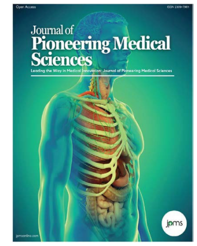
Journal of Pioneering Medical Sciences
Received: December 29, 2025 | Accepted: May 24, 2026 | Published: July 05, 2026
Volume 15, Issue 06, Pages 121-131

DOI https://doi.org/10.47310/jpms2026150616



Effects of Thymus vulgaris Essential Oil on Metabolic Parameters and Gut Microbiota in a Diabetic Rat Model

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Abstract **Background:** Medicinal plants, such as *Thymus vulgaris*, have garnered attention for their pharmacological properties, including their rich essential oils that demonstrate antioxidant and antimicrobial activities. **Objectives:** To explore the health benefits of *Thymus vulgaris* Essential Oil (TVEO) on gut microbiota and metabolic health in a diabetic rat model. Methods: Thirty adult male rats were acclimated and allocated into five groups, as control negative, Diabetic Control (DC), control positive (Empagliflozin), Low-Dose (LD) TVEO (50 mg/kg) and High-Dose (HD) TVEO (100 mg/kg). Treatments were given orally for four weeks, with weekly evaluations of blood glucose and body weight (BW). Finally, blood biochemical analyses, faecal cultures for bacterial identification and histopathological examinations of vital organs were done. **Results:** A significant reduction was detected in blood glucose levels, particularly in the HD-TVEO group. BW loss was less pronounced in treated rats than the DC. Biochemical analyses revealed lowered liver enzyme levels (ALT and AST) in all treatment groups, along with increased total protein and albumin in the HD group. The lipid profile showed significant decreases in triglycerides, total cholesterol and LDL, with an increase in HDL levels in the HD group. Haematological assessments revealed improvements in the absolute red cell distribution width counts. Additionally. CRP levels were potentially lowered in the HD group than the disease control. On the other hand, TVEO improved gut microbiota by reducing pathogenic bacteria and minimizing damage to the pancreas, liver and kidneys, thereby retaining normal tissue morphology and architecture. **Conclusions:** TVEO might act as a therapeutic agent in the management of diabetes, highlighting the multifaceted role in promoting metabolic health and suggesting its potential to serve as a natural adjunct to standard diabetes therapies.

Key Words Herbal Medicine, Essential Oil, Induced Diabetes, Gut Microbiota, Animal Model

INTRODUCTION

Diabetes Mellitus (DM) is increasingly recognized as a global health challenge among people of all age groups, regions and socioeconomic backgrounds. According to the International Diabetes Federation, an estimated 415 million people reported to have DM in 2020, which is projected to increase to >640 million by 2040 [1]. Nearly half of those affected are unaware of their condition, which increases the risk of developing severe complications [2]. The host gut is a complex ecosystem comprising of microbiome, cells and nutrients. There are approximately 100 trillion bacteria in the intestine, which form the gut microbiota, a composition of various bacterial species. These are taxonomically categorized by genus, family, order and phylum [3]. The composition of gut microbiota has been linked to various key features of metabolic syndrome, including obesity, DM, cardiovascular diseases and non-alcoholic steatohepatitis. Emerging research suggests that gut microorganisms may contribute to the low-grade inflammation commonly observed in these conditions, primarily through mechanisms related to impaired gut barrier function [4].

Studies have shown that managing DM can be supported through strategies that alter the gut microbiota. Approaches such as the use of probiotics, antidiabetic medications and specific dietary modifications positively influence the microbial balance in the gut, stimulating the growth of beneficial bacteria while destroying harmful strains. This shift in gut microbial composition may play a crucial role in improving metabolic function and regulating blood sugar levels [5]. Traditional herbal medicine has a long-standing history in treating various health conditions, particularly in managing metabolic disorders like DM. Despite its historical efficacy, modern scientific research has encountered obstacles in elucidating the specific active compounds and their mechanisms of action within herbal formulations [6]. Herbal medicines may help manage DM by interacting with the gut microbiota. They can restore microbial balance, reduce inflammation and be affected by gut bacteria in ways that influence their effectiveness. While the exact mechanisms are still being explored, studies suggest a strong link between gut health and the therapeutic potential of herbal treatments for DM [7].

Thymus vulgaris (TV), a medicinal plant with a pleasant aroma, contains essential oils rich in flavonoids recognized for their antioxidant and antimicrobial activities. Its traditional and modern uses suggest strong potential in developing natural therapeutic agents, particularly for gastrointestinal health [8]. However, up-to-date, there is no published data on the effect of TV Essential Oil (TVEO) on gut microbiota improvements in a diabetic rat model. Thus, we aimed to find the effects of TVEO on gut microbiota and metabolic health in streptozotocin (STZ)-induced diabetic rats.

METHODS

Study Design and Setting

This experimental study was done at the Animal House of the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq. The research involved thirty adult Sprague-Dawley rats, aged 8-10 weeks and weighted 150±20 g.

Induction of DM

DM was induced using a single injection of STZ intraperitoneally (50 mg/kg BW), prepared in 0.1 M citrate buffer (pH 4.5). Three days later, fasting Blood Glucose (BG) levels were tested and rats with FBG levels of ≥300 mg/dL were classified as DM [9].

Animal Grouping and Treatments

The animals were allocated to 5 groups (n = 6). Randomization and justification of sample size was derived from a previous report, which was found that a minimum of six animals per group was necessary to induce DM in rats and establish meaningful comparisons between groups after treatments with plant metabolites [9]. Group 1 (Control Negative; CN) was non-DM rats received distilled water, Group 2 (Disease Control; DC) was DM rats received distilled water, Group 3 (Control Positive; CP) was DM rats treated with empagliflozin (30 mg/kg), Group 4 was DM rats treated with LD-TVEO (50 mg/kg), Group 5 was DM rats treated with HD-TVEO (100 mg/kg). Treatments were administered orally, once daily using a force-feeding needle for 4 consecutive weeks, starting 3 days after the induction of DM.

Monitoring of FBG and BW

FBG levels were assessed weekly using the On Call Plus glucometer. BWs were recorded at baseline (day 0) and on days 7, 14, 21 and 28 to monitor changes throughout the study.

