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The protective effect of black pepper (*Piper nigrum*) on liver enzymes in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes mellitus (DM) is a global metabolic disorder that leads to various complications, including liver dysfunction. Streptozotocin (STZ)-induced diabetes in rats serves as a model to study diabetes-related liver injury, often evidenced by elevated liver enzyme levels. This study aimed to investigate the hepatoprotective effects of black pepper (*Piper nigrum*) aqueous extract on liver enzymes in STZ-induced diabetic rats. A controlled laboratory-based experimental study was conducted using eighteen male Wistar rats, divided into three groups: control (healthy), diabetic (induced by STZ), and black pepper-treated diabetic (50 mg/kg body weight for 28 days). Diabetes was induced with a single STZ injection (55 mg/kg). Liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), were assessed, and histological analysis was performed on liver tissues. the results showed significant elevations in liver enzymes were observed in the diabetic group compared to the control. Black pepper treatment significantly reduced ALT, AST, ALP, and GGT levels, with results showing normalization of liver function markers. Histological analysis revealed improved hepatic architecture in the treated group, with reduced signs of cellular stress and damage. Black pepper extract demonstrated significant hepatoprotective effects in STZ-induced diabetic rats, potentially due to its antioxidant and anti-inflammatory properties. These findings support the potential of black pepper as a complementary therapy for managing diabetes-induced liver dysfunction.

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INTRODUCTION

Diabetes mellitus (DM) is an increasingly important global health issue, currently affecting over 285 million people around the world. This number is projected to rise to 435 million by 2030 (Zhautikova *et al.*, 2022). DM is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from impaired insulin secretion, action, or both (Antar *et al.*, 2023; Lu *et al.*, 2024; Popoviciu *et al.*, 2023). In addition to its well-known effects on carbohydrate metabolism, diabetes has a negative impact on several organs, including the liver, which plays a crucial role in regulating glucose levels and detoxifying the body.

Hyperglycemia related to diabetes has been strongly associated with liver dysfunction. This connection involves mechanisms such as oxidative stress, inflammation, and disturbances in lipid metabolism (Ghosh *et al.*, 2015; Mohamed *et al.*, 2016). Disruptions in hepatic glucose production are a key factor in the pathophysiology of type 2 diabetes mellitus (T2DM) (Mori *et al.*, 2022; Solis-Herrera *et al.*, 2021). Elevated levels of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), are well-documented indicators of liver injury in diabetic patients (Ashoobi *et al.*, 2024; Thakur *et al.*, 2024). Diabetes mellitus is often accompanied by dyslipidemia, including elevated cholesterol and triglyceride levels, which further burden liver function and contribute to hepatic steatosis and inflammation. Since the liver is responsible for producing about 80% of the body's cholesterol, disturbances in lipid metabolism are closely linked to liver enzyme activity and diabetic complications (Patandung *et al.*, 2024; Valenti *et al.*, 2016). Therefore, developing therapeutic strategies to protect liver function is essential for managing diabetes and its complications.

In recent years, herbal medicine has received considerable attention for its potential role in managing diabetes and related liver conditions. Medicinal plants are often favored due to their availability, lower toxicity, and rich profiles of bioactive compounds (Hassan and Fadel, 2023; Salehi *et al.*, 2019). One notable example is black pepper (*Piper nigrum*), a common culinary spice that has demonstrated promising antidiabetic and liver-protective properties. The bioactive alkaloid piperine is primarily responsible for black pepper's pharmacological effects, including enhancement of insulin sensitivity, reduction of oxidative stress, and inhibition of hepatic inflammation (Balakrishnan *et al.*, 2023; Tiwari *et al.*, 2020).

Several preclinical studies have demonstrated that black pepper can modulate blood glucose levels and improve liver enzyme profiles in diabetic models (Dludla *et al.*, 2023; Mugundhan *et al.*, 2024). Therefore, the present study was designed to evaluate the hepatoprotective effects of black pepper aqueous extract in streptozotocin (STZ)-induced diabetic rats, using both biochemical markers (ALT, AST, ALP, and GGT) and histological analysis to assess structural hepatic integrity. This dual-approach investigation aims to provide comprehensive insight into the therapeutic potential of black pepper as a natural adjunct in managing diabetes-induced liver injury.

