 2).

highest AUC ($63,909\pm1,943\text{mg}\cdot\text{min/dL}$), compared with NC ($16,230\pm234\text{mg}\cdot\text{min/dL}$). DC animals showed severely elevated FBG at 0 min ($364.20 \pm 22.08 \text{ mg/dL}$), with further increases at 30 min ($575.20 \pm 16.05 \text{ mg/dL}$) and 60 min ($510.60 \pm 13.47 \text{ mg/dL}$), followed by only partial reduction at 120 min ($425.00 \pm 12.59 \text{ mg/dL}$), reflecting profound glucose intolerance and impaired clearance.

Metformin treatment significantly improved glucose clearance (AUC: $22,131 \pm 1,208 \text{ mg}\cdot\text{min/dL}$). All PTBP-treated groups showed significant attenuation of glucose excursions relative to DC ($p < 0.05$). PTBP-H demonstrated the most pronounced improvement, with the lowest AUC among treatment groups ($19,385\pm627\text{mg}\cdot\text{min/dL}$), comparable to MC. PTBP-W and PTBP-L exhibited intermediate improvements ($21,263\pm674$ and $20,805\pm816 \text{ mg}\cdot\text{min/dL}$, respectively). One-way ANOVA followed by Tukey's HSD post-hoc test confirmed highly significant inter-group differences for glucose concentrations at every time point and for all AUC parameters (F values ranging from 400.192 to 1220.453, $p < 0.001$). Overall, prophylactic TBP preserved glucose tolerance in a dose-dependent manner, with the high-dose extract exerting the strongest protective effect against HFHS

Table 2. Effects of PTBP on OGTT and area under the curve (AUC) groups at the EoE

Group	FBG (mg/dL) at different time interval				AUC at different time interval			
	0 min	30 min	60 min	120 min	0-30 min	30-60min	60-120min	0-120 min
NC (n=8)	84.7 $\pm$ 5.70 ^a	148.2 $\pm$ 6.03 ^a	129.25 $\pm$ 5.23 ^a	114.2 $\pm$ 4.27 ^a	4764.4 $\pm$ 164.89 ^a	4160.6 $\pm$ 95.19 ^a	7305 $\pm$ 203.47 ^a	16230 $\pm$ 234.31 ^a
DC (n=6)	363.83 $\pm$ 16.94 ^c	573.5 $\pm$ 14.95 ^d	507.33 $\pm$ 14.46 ^d	424.67 $\pm$ 11.29 ^d	16212.0 $\pm$ 403.1 ^d	16287.0 $\pm$ 401.85 ^d	27960.00 $\pm$ 726.47 ^d	63909.0 $\pm$ 1564.0 ^d
MC (n=8)	110.6 $\pm$ 7.71 ^b	223.4 $\pm$ 19.73 ^b	176.63 $\pm$ 19.22 ^b	138.7 $\pm$ 5.7 ^b	6669.4 $\pm$ 234.85 ^b	6000.0 $\pm$ 530.39 ^b	9461.3 $\pm$ 671.96 ^b	22130.6 $\pm$ 1208.14 ^b
PTBP-W (n=6)	122.3 $\pm$ 7.20 ^b	205.2 $\pm$ 6.11 ^b	165.5 $\pm$ 5.99 ^b	133.00 $\pm$ 6.3 ^b	6747.5 $\pm$ 288.60 ^b	5560 $\pm$ 159.22 ^b	8955 $\pm$ 306.38 ^b	21262.5 $\pm$ 674.28 ^b
PTBP-H (n=6)	114.7 $\pm$ 9.22 ^b	185.7 $\pm$ 5.43 ^b	147 $\pm$ 6.60 ^b	125.33 $\pm$ 7.3 ^b	6225 $\pm$ 294.40 ^b	4990 $\pm$ 163.13 ^b	8170 $\pm$ 380.74 ^b	19385 $\pm$ 626.54 ^b
PTBP-L (n=7)	131.6 $\pm$ 7.70 ^b	182.7 $\pm$ 9.62 ^b	158.4 $\pm$ 7.35 ^b	141.6 $\pm$ 6.68 ^b	6687.9 $\pm$ 300.43 ^b	5117.2 $\pm$ 235.02 ^b	9000 $\pm$ 396.48 ^b	20805 $\pm$ 815.86 ^b
F (8,52)*	400.192	702.512	520.113	907.925	812.294	805.553	938.662	1220.453
p-value*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*Values are expressed as Mean $\pm$ SD. *Statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey's HSD post-hoc test for multiple comparisons.