Blood Sample Collection

On day 29 of the experiment, all rats were deeply anaesthetized using a mixture of xylazine (5 mg/kg) and ketamine (75 mg/kg) intraperitoneally. Once the animals were fully anaesthetized, blood (5-7 mL) was collected via cardiac puncture and one-third of it was immediately transferred to sterile tubes containing specific anticoagulants for haematological analysis. After 30 minutes, another 2/3 was centrifuged at 3500 rpm for 10 minutes at 4°C. The serum was collected and kept at -20°C for subsequent use.

Faecal Sample Collection

Faecal samples were collected from the rat's gut immediately after excision and placed into sterile containers. The samples were processed within one hour of collection to ensure the viability of bacteria for gut microbiota analysis using anaerobic culture techniques. Briefly, faecal samples were inoculated onto MacConkey agar, blood agar and S-S agar. Plates were incubated in an anaerobic jar at 37°C for two days. Then, isolated colonies were identified using the VITEK and BD Phoenix systems, which provide rapid identification and antimicrobial susceptibility testing.

Biochemical and Haematological Analyses

Serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total Protein (TP), albumin, C-Reactive Protein (CRP), urea, creatinine and Total Serum Bilirubin (TSB) were estimated. Total Cholesterol (TC), triglycerides (TG), High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) levels were also analyzed using enzymatic methods. A Complete Blood Count (CBC) includes haemoglobin (Hb), Red Blood Cell (RBC), hematocrit (HCT) and platelet (PLT) count, which were assessed using an automated haematological analyzer.

Histopathological Examination

Tissue samples from the pancreas, liver and kidneys were collected directly post-euthanasia for histopathological analysis. The tissues were fixed in 10% formaldehyde solution for 24 hours, processed through a graded series of ascending/descending ethanol concentrations, cleared by xylene in three successive stages, embedded in molten paraffin wax, sectioned (5 μm width) using a microtome, tissue slices were floated on a warm water bath at 50°C to eliminate folds or wrinkles, placed on glass slides, dried on a heated tissue slide warmer, stained with freshly prepared hematoxylin and eosin (H & E), mounted with DPX and cover-slipped. Finally, slides were examined under a normal light microscope equipped with digital camera. Then, lesion scoring was performed semi-quantitatively through image analysis software (AmScope, version 3.7) and quantified data were interpreted using a standardized lesion scoring system, in which lesions from 0-10% was considered negative, 10-25% as mild, 25-50% as moderate, 50-75% as severe and 75-100% as critical severity.

Statistical Analysis

Data were analyzed using the SPSS (Chicago, IBM, USA, Version 26). Kolmogorov-Smirnov test was used to assess the normality of the data. Independent Sample t-tests and Mann-Whitney U tests were used to compare the differences of parametric and non-parametric variables between the groups. Whereas ANOVA test was used for comparisons across the different time points for BW and BG levels. A $p \leq 0.05$ was set as significant.

RESULTS

BW Analysis

Significant variations ($p \leq 0.05$) in BW were observed among the experimental groups during the 28-day treatment period. The CN, DC and CP groups exhibited significant increases in BW ($p \leq 0.05$). However, the groups treated with TVEO (50 and 100 mg/kg) did not show substantial BW changes ($p \geq 0.05$) (Table 1).

BG Level

BG levels were significantly ($p \leq 0.05$) diminished in the CP, LD and HD-TVEO groups than baseline and the DC group. In contrast, it significantly increased ($p \leq 0.05$) in the DC group than the CN (Table 2).

Liver and Renal Function Tests

TVEO, particularly at 100 mg/kg, resulted in significant improvements ($p \leq 0.05$) in liver enzyme markers. Additionally, it was observed that TVEO, especially at the HD, enhanced liver protein synthesis. Renal function parameters remained within physiological norms, although urea levels were mildly elevated in the HD-TVEO group (Table 3).

Lipid Profile

TVEO significantly ($p \leq 0.05$) improved lipid profile parameters, including decreased TG, TC and LDL, especially in the HD-TVEO group than the DC group, while the HDL level was significantly increased in both doses of TVEO group ($p \leq 0.05$) than the DC group (Table 4).

Electrolyte Levels

Both doses of TVEO had no significant alterations on serum sodium, potassium and chloride, suggesting no modulatory effects on electrolyte balance (Table 5).

Hematological Parameters

Significant ($p \leq 0.05$) improvements were observed in RDWa in both doses of TVEO (Table 6a-b).

Gut Microbiota Modulation

A total of 16 positive gut samples of 5 different bacterial species were detected, with most isolates (56.25%) found in DC, followed by CN (25%) and CP (18.57%), while no isolates were found TVEO treated groups. Among species, *E. coli* was more prevalent (n = 6), then *P. vulgaris* (n = 5), *S. boydii* and *P. penneri* (n = 2 each) and *Klebsiella* species (n = 1) (Table 7).

Table 1: Mean Body Weight of Rats Treated with Various Agents During the Experimental Period

Study Group	Body weight (gram) (Mean±SD)					p-value
	Day 0	Day 7	Day 14	Day 21	Day 28	
CN	232±10.05	234±3.81	254.2±19.23	282.2±27.59	288.4±21.87	0.008*
DC	194.6±15.41	196±20.41	203.5±15.93	219.25±17.56	245.75±7.04	0.040*
CP(Empagliflozin)	199.8±12.52	186.4±11.55	199.5±9.75	206.6±24.90	272.6±30.63	0.019*
TVEO (50 mg/kg)	191.6±27.24	171.75±33.12	156.25±37.63	216±16.97	212.5±9.19	0.437
TVEO(100 mg/kg)	200.4±23.22	170.6±24.10	170.6±18.12	189.5±43.31	195.25±46.59	0.221

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, *Significant difference using repeated measure ANOVA test

Table 2: Blood Glucose Levels of Experimental Rats During the Study

Study Group	Random Blood Glucose (mg/dL) (Mean±SD)					
	Zero day	7th day	14th day	21st day	28th day	p-value
CN	75.80±7.50	78±7.25	81.80±6.94	114.20±30.46	88±6.78	0.047*
DC	73.20±7.60	64±15.77	135.25±56.42	412.75±173.05	343.75±158	0.014*
CP(Empagliflozin)	78.80±5.54	81±16.63	117±10.13	242.75±98.28	121.25±69.12	0.04*
TVEO (50 mg/kg)	86.40±11.10	299.5±114.12	346.75±212.25	450±14.14	400.50±13.44	0.041*
TVEO(100 mg/kg)	85.80±10.18	297.6±87.51	309.60±113.93	300.20±49.90	249±45.03	0.019*