MATERIALS AND METHODS

Study design

A controlled laboratory-based experimental study was conducted at the King Fahad Medical Research Center (KFMRC), King Abdulaziz University (KAU), Jeddah, Saudi Arabia. The study utilized a rodent model to investigate the potential hepatoprotective effects of black pepper (*Piper nigrum*) aqueous extract in streptozotocin (STZ)-induced diabetic rats. Following acclimatization, animals were randomly allocated to experimental groups. All procedures were performed under established institutional laboratory protocols and ethical guidelines.

Animals and experimental groups

Eighteen adult male Wistar rats (150–200 g) were used and housed under standard laboratory conditions (22 ± 2°C, 60% humidity, 12-hour light/dark cycle), with free access to food and water.

After a 7-day acclimatization period, rats were randomly assigned to three groups (n = 6 per group):

1. Group 1 (Control): Healthy rats receiving no treatment.
2. Group 2 (Diabetic): Rats induced with diabetes using streptozotocin (STZ).
3. Group 3 (Black pepper treated): Diabetic rats treated with black pepper aqueous extract (50 mg/kg body weight) orally for 28 days.

Diabetes induction

Streptozotocin (STZ) [S0130-1G, N-(Methylnitrosocarbamoyl)-α-D-glucosamine] was purchased from Sigma-Aldrich (St. Louis, MO, USA) through Alrowad Medical Supplies, a local distributor based in Jeddah, Saudi Arabia. The compound was freshly dissolved in 0.1 M citrate buffer (pH 4.5) immediately before use.

Diabetes was induced in Groups 2 and 3 by a single intraperitoneal injection of STZ at a dose of 55 mg/kg body weight. After 7 days, fasting blood glucose levels were measured using a glucometer, and rats with blood glucose levels exceeding 200 mg/dL were considered diabetic, as previously described (Clim *et al.*, 2024) (Fig. 1).

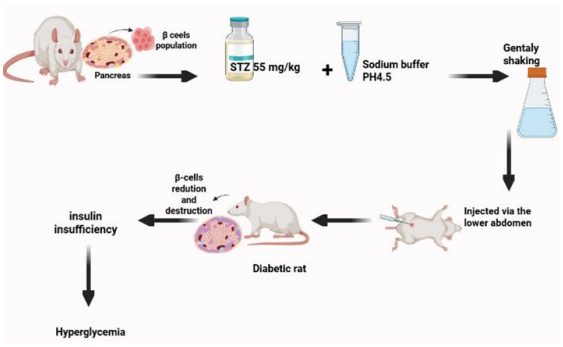

Fig. 1. Scheme showing damage and reduction in β cells by STZ (Done by researcher).

Black pepper extract preparation

Black pepper seeds were purchased from iHerb(USA), washed, dried, and ground. An aqueous extract was prepared by soaking 100 g of powder in 1,000 mL of distilled water for 24 hours, followed by filtration. The extract was administered daily to Group 3 via oral gavage.

Sample collection and liver enzyme analysis

At the end of the experimental period, rats were fasted overnight and anesthetized using isoflurane. Blood was collected via retro-orbital puncture, centrifuged at 3500 rpm for 15 minutes at 4°C, and serum was stored at –80°C.

According to the manufacturer's instructions, liver function markers, including ALT, AST, ALP, and GGT, were analyzed using ELISA kits (Spectrum Diagnostics, Egypt) (Fig. 2).

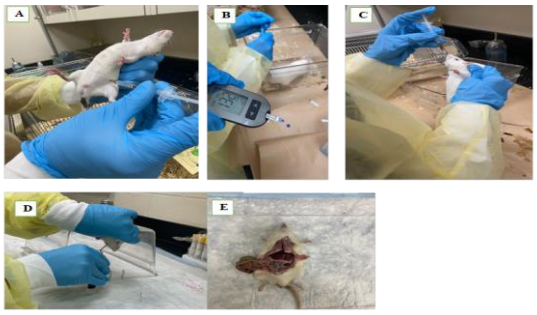

Fig. 2. Diabetes induction, dose administration, and sample collection

Histological examination

Following blood collection, animals were euthanized, and liver tissue specimens were dissected. Organs were immediately fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned for histopathological analysis. Sections (5 µm thick) were stained with hematoxylin and eosin (HandE) and examined using a light microscope at the Histology Unit, Faculty of Medicine, King Abdulaziz University. Histological evaluations were conducted to assess structural alterations and potential protective effects of black pepper extract on liver tissue.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Comparisons were made using independent samples t-tests between:

1. Group 1 vs. Group 2: to evaluate liver damage in diabetic rats.
2. Group 2 vs. Group 3: to assess the protective effect of black pepper.