^{a,b,c,d}Values in the same column with different superscript letters are significantly different (Tukey's HSD test, $p < 0.05$).

Pancreatic Histopathology and Islet Architecture

Semi-quantitative histopathological scoring (0–3 scale) at the EoE revealed significant structural alterations in pancreas of DC compared with NC group (Table 3). Significant

differences were observed among the groups for pancreatic acinar changes ($p = 0.036$), insulinitis ($p < 0.001$), islet necrosis ($p < 0.001$), and islet atrophy ($p < 0.001$). The DC group exhibited the highest median scores for all

histopathological parameters, indicating marked pancreatic injury, whereas the NC group showed normal pancreatic architecture with median scores of zero. The MC and PTBP treated groups generally demonstrated lower histopathological scores than the DC group, suggesting attenuation of pancreatic damage. Among the PTBP-treated groups, the PTBP-W and PTBP-H showed minimal exocrine changes and comparatively lower grades of insulitis, islet necrosis, and islet atrophy, while the PTBP-L exhibited relatively higher histopathological scores.

Table 3. Effects of prophylactic TBP on histopathological changes among groups at EoE

Experimental Group	Semi-quantitative grading of Pancreas [Median (IQR: Q1 – Q3)] (0 = normal, 1 = mild, 2 = moderate, 3 = severe changes)			
	Exocrine Pancreas		Endocrine Pancreas	
	Pancreatic Acini	Insulitis	Islet Necrosis	Islet Atrophy
NC (n=8)	0.00 (0.0 - 0.0) ^a	0.0 (0.0 - 0.0) ^a	0.0 (0.0 - 0.0) ^a	0.0 (0.0 - 0.0) ^a
DC (n=6)	1.00 (0.5 - 1.5) ^b	2.0 (1.5 - 3.0) ^c	2.25 (2.0 - 3.0) ^c	2.25 (2.0 - 3.0) ^c
MC (n=8)	0.00 (0.0 - 0.25) ^{ab}	0.50 (0.0 - 1.0) ^{ab}	1.00 (0.0 - 1.0) ^{ab}	1.0 (0.25 - 1.0) ^{ab}
PTBP-W (n=6)	0.00 (0.0 - 0.25) ^{ab}	0.0 (0.0 - 1.0) ^{ab}	1.0 (0.0 - 1.0) ^{ab}	0.5 (0.0 - 1.0) ^{ab}
PTBP-H (n=6)	0.00 (0.0 - 0.25) ^{ab}	0.0 (0.0 - 1.0) ^{bc}	1.0 (0.75 - 1.0) ^b	1.0 (0.0 - 1.0) ^{ab}
PTBP-L (n=7)	1.00 (0.0 - 1.0) ^{ab}	1.0 (1.0 - 1.25) ^{ab}	1.0 (1.0 - 2.0) ^{bc}	1.0 (1.0 - 2.0) ^b
F (8,52)*	2.273	7.384	10.082	8.183
p-value*	0.036	<0.001	<0.001	<0.001

Notes: Values are expressed as Median (IQR: Q1 - Q3) *Statistical significance was determined using Kruskal-Wallis followed by Dunn's post-hoc test with Bonferroni correction.^{a,b,c} Means within a column sharing a common superscript letter are not significantly different ($p > 0.05$). Different superscript letters within a column indicate statistically significant differences between groups ($p < 0.05$).

Histopathological evaluation of the islets of Langerhans across experimental groups revealed well-defined, densely cellular islets with clear boundaries in NC group. DC group showed severe islet shrinkage, reduced cellularity, vacuolation, and peri-insular inflammation. MC group demonstrated partial restoration of islet architecture, while PTBP groups showed dose-dependent protection. PTBP-H exhibited the most preserved islet morphology, closely resembling NC, with maintained cellular density and minimal necrosis. PTBP-W and PTBP-L groups showed intermediate improvements with partial preservation of islet integrity relative to DC (Figure 2).