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, *Significant difference using repeated measure ANOVA test

Table 3: Liver and Renal Function Tests Among Study Groups

Study Group	Biochemical Parameter (Mean±SD)								
	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein	Albumin	CRP	Urea (mg/mL)	Creatinine (μG/mL)	TSB
CN	138.67±32.35	58.87±16.85[a]	185.67±70.61[a]	3.84±0.14	6.70±0.4[a]	0.05±0.02	34.27±4.58[a]	0.28±0.01	0.06±0.01
DC	158.52±11.46	303.35±54.6[b]	494.50±58.54[b]	3.58±0.23	5.92±0.1[b]	0.03±0.03	42.68±3.56[b]	0.29±0.04	0.07±0.05
CP (Empagliflozin)	119.33±7.77[a]	91.10±25.70[a]	504.33±241.21	4.03±0.2[a]	6.36±0.41	0.02±0.01	40.27±5.75	0.30±0.03	0.08±0.02
TVEO (50 mg/kg)	287.00±5.66[a]	203.50±6.36[a]	737.00±127.72[a]	3.31±0.29	6.74±0.68	0.04±0.01	44.95±23.12	0.32±0.04	0.08±0.03
TVEO (100 mg/kg)	139.55±43.65	81.25±29.79[a]	505.25±395.34	3.09±0.67	7.15±0.2[a]	0.06±0.0[a]	62.53±16.9[a]	0.28±0.03	0.22±0.31

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TP: Total protein, CRP: C-reative protein, TSB: Total serum bilirubin, *Significant difference using repeated measure ANOVA test

Table 4: Lipid Profile Levels Across Experimental Groups of Treated Rats

Study Group	Lipid profile (Mean±SD)			
	TC	TG	LDL	HDL
CN	58.17±4.72	45.37±5.38[a]	17.53±5.68	31.83±1.07
DC	59.43±5.64	106.98±15.56[b]	14.03±2.49	33.18±1.56
CP (Empagliflozin)	50.33±3.47	104.27±38.77	9.94±0.88	30.37±2.31
TVEO (50 mg/kg)	71.60±12.16	100.70±32.95	17.10±8.91	40.40±5.23[a]
TVEO(100 mg/kg)	92.65±14.34[a]	85.85±8.28[a]	27.18±5.42[a]	56.25±1.95[a]

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, a: p≤0.05 when compared to diabetic control group and b: p≤0.05 compared to the control negative group using Independent sample t-test

Table 5: Serum Electrolyte Levels Among Rats of the Study Groups

Study Groups	Serum Electrolytes (Mean±SD)		
	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L
CN	137.07±1.12	8.11±1.31	100.97±1.32[a]
DC	134.88±1.18	6.15±1.25	96.65±1.96[b]
CP (Empagliflozin)	136.47±1.34	6.38±1.37	98.20±1.00
TVEO (50 mg/kg)	130.55±1.06[a]	7.62±0.69	93.00±1.27
TVEO(100 mg/kg)	35.43±2.96[a]	35.43±2.96[a]	96.18±1.77

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, a: p≤0.05 when compared to the diabetic control group, and b: p≤0.05 compared to the control negative group using the Independent sample t-test

Table 6a: Haematological Parameters of the Experimental Groups

Study Group	Hematological parameters (Mean±SD)										
	WBC	LYM (10^9/L)	MID (10^9/L)	GRA (10^9/L)	Hb (g/dL)	RBC (10^{12}/L)	MCV (fl)	HCT (%)	RDW (fl)	PLT (10^9/L)	PCT
CN	20.67±8.25	18.13±7.80	1.30±0.44	1.23±0.51	17.77±0.55[a]	8.91±0.33	57.93±1.61	51.63±2.34	37.73±3.12	672.67±17.04	0.49±0.05
DC	10.48±4.95	8.18±4.46	1.03±0.34	1.28±0.79	16.45±0.60[b]	8.34±0.44	56.53±2.16	47.13±2.60	36.25±1.77	792.25±88.28	0.57±0.05
CP (Empagliflo zin)	10.60±2.48	7.53±1.33	1.30±0.44	1.77±0.75	17.67±0.31[a]	8.56±0.31	59.70±1.25	51.07±0.81	37.50±2.07	570.67±9.82[a]	0.42±0.03[a]
TVEO (50 mg/kg)	10.75±0.21	8.60±0.00	1.10±0.42	1.05±0.21	17.20±0.42	8.43±0.11	59.65±2.05	50.25±2.33	44.30±0.28	735.00±22.63	0.55±0.01
TVEO (100 mg/kg)	13.50±8.17	9.58±5.52	1.63±1.23	2.30±0.35	14.90±2.82	7.80±1.33	54.38±3.27	42.60±8.67	41.78±6.32[a]	677.25±339.02	0.50±0.26

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, a: p≤ 0.05 when compared to the diabetic control group and b: p≤ 0.05 compared to the control negative group using the Independent sample t-test (MCV, PLT and PCT) and the Mann-Whitney U-test (Others)

Table 6b: Haematological Parameters of the Experimental Groups

Study Group	Hematological parameters (Mean±SD)										
	Lymp (%)	Mid (%)	Gran (%)	MCH (pg)	MCHC (g/dl)	RDW (%)	MPV (fl)	PDW (fl)	PDW (%)	P-LCR (%)	P-LCC (10^9/L)
CN	86.67±3.86	6.73±1.22	6.60±2.65	19.97±0.76	34.43±0.75	15.43±0.91	7.27±0.47	9.47±0.47	37.00±0.40[a]	8.50±2.55	57.00±18.52
DC	75.85±10.52	10.93±3.10	13.23±7.87	19.73±0.39	34.93±0.87	15.33±0.71	7.15±0.17	9.28±0.21	36.25±0.31[b]	7.53±1.04	58.50±4.51
CP (Empagliflozin)	70.73±4.57	12.60±1.65	16.67±3.25	20.63±0.38[a]	34.57±0.21	14.60±0.87	7.43±0.38	9.60±0.46	37.37±0.60[a]	9.47±1.92	53.67±11.37
TVEO (50 mg/kg)	79.40±2.12	10.40±3.82	10.20±1.70	20.40±0.28	34.25±0.64	18.05±0.78[a]	7.50±0.28	9.65±0.35	36.50±0.14	9.65±1.48	70.00±2.83[a]
TVEO(100 mg/kg)	72.38±9.25	11.38±2.80	16.25±6.59	19.05±1.10	35.05±0.65	19.35±1.36[a]	7.33±0.15	9.65±0.25	37.70±1.37	9.35±1.48	63.50±36.21