Before ANOVA, Levene's test for homogeneity of variances was performed. Based on the outcome:

1. Welch's ANOVA was applied when variances were unequal.
2. Standard one-way ANOVA was used when the assumption was met.

Effect sizes were calculated using Eta-squared and Cohen's d to determine the practical significance of group differences.

Statistical significance was set at $p \leq 0.05$. Analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA).

Ethics approval

All animal procedures were conducted following institutional guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia (Ref No. ACUC-24-09-07, dated September 4, 2024). Efforts were made to minimize animal suffering, and all interventions were carried out under professional supervision.

RESULTS

Inferential statistics

Inferential analysis revealed statistically significant differences among the three groups across all measured liver enzyme parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Descriptive statistics for each group are summarized in Table 1.

Levene's test showed that the assumption of equal variances was violated for ALT ($p = 0.025$) and GGT ($p = 0.009$); thus, Welch's ANOVA was applied for these enzymes. For AST and ALP, the homogeneity assumption was met ($p > 0.05$), and one-way ANOVA was used. As shown in Table 2, the differences between groups were statistically significant for all enzymes ($p < 0.001$).

Table 1. Descriptive statistics of liver enzyme levels (Mean ± SD, n = 6)

Enzyme	Group	Mean ± SD	Min – Max	95% CI
ALT	Control	13.67 ± 1.35	11.9–15.6	12.25 – 15.08
	Diabetic	17.75 ± 4.52	12.5–24.4	13.01 – 22.49
	Treated	65.52 ± 8.43	53.0–77.0	56.67 – 74.36
AST	Control	17.20 ± 2.33	14.6–21.0	14.75 – 19.65
	Diabetic	24.82 ± 8.31	16.0–38.2	16.09 – 33.54
	Treated	85.60 ± 8.75	70.0–97.0	76.42 – 94.78
ALP	Control	44.67 ± 5.13	40–54	39.29 – 50.05
	Diabetic	43.93 ± 3.91	40–50	39.83 – 48.04
	Treated	139.00 ± 9.12	124–147	129.43 – 148.57
GGT	Control	14.90 ± 1.86	12.5–17.4	12.94 – 16.86
	Diabetic	16.20 ± 1.15	14.5–17.1	15.00 – 17.40
	Treated	46.53 ± 6.70	35.9–54.0	39.50 – 53.56

Table 2. ANOVA and Levene's test results with effect sizes

Enzyme	Levene's p	ANOVA method	F value	*p*-value	Eta² (Effect size)
ALT	0.025	Welch ANOVA	160.45	<0.001	0.955
AST	0.193	One-way ANOVA	167.44	<0.001	0.957
ALP	0.085	One-way ANOVA	431.27	<0.001	0.983
GGT	0.009	Welch ANOVA	116.11	<0.001	0.939

The effect sizes (Eta-squared) ranged from 0.939 to 0.983, indicating a large practical significance of group differences. These results suggest that streptozotocin-induced diabetes significantly altered liver enzyme levels and that the black pepper extract treatment partially ameliorated these effects in the treated group.

Comparison between the healthy control and diabetic group

Independent samples t-tests were conducted to compare liver enzyme levels between the healthy control group and the diabetic group. The results revealed a statistically significant increase in all

measured enzymes in the diabetic group, indicating notable hepatic dysfunction (Table 3).

All enzymes were significantly elevated in the diabetic group ($p < 0.001$), with very large effect sizes (Cohen's $d > 4$), indicating substantial liver injury (Fig. 3).