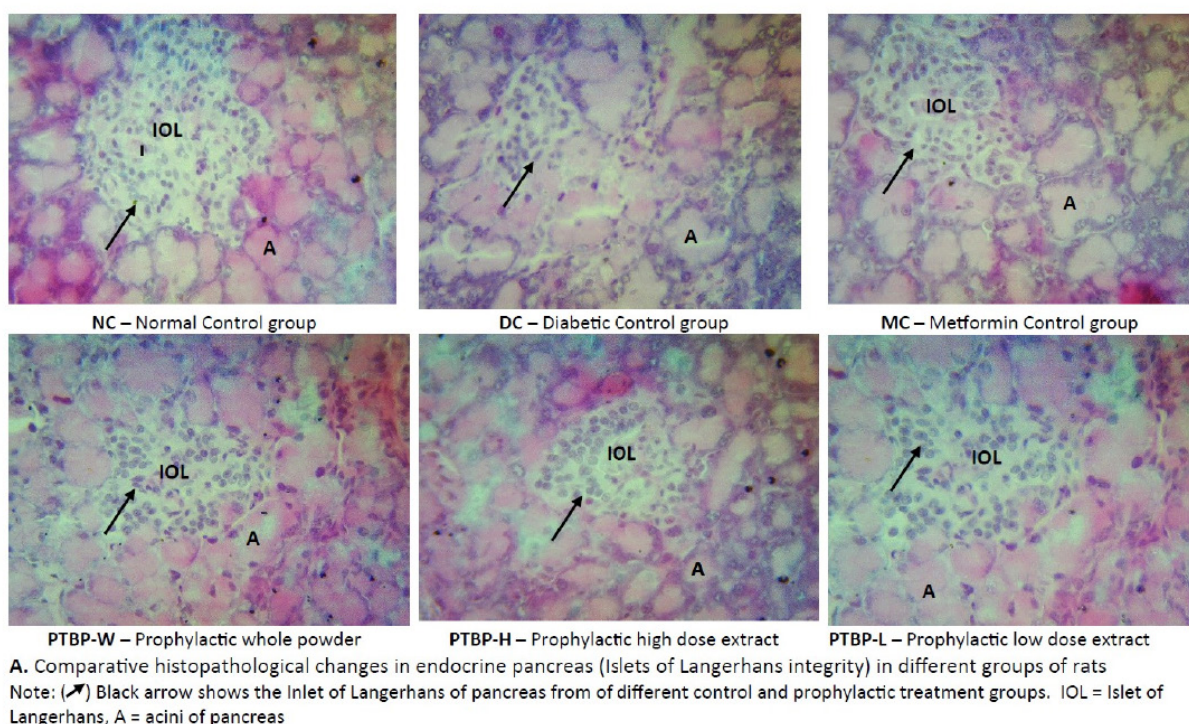


Figure 2. Comparative histopathological changes in endocrine pancreas among groups

DISCUSSION

The present study demonstrates that prophylactic TBP administration confers substantial metabolic and structural protection against HFHS diet and MLM-ALX-induced diabetic rats. Integrating biochemical, functional, and histopathological data, the findings collectively support a preventive role of TBP against progressive dysglycaemia and pancreatic injury.

Baseline homogeneity across all experimental groups confirms that subsequent metabolic divergence reflects the effects of dietary challenge, alloxan exposure, and prophylactic intervention rather than pre-existing variability. Body weight, FBG, FSI, and HbA1c values at baseline fell within established physiological ranges for adult male rats,¹¹ consistent with prior metabolic studies using this strain.¹² This methodological rigour strengthens causal interpretation of the prophylactic outcomes observed at study end.

Progressive weight loss in DC group following alloxan exposure represents a classical feature of insulin-deficient diabetes, driven by enhanced proteolysis, lipolysis, and negative energy balance.^{13,14} All treated groups maintained body weight and avoided the severe catabolic state seen in DC group. Preservation of body mass in prophylactic groups suggests improved metabolic efficiency and partial maintenance of insulin action. Comparable protective effects of curcumin on diabetes-associated weight loss have been reported in HFHS and chemical induction models, where curcumin supplementation attenuated muscle wasting and adipose tissue depletion by improving insulin signalling and reducing oxidative stress.¹⁵