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil, a: p≤ 0.05 when compared to the diabetic control group and b: p≤ 0.05 compared to the control negative group using the Independent sample t-test

Table 7: Detected Gut Microbiota Among Rats of the Study Groups

Study Group	Bacterial Species Number (Percentages)					
	Proteus penneri (n = 2)	*Escherichia coli* (n = 6)	Klebsiella species (n = 1)	*Proteus vulgaris* (n = 5)	*Shigella boydii* (n = 2)	Total (n = 16)
CN	1 (50)	1 (16.66)	0 (0.0)	2 (40)	0 (0.0)	4 (25)
DC	1 (50)	3 (50)	1 (100)	2 (40)	2 (100)	9 (56.25)
CP (Empagliflozin)	0 (0.0)	2 (33.33)	0 (0.0)	1 (20)	0 (0.0)	3 (18.75)
TVEO (50 mg/kg)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TVEO (100 mg/kg)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

CN: Control negative, CP: Control positive, DC: Disease control, TVEO: *Thymus vulgaris* essential oil

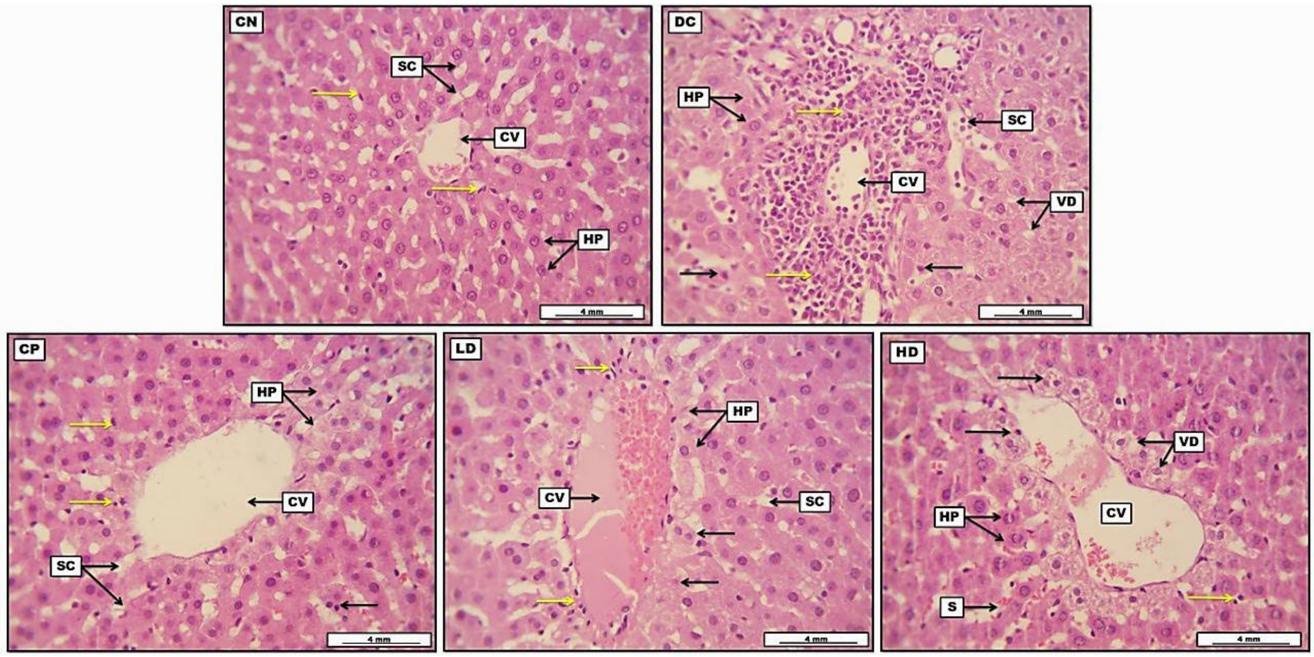

Figure 1: Photomicrograph of Liver Sections from the Control Negative (CN) Group Exhibited No Significant Morphological Alterations, Characterized by the Typical Arrangement of Hepatocytes (HP) Surrounding the Slightly Congested Central Vein (CV). The Sinusoidal Capillaries (SC) Appear Typically Normal, Accompanied by a Mild Infiltration of Hepatic Kupffer Cells (yellow arrow). The Disease Control (DC) Group Showed Significantly Distributed Perivascular Cuffing of Leukocytes (Yellow Arrows) Around the CV. Most HP Displayed Significant Vacuolar Degeneration (VD), Evident by Their Pale Cytoplasm and Swollen Cells

Additionally, most SC dilated and engorged with inflammatory cells; moreover, the presence of pyknotic necrotic cells in the given section (black arrow). The control positive (CP) group revealed marked centrilobular cellular injury in HP, prominently indicated by extensive VD. Other hepatocytes displayed eosinophilic cytoplasm with pyknotic or condensed nuclei (indicated by the black arrow). Furthermore, the CV was distinctly dilated with some other SC and moderately infiltrated with Kupffer cells (yellow arrows). Low dose (LD) Thymus Vulgaris Essential Oil (TVEO) group demonstrated many centrilobular HP with significant cellular swelling apparent by VD, together with other necrotic hepatocytes (black arrows) around the dilated and engorged CV, with a mild to moderate degree of perivascular cuffing of leukocytes (yellow arrows). The section also displays some dilated and mildly congested SC. The high-dose (HD) TVEO group showed moderate centrilobular hydropic VD. The remaining HP maintain a typical centrilobular arrangement without evident morphological alterations except for low-grade cytoplasmic eosinophilia. Some other hepatocytes exhibit fragmented cytoplasm accompanied by pyknotic nuclei. The SC and the CV displayed visible dilation with low-grade Kupffer cells infiltration (yellow arrow). H&E, Scale bar of 4 mm

Table 8: Micromorphological Semi-Quantitative Assay of Liver Sections

Study Group	Vacuolar Degeneration (Mean% %)	Cellular Swelling (Mean% %)	Inflammatory Cells (Mean% %)	Lesion Scoring (0 -100%)	Lesion Grading
CN	3.16$^{A\#}$	6.47^{A}	9.58^{A}	0-10	No Lesion
DC	79.22^{E}	84.39^{E}	92.73^{E}	75-100	Critical
CP (Empagliflozin)	41.63^{C}	49.32^{C}	44.87^{C}	25-50	Moderate
TVEO (50 mg/kg)	68.45^{D}	71.46^{D}	59.34^{D}	50-75	Severe
TVEO (100 mg/kg)	56.21^{D}	59.32^{D}	50.61^{D}	50-75	Severe