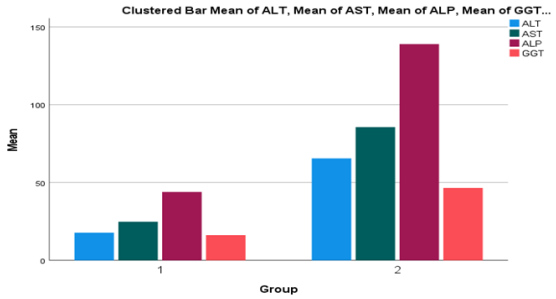

Fig. 3. Comparison of liver enzyme levels between the control and diabetic groups. All enzymes show significant elevations in the diabetic group ($p < 0.001$).

These findings confirm that diabetes induces substantial liver enzyme elevations, reflective of hepatocellular injury and dysfunction.

Comparison between the diabetic group and black pepper-treated group

Independent samples t-tests revealed significant reductions in liver enzyme levels in the black pepper-treated group compared to the untreated diabetic group. The treatment with black pepper was associated with substantial normalization of liver function markers.

The black pepper-treated group showed significantly lower liver enzyme levels compared to the diabetic group ($p < 0.001$), with large effect sizes (Cohen's d > 4), indicating a strong normalization of liver biomarkers following treatment (Table 4, Fig. 4).

Table 3. Comparison of liver enzyme levels between healthy controls and diabetic groups

Enzyme	Group 1 (Healthy control) Mean ± SD	Group 2 (Diabetic) Mean ± SD	t (df)	*p*-value	Cohen's d
ALT	17.75 ± 4.52	65.52 ± 8.43	-12.24 (10)	< 0.001	6.76
AST	24.82 ± 8.31	85.60 ± 8.75	-12.34 (10)	< 0.001	8.53
ALP	43.93 ± 3.91	139.00 ± 9.12	-23.46 (10)	< 0.001	7.02
GGT	16.20 ± 1.15	46.53 ± 6.70	-10.93 (10)	< 0.001	4.81

Table 4. Comparison of liver enzyme levels between diabetic and black pepper-treated groups

Enzyme	Group 2 (Diabetic) Mean ± SD	Group 3 (Black pepper treated) Mean ± SD	t (df)	*p*-value	Cohen's d
ALT	65.52 ± 8.43	13.67 ± 1.35	14.88 (10)	< 0.001	6.03
AST	85.60 ± 8.75	17.20 ± 2.33	18.51 (10)	< 0.001	6.40
ALP	139.00 ± 9.12	44.67 ± 5.13	22.09 (10)	< 0.001	7.40
GGT	46.53 ± 6.70	14.90 ± 1.86	11.14 (10)	< 0.001	4.92

Histological findings

Microscopic examination of liver sections stained with hematoxylin and eosin (HandE) provided supportive evidence for the biochemical findings (Fig. 5).

Group 1 (Control): Liver sections displayed normal hepatic architecture with well-organized hepatocyte cords radiating from a central vein. Sinusoids appeared open and regular, and hepatocytes exhibited normal polygonal shapes and vesicular nuclei without signs of cytoplasmic vacuolation or inflammation.

Group 2 (Diabetic Control): Moderate distortion of lobular structure was observed. Hepatocyte cords were disorganized, and sinusoidal dilatation was evident. Hepatocytes near the central vein exhibited mild cytoplasmic vacuolation and occasional nuclear condensation, indicative of early cellular stress and hepatocellular injury.

Group 3 (Black pepper treated): Liver histology revealed marked improvement in structural integrity compared to the diabetic group. Hepatocyte cords were better organized, sinusoidal spaces were more regular, and cytoplasmic vacuolation was minimal. The architecture was largely preserved, suggesting a protective effect of black pepper against STZ-induced hepatic damage.