Marked hyperglycaemia, elevated HbA1c, and reduced FSI in DC group confirm successful induction of a severe diabetic phenotype.¹⁶ Alloxan induces selective β -cell necrosis through ROS generation following GLUT2-mediated intracellular uptake.⁵ The combination of HFHS diet and alloxan recapitulates key pathophysiological features of T2DM with β -cell failure, encompassing both peripheral insulin resistance and direct pancreatic β -cell toxicity.^{17,18} Prophylactic TBP administration significantly reduced FBG and HbA1c; the high-dose extract group achieved glycaemic control comparable to

metformin. The sustained reduction in HbA1c reflects durable glycaemic improvement over multiple weeks rather than acute glucose-lowering. Improved FSI levels across all TBP-treated groups further suggest preservation of functional β -cell mass.

OGTT findings provide functional corroboration of preserved glucose handling in TBP-treated rats. DC animals exhibited profound glucose intolerance with sustained hyperglycaemia throughout the test period and markedly elevated AUC values. Prophylactic TBP treatment, particularly at the higher extract dose, significantly reduced peak glucose excursions and facilitated faster return toward baseline. AUC values in the PTBP-H group closely approximated those of MC rats, indicating preserved dynamic insulin responsiveness and peripheral glucose uptake. Similar improvements in OGTT parameters have been reported for curcumin supplementation in diet-induced insulin resistance models.¹⁹

The glycaemic benefits of TBP are underpinned by several complementary mechanisms. Curcumin exerts potent antioxidant and free radical scavenging activity, directly counteracting alloxan-induced oxidative damage.^{19,20} Moreover, curcumin modulates Nrf2 signalling and suppresses NF- κ B-mediated inflammatory pathways, mechanisms that collectively support β -cell survival.²¹ The enhanced efficacy observed in extract-treated groups relative to whole powder likely reflects superior bioavailability, further augmented by piperine-mediated inhibition of hepatic and intestinal glucuronidation.²²

Histopathological analysis provides direct structural evidence corroborating the biochemical and functional outcomes. DC groups showed extensive pancreatic damage, including islet shrinkage, β -cell necrosis, and inflammatory cell infiltration, lesions characteristic of combined metabolic stress and chemical β -cell toxicity. Prophylactic TBP significantly reduced these pathological changes. PTBP-H demonstrated near-normal islet architecture with reduced necrosis and inflammation scores, surpassing even metformin on some histological parameters. This finding emphasizes the capacity of turmeric to protect pancreatic microarchitecture when administered prior to diabetogenic insult.

The dose-dependent histological protection aligns with experimental evidence showing that curcumin preserves islet morphology, reduces macrophage infiltration, and limits cytokine-mediated β -cell apoptosis.^{10,20} Protection of exocrine acini further implies a broader cytoprotective effect, possibly mediated through suppression of oxidative stress and microvascular congestion within the pancreatic parenchyma.^{8,21,22}

Some of the limitations of the present study deserve acknowledgement. The nine-week observation period precludes assessment of long-term efficacy and safety. Dose optimisation was limited, and the molecular mechanisms of β -cell protection were not investigated. Being a preclinical rat model, the findings may not fully translate to human type 2 diabetes. Further mechanistic and clinical studies are needed.

CONCLUSION

Prophylactic supplementation with turmeric co-administered with black pepper (TBP) preserved endocrine pancreatic structure and improved the glycaemic profile in an HFHS diet and alloxan-induced T2DM. These functional improvements were significantly associated with structural protection of pancreatic islets, particularly in rats receiving prophylactic high-dose extract followed by prophylactic whole powder. These findings suggest that prophylactic TBP supplementation has potential to delay diabetes progression through structural and functional protection of pancreatic tissue, supporting further mechanistic and translational investigations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualisation: Kapil Amgain and Shamima Abdul Rahman; Methodology: Kapil Amgain, Siti Munirah Md Noh, and Shamima Abdul Rahman; Data Collection: Kapil Amgain; Formal Analysis: Kapil Amgain, Shamsher Shrestha, and Bijaya Aryal; Writing – Original Draft: Kapil Amgain; Writing – Review & Editing: Siti Munirah Md Noh, Shamsher Shrestha, Bijaya Aryal, and Shamima Abdul Rahman; Supervision: Shamima Abdul Rahman.

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