#Statistical comparison among groups; mean values with different capital letters have significant differences (p<0.05), CN: Control negative, CP: Control positive, DC: Disease control, TVEO: Thymus vulgaris essential oil

Histopathological Examination

Liver: All treatment groups demonstrated a noticeable improvement in lesion severity, with a significant reduction (p<0.05) in lesion scores than the untreated DC group (Table 8). Liver sections from the CN group exhibited preserved histological integrity, characterized by a typical hepatic architecture and regular radial arrangement of hepatocytes surrounding the central vein (Figure 1-CN). In contrast, DC showed pronounced hepatic injury, evidenced by severe vacuolar degeneration and prominent cellular swelling accompanied by severe perivascular cuffing of leukocytes (Figure 1-DC). Treatment with TVEO at both doses resulted in a significant (p<0.05) reduction in lesion scores than the DC group. Furthermore, CP group exhibited a notably greater reduction in hepatic damage, as reflected by a moderate degree of vacuolar degeneration, relative cellular swelling and diminished inflammatory cell infiltration when than the severe lesions observed in the DC, LD and HD groups.

Kidneys

Animals in the CP and HD-TVEO group, exhibited a significant (p<0.05) reduction in the extent of tubular epithelial cell swelling and degenerative changes.

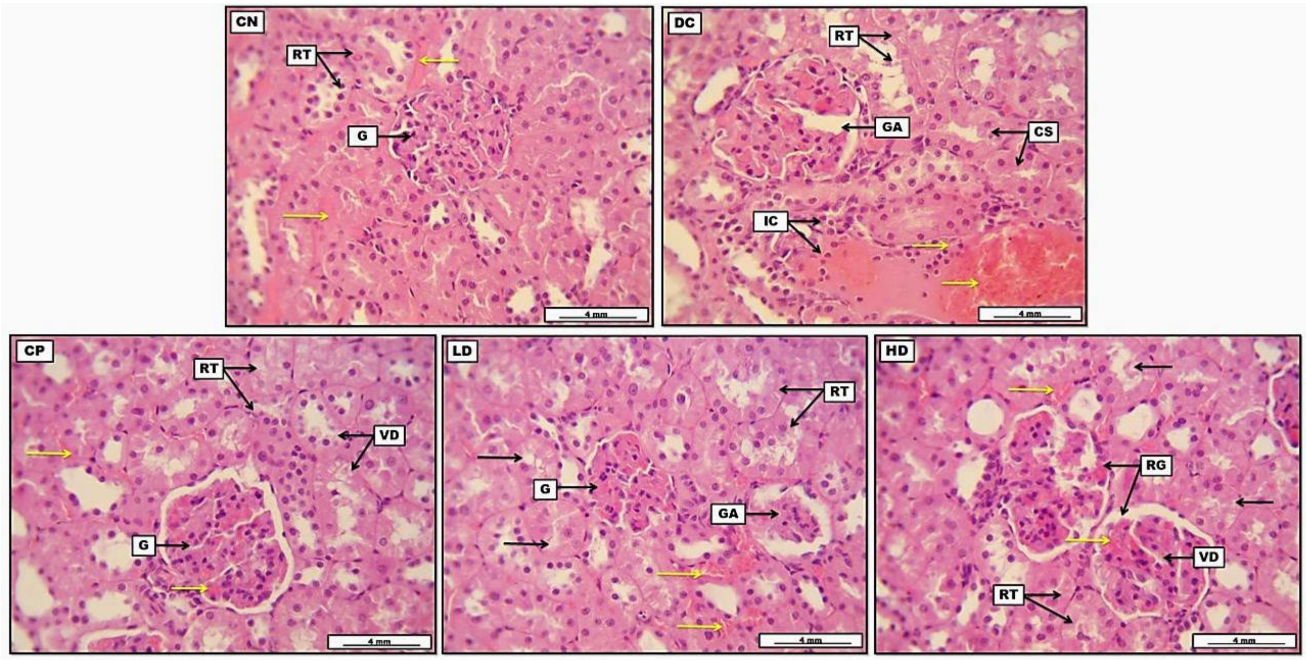

Figure 2: Photomicrograph of Kidney Sections from the Control Negative (CN) Group Exhibited No Morphological Alterations, with a Well-Preserved Glomerular Architecture (G). Renal Tubules (RT) Appear Mildly Dilated, Lacking Significant Pathological Lesions. Additionally, Occasional Acidophilic Proteinaceous Material is Observed Within the Interstitial Vasculature (Yellow Arrows). Disease Control (DC) Group Revealed Pronounced and Extensive Cloudy Swelling (CS) of the RT with Distinct Eosinophilic Hyaline Casts Within the Tubular Lumina. Marked Glomerular Atrophy (GA) and Degeneration are Evident, Along with Severe and Significant Vascular Congestion (Indicated by Yellow Arrows), As Well As a Precise Perivascular Distribution of Inflammatory Cells (IC). The Control Positive (CP) Group Demonstrates Marked Cellular Swelling and Vacuolar Degeneration (VD) Within the RT. Vascular Congestion is Evident Within the Interstitial and Glomerular Microcirculation (Yellow Arrows). Additionally, the Glomeruli (G) Display Mild Atrophic Changes. Low Dose (LD) Thymus Vulgaris Essential Oil (TVEO) Group Displayed Severe and Significant GA, Accompanied by Obvious Vascular Congestion Within the Interstitial Vasculature (Yellow Arrows) and the Glomerular Capillaries (G). The Section Also Reveals Significant VD (Black Arrows) Within the RT, Enclosing Some Acidophilic Amorphous Proteinaceous Substances. The High-Dose (HD) TVEO Group Showed a Moderate Grade of Vascular Congestion Within the Renal Interstitium and Renal Glomerular (RG) Microcirculation (Yellow Arrows), with the Presence of VD in the Renal Glomeruli. Additionally, the Lining of the RT Appeared Swollen and Vacuolated, Accompanied by the Presence of Eosinophilic Protein Casts in Their Lumina (Indicated by Black Arrows). H&E, Scale Bar of 4 mm