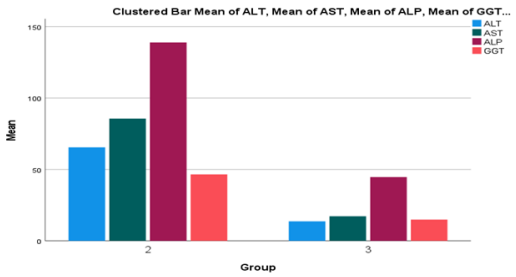

Fig. 4. Comparison of liver enzyme levels between diabetic and treated groups. Significant reductions were observed in all enzymes after treatment with black pepper ($p < 0.001$)

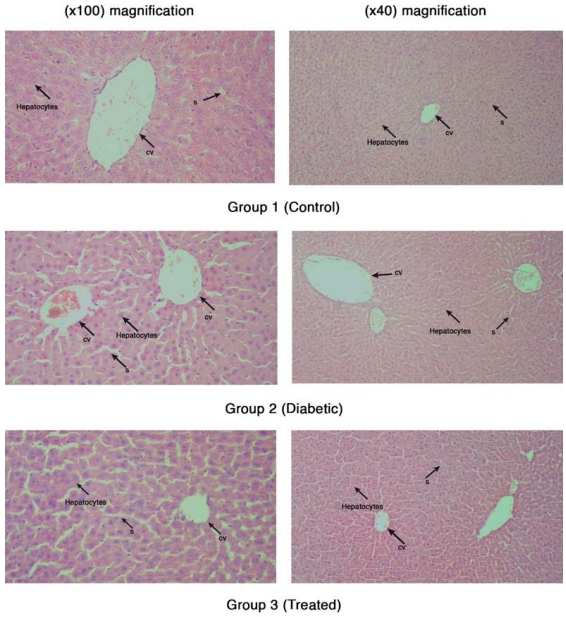

Fig. 5. Histological examination of liver tissue (H&E staining, ×40 and ×100 magnifications)

These histological findings reinforce the biochemical results, demonstrating that black pepper extract exerts a notable hepatoprotective effect in STZ-induced diabetic rats by restoring liver tissue architecture and reducing histological markers of damage.

DISCUSSION

This study investigated the hepatoprotective effects of black pepper (*Piper nigrum*) aqueous extract in streptozotocin (STZ)-induced diabetic rats, focusing on both biochemical liver enzyme markers (ALT, AST, ALP, and GGT) and histopathological changes.

The results demonstrated that diabetes significantly elevated the serum levels of all tested liver enzymes compared to healthy controls. Specifically, diabetic rats exhibited marked increases in ALT, AST, ALP, and GGT, well-known biomarkers of hepatic injury. These findings are consistent with prior research indicating that STZ-induced hyperglycemia leads to hepatocellular damage and altered membrane permeability, resulting in enzyme leakage into the bloodstream (Ghosh *et al.*, 2015; Lateef *et al.*, 2024; Samy *et al.*, 2023).

ALT and AST are key indicators of hepatic parenchymal injury, while ALP and GGT are primarily associated with cholestasis or biliary obstruction (Lee *et al.*, 2023; Thakur *et al.*, 2024). The observed elevation in these enzymes among diabetic rats suggests not only cellular damage in hepatocytes but also potential biliary dysfunction. Previous studies have confirmed the role of oxidative stress and inflammation in diabetes-induced liver injury (Mohamed *et al.*, 2016; Weinberg Sibony *et al.*, 2024), which supports the findings of elevated liver enzyme activity in the diabetic group.

Histological findings complemented the biochemical results. Diabetic rats exhibited disorganized hepatic cords, sinusoidal dilatation, and signs of cellular stress, including cytoplasmic vacuolation and nuclear condensation. These structural changes reflect early stages of diabetic liver injury and are in line with reported histopathological features of STZ-induced hepatotoxicity (Abuduyimiti *et al.*, 2024; Mahfoz and Gawish, 2022).

Importantly, oral administration of black pepper extract for 28 days significantly attenuated these pathological elevations. Rats treated with black pepper showed enzyme levels that were statistically and biologically closer to those of the healthy control group, indicating a restorative and protective effect. These effects may be attributed to the antioxidant and anti-inflammatory properties of piperine, the primary active alkaloid in black pepper, which has been shown to modulate oxidative stress, enhance antioxidant defense systems, and reduce hepatic inflammation (Balakrishnan *et al.*, 2023; Kumar *et al.*, 2015; Tiwari *et al.*, 2020).