Table 9: Micromorphological Semi-Quantitative Assay of Kidney Sections

Study Group	Vacuolar Degeneration (Mean% %)	Cellular Swelling (Mean% %)	Vascular Congestion (Mean% %)	Lesion Scoring (0 -100%)	Lesion Grading
CN	7.25$^{A\#}$	8.79^{A}	9.57^{A}	0-10	No Lesion
CD	64.38^{D}	68.81^{D}	75.23^{D}	50-75	Severe
CP (Empagliflozin)	32.87^{C}	36.67^{C}	35.24^{C}	25-50	Moderate
TVEO (50 mg/kg)	42.61^{C}	46.25^{C}	38.82^{C}	25-50	Moderate
TVEO (100 mg/kg)	36.82^{C}	40.38^{C}	33.94^{C}	25-50	Moderate

The area of vascular congestion is estimated in (μm) of the distributed congested areas from dilated interstitial blood vessels. #Statistical comparison among groups: Mean values with different capital letters have significant differences (p<0.05), CN: Control negative, CP: Control positive, DC: Disease control, TVEO: Thymus vulgaris essential oil

Additionally, these groups showed a decrease in the area of vascular congestion within the renal interstitial tissue, contributing to an overall decline in lesion severity scores when than the DC group (Figure 2, panels DC, CP and HD). Although rats in the LD-TVEO treatment group also demonstrated appreciable amelioration in lesion severity relative to the DC group, the improvements were more substantial in the CP and HD groups. Despite these differences, all treatment groups presented lesion scores within the moderate severity range (Table 9), reflecting varying degrees of therapeutic efficacy. In contrast, the DC group revealed extensive tubular epithelial vacuolar degeneration, indicative of severe renal injury. Meanwhile, the CN group retained normal renal histoarchitecture, characterized by intact tubular and glomerular morphology, thereby serving as a benchmark for a healthy renal tissue template.

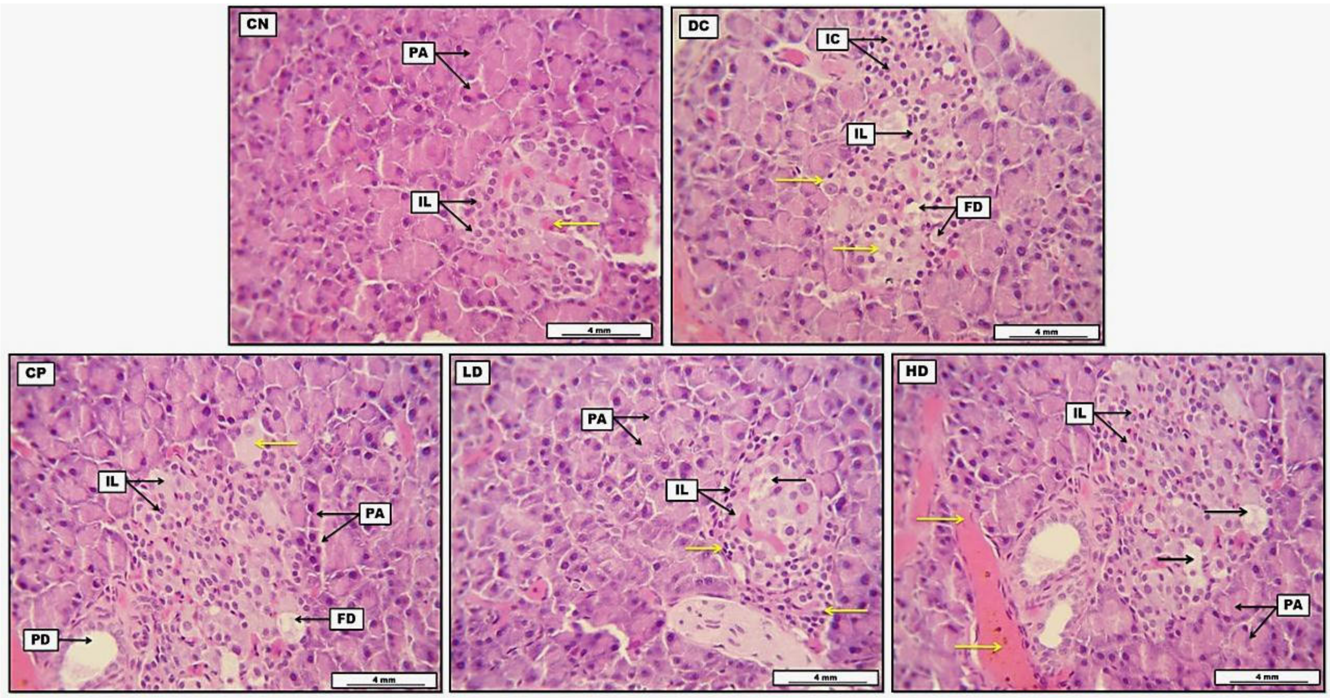

Figure 3: Photomicrograph of Pancrease from Control Negative (CN) Group Exhibits a Normal Morphological Appearance of the Pancreatic Acinar Structures (PA), With a Well-Preserved and Characteristic Organization of the Islets of Langerhans (IL), as Indicated by Their Typical Cellular Arrangement, With the Presence of Mild to Low Grade Vascular Engorgement Within the Islet's Microcirculations (Yellow Arrow). The Disease Control (DC) Group Reveals Significant Fatty Degeneration (FD), Which Diffusely Affects the IL Parenchymal Tissues. Moreover, Many Other Endocrine Cells Exhibit Significant Cellular Swelling, as Evidenced by Their Pale, Enlarged Cytoplasm (Yellow Arrows). Additionally, the Section Presents Considerable Infiltration of Inflammatory Cells (IC) Within the Mesenchymal Tissue and Around the IL. The Control Positive (CP) Group Shows a Moderate Degree of FD, Accompanied by Significant Cellular Swelling (Yellow Arrow) Within the Pancreatic Islets, As Well as Low-Grade Vascular Congestion Within the IL Microcirculation. Furthermore, the Section Also Displays the Presence of Dilated Pancreatic Duct (PD) With Some Deeply Stained Pancreatic Acini (PA). The Low Dose (LD) Thymus Vulgaris Essential Oil (TVEO) Group Demonstrates Significant to Severe Degenerative Changes in the Morphology of the IL, Revealing Significant Structural Atrophy Accompanied by Some Cellular Swelling (Black Arrow). Additionally, The Section Shows Moderate Infiltration of Inflammatory Cells, Particularly Around the IL (Yellow Arrow); Moreover, PA Exhibits a Pale Cytoplasmic Appearance. The High-Dose (HD) TVEO Group Demonstrates Notable Morphological Regeneration of the IL and the PA. Mild Fatty Degeneration is Observed Within the Glandular Epithelial Cells (Black Arrows) of IL. Additionally, Well-Defined Pancreatic Ducts are Visible, With the Occurrence of Prominent Longitudinally Sectioned Vascular Congestion (Yellow Arrows). H&E, Scale bars of 4 mm

Table 10: Micromorphological Semi-Quantitative Assay of Pancreas Sections

Study Group	Fatty Degeneration (Mean% %)	Cellular Swelling (Mean% %)	Inflammatory Cells (Mean% %)	Lesion Scoring (0 -100%)	Lesion Grading
CN	$3.52^{A\#}$	4.81^{A}	6.48^{A}	0-10	No Lesion
CD	67.24^{D}	71.09^{D}	74.53^{D}	50-75	Severe
CP (Empagliflozin)	34.57^{C}	38.64^{C}	31.96^{C}	25-50	Moderate
TVEO (50 mg/kg)	59.44^{D}	60.39^{D}	63.84^{D}	50-75	Severe
TVEO (100 mg/kg)	37.69^{C}	35.4^{C}	33.97^{C}	25-50	Moderate

#Statistical comparison among groups: Mean values with different capital letters have significant differences (p<0.05), CN: Control negative, CP: Control positive, DC: Disease control, TVEO: Thymus vulgaris essential oil

Pancreas

Table 10 presents the morphometric quantitative evaluation of pancreatic histopathology, with a focus on the islets of Langerhans. Rats in HD-TVEO and CP groups exhibited significant ($p<0.05$) reductions in the mean percentage of fatty degeneration and cellular swelling within the endocrine glandular structures of the islets. Furthermore, both groups demonstrated a marked attenuation of inflammatory cell infiltration within the interstitial pancreatic tissue than the DC group. These histopathological improvements corresponded to a shift toward moderate lesion severity grades (Figure 3, panels DC, CP and HD). In contrast, the LD-TVEO group showed a discernible, although less pronounced, numerical reduction in lesion severity relative to the DC group. However, the overall lesion grading in this group remained within the severe category, suggesting only partial histological recovery. For reference, pancreatic sections from the CN group retained their intact histological architecture, with normal cellular organization in both the exocrine and endocrine compartments of the parenchyma.

DISCUSSION

The progression of DM can cause significant tissue or vascular damage, resulting in serious complications. Treatments primarily aim to preserve life, alleviate symptoms, preventing long-term complications and enhancing longevity by managing risk factors [10]. Traditional herbal remedies have been used to help manage DM and its complications. There has been growing scientific interest in their potential to support gut health by influencing the microbiota [11]. Thus, this study aimed to investigate the effects of TVEO on gut microbiota, glycemic control, biochemical tests and haematological markers in STZ-induced DM rats.

In this study, STZ-induced DM typically results in significant BW loss in untreated DC rats due to increased muscle wasting and reduced tissue protein content caused by insulin deficiency. However, rats treated with TVEO, especially at 100 mg/kg, exhibited less BW loss than DC. This suggests a potential protective effect of TVEO against catabolic wasting. The mitigation of BW loss in the treated groups may be attributed to improvements in insulin sensitivity and energy metabolism. These results support earlier studies suggesting that plant-derived essential oils containing flavonoids and phenolic compounds may preserve lean mass by modulating glucose and lipid metabolism [12,13].

Also, in this study, untreated DM rats showed a significant elevation in FBG throughout the experimental period, which is in agreement with other studies reporting that STZ causes selective destruction of pancreatic β-cells, leading to diminished insulin secretion and sustained hyperglycemia [9]. In contrast, rats treated with TVEO, particularly at a dose of 100 mg/kg, exhibited a significant reduction in FBG on day 28 of treatment that was comparable to that of empagliflozin treated group. This sodium-glucose cotransporter-2 inhibitor is widely used in clinical settings for lowering BG by promoting renal glucose excretion [14]. The observed hypoglycemic effect of TVEO may be explained by several potential mechanisms, supported by emerging scientific literature. One of the significant effects contributing to reduced BG is the enhancement of peripheral glucose uptake in insulin-sensitive tissues, such as skeletal muscle and adipose tissue. Thymol, one of the principal bioactive compounds in TVEO, has been shown to increase the activity of key glycolytic enzymes, including hexokinase and phosphofructokinase, thereby facilitating glucose utilization at the cellular level [15]. Another critical mechanism is that thymol and carvacrol have been shown to downregulate the expression of gluconeogenic enzymes (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase), leading to decreased hepatic glucose output, that contributes directly to the reduction in FBG [16]. TVEO is also known for its antioxidant properties, as thymol and carvacrol exert protective effects on pancreatic tissue by neutralizing reactive oxygen species, thereby partially restoring β-cell and insulin secretory capacity [14].

In DM, liver enzyme elevation often indicates hepatocellular injury due to oxidative stress and fat accumulation. In this study, serum ALT and AST were significantly elevated in the DC group. However, both doses of TVEO, especially 100 mg/kg, significantly reduced ALT and AST levels, suggesting hepatoprotective effects and reduced hepatic injury, which is consistent with the previous study's outcome [14]. Similarly, Grespan *et al.* [17] investigated the hepatoprotective effect of TVEO in an acetaminophen-induced liver injury model in mice. Pretreatment with TVEO resulted in significant reductions in serum ALT and AST levels and mitigated histopathological liver damage that may be attributed to the antioxidant properties of TVEO. Furthermore, the HD-TVEO exhibited a significant increase in albumin and TP levels, implying improved liver synthetic function. These results may be due to the antioxidant and anti-inflammatory properties of TVEO.

Moreover, an unexpected observation was the elevated serum urea in the HD-TVEO group than CN and DC groups that could suggest mild renal stress or increased protein catabolism that might be due to few underlying physiological processes or bioactivity of phytochemicals in the TVEO group and elevated nitrogenous waste production without concurrent compromise of glomerular filtration function. The DC group showed decreased serum albumin and TP, signs of hepatic dysfunction. In contrast, the HD-TVEO group showed significant increases in both parameters, indicating improved liver synthetic capacity, which might be attributed to thymol's anti-inflammatory and antioxidant properties, which reduce hepatic stress and restore protein synthesis [18].