The significant reductions in ALT and AST suggest that black pepper exerts a protective effect on hepatocytes, while the normalization of ALP and GGT further indicates improved biliary function. These results are supported by studies showing that piperine enhances hepatic glutathione levels, inhibits lipid peroxidation, and restores enzyme activity in diabetic models (Arcaro *et al.*, 2014; Choi *et al.*, 2013).

Furthermore, the large effect sizes observed in the statistical analysis (Cohen's d > 4 for all comparisons) provide strong evidence of the clinical relevance and robustness of the findings. Notably, the use of both Welch's ANOVA and t-tests with Levene's correction ensured statistical rigor despite heterogeneity of variances in some parameters.

Moreover, histological examination confirmed the protective role of black pepper. In treated rats, liver architecture was preserved, with improved hepatocyte morphology and reduced cytoplasmic vacuolation compared to untreated diabetic animals. Sinusoidal structure and central vein morphology were also more regular. This structural normalization supports the biochemical evidence of hepatic protection and highlights black pepper's role in enzyme modulation and in maintaining tissue integrity.

Several previous studies have similarly reported improvements in liver function markers following administration of piperine or black pepper extract in diabetic or high-fat diet models (Dludla *et al.*, 2023; Obia and Emmanuel, 2025; Zuvairiya *et al.*, 2024). However, the current study advances this knowledge by demonstrating both quantitative enzyme normalization and qualitative histological recovery, supported by robust statistical analysis. The alignment of biochemical and histological findings offers powerful corroboration, enhancing the overall validity of the results.

CONCLUSION

The aqueous extract of black pepper significantly reduced elevated liver enzyme levels in STZ-induced diabetic rats, indicating a potent hepatoprotective effect. This protective benefit is likely attributed to the antioxidant and anti-inflammatory properties of piperine, the active compound in black pepper. Furthermore, histological findings corroborate the biochemical data, showing the restoration of liver architecture and a reduction in signs of hepatocellular damage in treated animals. These findings provide robust evidence supporting the potential use of black pepper as a complementary therapy for liver complications associated with diabetes, highlighting its therapeutic promise for improving liver function and mitigating diabetic-induced hepatic damage.

RECOMMENDATION(S)

Future research should expand on this study by exploring different doses and durations of black pepper extract to identify optimal therapeutic levels and duration, while isolating and characterizing its active compounds, particularly piperine and related phytochemicals, to better understand their specific mechanisms. Molecular investigations assessing oxidative stress and inflammatory markers would help clarify the biochemical pathways involved, and histochemical or ultrastructural analyses could reveal deeper cellular restoration effects. Comparative studies with standard hepatoprotective or antidiabetic drugs, along with toxicological and pharmacokinetic evaluations, are essential to confirm safety and efficacy. Translational research, including clinical trials, should be pursued to validate these findings in humans. Additionally, examining synergistic combinations with other natural antioxidants and assessing effects on other diabetes-affected organs could broaden therapeutic potential and establish black pepper as a promising complementary treatment for diabetic liver dysfunction.

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REFERENCES

Abuduyimiti T, Goto H, Kimura K, Oshima Y, Tanida R, Kamoshita K, Leerach N, Abuduwaili H, Oo HK, Li Q, Galicia-Medina CM, Takayama H, Ishii K, Nakano Y, Takeshita Y, Iba T, Naito H, Honda M, Harada K, Takamura T. 2024. Diabetes accelerates steatohepatitis in mice: Liver pathology and single-cell gene expression signatures. The American Journal of Pathology **194**(5), 693–707. https://doi.org/10.1016/j.ajpath.2024.01.007

Antar SA, Ashour NA, Sharaky M, Khattab M, Ashour NA, Zaid RT, Roh EJ, Elkamhawy A, Al-Karmalawy AA. 2023. Diabetes mellitus: Classification, mediators, and complications; a gate to identify potential targets for the development of new effective treatments. Biomedicine & Pharmacotherapy **168**, 115734. https://doi.org/10.1016/j.biopha.2023.115734

Arcaro CA, Gutierres VO, Assis RP, Moreira TF, Costa PI, Baviera AM, Brunetti IL. 2014. Piperine, a natural bioenhancer, nullifies the antidiabetic and antioxidant activities of curcumin in streptozotocin-diabetic rats. PloS One **9**(12), e113993. https://doi.org/10.1371/journal.pone.0113993