However, serum creatinine remained within normal range across all groups, with no significant differences. This pattern is consistent with another study, which reported that higher doses of thyme oil slightly increased urea levels but creatinine remained unchanged, indicating preserved kidney function and filtration capacity. Thus, they concluded that thyme oil at appropriate doses did not exhibit nephrotoxic effects [19]. However, prolonged exposure may challenge renal detoxification pathways, necessitating further safety profiling [20].

Furthermore, rats treated with TVEO, particularly at 100 mg/kg, showed significant reductions in TG, TC and LDL, while HDL improved than the DC group. These findings suggest that TVEO has a favourable modulation of lipid metabolism that may be attributed to improved insulin sensitivity, suppression of hepatic lipogenesis, enhanced lipoprotein lipase activity and reduced oxidative degradation of lipids. These results are supported by Moussa *et al.* [21], who demonstrated that TV seed extract improved lipid profiles and insulin sensitivity in obese rats by reducing hepatic oxidative stress and regulating key lipid metabolism pathways. Therefore, the improvements in lipid parameters in TVEO-treated groups reinforce the cardiovascular protective potential of TV in diabetic conditions.

Diabetes often leads to haematological alterations due to chronic hyperglycemia, oxidative stress and low-grade systemic inflammation [22-24]. In this study, treatment with TVEO resulted in improvements in absolute red cell distribution width (RDWa) count, particularly in the HD group. These changes suggest a protective and restorative effect of TVEO on the hematopoietic system. These findings align with those of Muhanna *et al.* [25], who reported that TV ameliorated haematological disturbances in rats induced by oxidative stress, restoring normal Hb and PLT counts. The Hb levels were significantly lower in the DC than the CP group, indicating that DM often leads to anaemia due to chronic hyperglycemia and oxidative stress, which impair erythropoiesis [22]. The restoration of some hematological values in the TVEO-treated groups suggests that its antioxidant properties may mitigate oxidative damage, thereby enhancing RBC production [25]. The RDWa was significantly elevated in the HD-TVEO group than the DC group, which may suggest improved iron metabolism and enhanced erythropoiesis [23]. PLT counts were significantly higher in the CP than in the DC group. Diabetes is frequently associated with impaired thrombopoiesis and altered PLT function [22]. MCV remained stable in the DC group but showed slight variations in the TVEO groups. This stability indicates that TVEO may help normalize RBC indices affected by DM-induced oxidative stress [22]. MCH levels were significantly higher in the PC than the DC group, suggesting improved Hb synthesis in response to effective glycemic control [23]. PDW showed substantial increases in the TVEO groups than the DC group, indicating enhanced PLT activity and potential benefits in preventing complications associated with DM [25].

Electrolyte imbalances are frequently observed in DM, primarily due to osmotic diuresis, insulin deficiency and altered renal handling of ions. Sodium and potassium disturbances are widespread and may contribute to cardiovascular and neuromuscular complications. In this study, TVEO resulted in non-significant alterations in sodium and potassium levels in the HD group. These shifts suggest a non-modulatory effect of TVEO on electrolyte balance that are not in agreement with Khan *et al.* [6], who reported potential sodium and potassium disturbances in diabetic patients, emphasizing the role of metabolic correction in restoring electrolyte homeostasis. CRP is a well-established biomarker of systemic inflammation, often elevated in DM due to chronic low-grade inflammatory responses. In this study, CRP was significantly elevated in the DC group, reflecting underlying systemic inflammation associated with hyperglycemia-induced oxidative stress. However, the HD-TVEO showed elevated CRP but still lower than that of DC, suggesting an anti-inflammatory benefit, albeit with a possible immune-stimulatory response at higher concentrations that potentially mediated by its thymol content, which can inhibit nuclear factor kappa B (NF-κB) signaling [26]. For instance, thymol, in combination with silibinin, inhibited the NF-κB and mitogen-activated protein kinase pathways in lipopolysaccharide-induced RAW264.7 cells, leading to a decrease in the production of nitric oxide, tumour necrosis factor-alpha and interleukin-6 [27].

There is recognition of the gut microbiota's role in metabolic regulation, insulin sensitivity and inflammation [28]. In this study, the TVEO inhibited the growth of pathogenic bacteria in the rats' guts (*E. coli*, *S. boydii*, *Proteus* species and *Klebsiella* species). In the same manner, TVEO has positively modulated gut microbial composition by increasing beneficial bacterial populations (*Lactobacillus* and *Bifidobacterium*) and suppressing pathogenic organisms (*Clostridium* species and *E. coli*) [5,29-31]. Through these microbial shifts, TVEO may reduce systemic endotoxemia, enhance gut barrier function and modulate incretin hormone secretion; factors that collectively contribute to improved glucose homeostasis [32].

Furthermore, this studys' histopathological analysis showed that both doses of TVEO reduced hepatic, renal and pancreatic damage caused by hyperglycemia than DC. Despite this improvement, the lesion scores in these groups remained within the severe grade category, indicating partial but not complete recovery of the liver. Similarly, another study found that TVEO reduced degeneration of interleukin and improved the liver and kidney tissues of treated rats, indicating reduced hepatocellular and renal damage [14,33,34].

The limitations of the study was that gut microbiota modulation and systemic metabolic improvement were not sufficiently supported by functional analyses. Also, the microbiota assessment was based on culture-dependent methods and identification of a small number of bacterial species did not adequately capture gut microbial diversity or functional changes.

CONCLUSIONS

The TVEO, particularly at the high dose, improved BG levels, lipid profiles and liver enzymes. It also exerted beneficial anti-inflammatory effects, evidenced by partial reductions in CRP levels. Haematological improvements, such as elevated Hb and PLT counts, suggest hematopoietic restoration. TVEO favorably modulated gut microbiota, increasing microbial diversity and improving metabolic outcomes. While HD-TVEO slightly increased serum urea, creatinine remained normal, indicating preserved renal function. These findings support the use of TVEO as a multi-targeted supplement in DM management.

Acknowledgement

The authors would like to appreciate the authorities of the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq, for their support and assistance with this study.

Ethical Consideration

The study protocol received approval from the Scientific and Ethical Committees of the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq (No. 27, on October 01, 2024). All procedures adhered to the ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guidelines to minimize pain and distress to the studied animals.

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