Ashoobi MT, Joukar F, Mojtahedi K, Maroufizadeh S, Javid M, Parvaneh A, Zeinali T, Faraji N, Naghipour M, Mansour-Ghanaei F. 2024. Elevated liver enzymes and diabetes in the PERSIAN Guilan cohort study. Caspian Journal of Internal Medicine **16**(1), 73–82. https://doi.org/10.22088/cjim.16.1.73

Balakrishnan R, Azam S, Kim IS, Choi DK. 2023. Neuroprotective effects of black pepper and its bioactive compounds in age-related neurological disorders. Aging and Disease **14**(3), 750–777. https://doi.org/10.14336/AD.2022.1022

Choi S, Choi Y, Choi Y, Kim S, Jang J, Park T. 2013. Piperine reverses high fat diet-induced hepatic steatosis and insulin resistance in mice. Food Chemistry **141**(4), 3627–3635. https://doi.org/10.1016/j.foodchem.2013.06.028

Clim A, Maranducă MA, Pinzariu AC, Faur IF, Huzum B, Hăncianu M, Szilagyi A, Tamba BI, Șerban DN, Șerban IL. 2024. Dosage of streptozotocin for inducing models of diabetes mellitus in obesity rats with endothelial dysfunction. Farmacia **72**(2), 448–455. https://doi.org/10.31925/farmacia.2024.2.22

Dludla PV, Cirilli I, Marcheggiani F, Silvestri S, Orlando P, Muvhulawa N, Moetlediwa MT, Nkambule BB, Mazibuko-Mbeje SE, Hlengwa N, Hanser S, Ndwandwe D, Marnewick JL, Basson AK, Tiano L. 2023. Bioactive properties, bioavailability profiles, and clinical evidence of the potential benefits of black pepper (*Piper nigrum*) and red pepper (*Capsicum annum*) against diverse metabolic complications. Molecules **28**(18), 6569. https://doi.org/10.3390/molecules28186569

Ghosh S, Bhattacharyya S, Rashid K, Sil PC. 2015. Curcumin protects rat liver from streptozotocin-induced diabetic pathophysiology by counteracting reactive oxygen species and inhibiting the activation of p53 and MAPKs mediated stress response pathways. Toxicology Reports **2**, 365–376. https://doi.org/10.1016/j.toxrep.2014.12.017

Hassan HY, Fadel HK. 2023. Comparison between the efficacy of *Nigella sativa* aqueous extract and its oil on methimazole-induced hypothyroidism in albino mice. Jordan Journal of Pharmaceutical Sciences **16**(1), 103–111. https://doi.org/10.35516/jjps.v16i1.1072

Kumar S, Malhotra S, Prasad AK, Van der Eycken EV, Bracke ME, Stetler-Stevenson WG, Parmar VS, Ghosh B. 2015. Anti-inflammatory and antioxidant properties of Piper species: a perspective from screening to molecular mechanisms. Current Topics in Medicinal Chemistry **15**(9), 886–893. https://doi.org/10.2174/1568026615666150220120651

Lateef MAM, Abdulhadi HL, Ali LH. 2024. Study of the therapeutic efficacy of pumpkin seed in improving the liver's activity in rats with STZ-induced diabetes. Frontiers in Health Informatics **13**(3), 5336–5351.

Lee AS, Persoff J, Lange SM. 2023. Liver function tests. Mayo Clinic Medical Manual, 373–387. https://doi.org/10.1201/b14283-48

Lu X, Xie Q, Pan X, Zhang R, Zhang X, Peng G, Zhang Y, Shen S, Tong N. 2024. Type 2 diabetes mellitus in adults: pathogenesis, prevention and therapy. Signal Transduction and Targeted Therapy **9**(1), 262. https://doi.org/10.1038/s41392-024-01951-9

Mahfoz AM, Gawish AY. 2022. Insight into the hepatoprotective, hypolipidemic, and antidiabetic impacts of aliskiren in streptozotocin-induced diabetic liver disease in mice. Diabetology & Metabolic Syndrome **14**(1), 163. https://doi.org/10.1186/s13098-022-00935-5

Mohamed J, Nazratun Nafizah AH, Zariyantey AH, Budin SB. 2016. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. Sultan Qaboos University Medical Journal **16**(2), e132–e141. https://doi.org/10.18295/squmj.2016.16.02.002

Mori Y, Duru OK, Tuttle KR, Fukuma S, Taura D, Harada N, Inagaki N, Inoue K. 2022. Sodium-glucose cotransporter 2 inhibitors and new-onset type 2 diabetes in adults with prediabetes: systematic review and meta-analysis of randomized controlled trials. The Journal of Clinical Endocrinology and Metabolism **108**(1), 221–231. https://doi.org/10.1210/clinem/dgac591

Mugundhan V, Priya V, Jayaraman S, Gayathri R, Kavitha S. 2024. Effect of piperine on IR/IRS-1/AKT signaling molecules in high-fat diet and sucrose-fed type 2 diabetic rat. Texila International Journal of Public Health **2024**(Special Issue-1), 136–145. https://doi.org/10.21522/TIJPH.2013.SE.24.01.Art018

Obia O, Emmanuel F. 2025. Effect of oral administration of common pepper types on the liver enzymes of Wistar rats fed with high-fat diet. East African Scholars Journal of Medical Sciences **8**, 92–95. https://doi.org/10.36349/easms.2025.v08i03.001

Patandung G, Wahyudin E, Yustisia I, Kabo P, Djabir YY, Sulfahri, Zulfiah. 2024. Potential of black pepper extract (*Piper nigrum* L) as antidiabetic and anticholesterol in alloxan and propylthiouracil (PTU) induced Wistar rats. Pakistan Journal of Life and Social Sciences **22**(2), 20289–20301. https://doi.org/10.57239/pjlss-2024-22.2.001487

Popoviciu MS, Paduraru L, Nutas RM, Ujoc AM, Yahya G, Metwally K, Cavalu S. 2023. Diabetes mellitus secondary to endocrine diseases: an update of diagnostic and treatment particularities. International Journal of Molecular Sciences **24**(16), 12676. https://doi.org/10.3390/ijms241612676

Salehi B, Ata A, Anil Kumar NV, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, Abdulmajid Ayatollahi S, Tsouh Fokou PV, Kobarfard F, Amiruddin Zakaria Z, Iriti M, Taheri Y, Martorell M, Sureda A, Setzer WN, Durazzo A, Lucarini M, Santini A, Capasso R, Sharifi-Rad J. 2019. Antidiabetic potential of medicinal plants and their active components. Biomolecules **9**(10), 551. https://doi.org/10.3390/biom9100551

Samy JVRA, Kumar N, Singaravel S, Krishnamoorthy R, Alshuniaber MA, Gatasheh MK, Venkatesan A, Natesan V, Kim SJ. 2023. Effect of prunetin on streptozotocin-induced diabetic nephropathy in rats: a biochemical and molecular approach. Biomolecules & Therapeutics **31**(6), 619–628. https://doi.org/10.4062/biomolther.2023.068

Solis-Herrera C, Triplitt C, Cersosimo E, DeFronzo RA. 2021. Pathogenesis of type 2 diabetes mellitus.

Thakur S, Kumar V, Das R, Sharma V, Mehta DK. 2024. Biomarkers of hepatic toxicity: an overview. Current Therapeutic Research **100**, 100737. https://doi.org/10.1016/j.curtheres.2024.100737

Tiwari A, Mahadik KR, Gabhe SY. 2020. Piperine: a comprehensive review of methods of isolation, purification, and biological properties. Medicine in Drug Discovery **7**, 100027. https://doi.org/10.1016/j.medidd.2020.100027

Valenti L, Bugianesi E, Pajvani U, Targher G. 2016. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? Liver International **36**(11), 1563–1579. https://doi.org/10.1111/liv.13185

Weinberg Sibony R, Segev O, Dor S, Raz I. 2024. Overview of oxidative stress and inflammation in diabetes. Journal of Diabetes **16**(10), e70014. https://doi.org/10.1111/1753-0407.70014

Zhautikova S, Khamida A, Zhienbayeva K, Suleimenova B, Talaspekova Y, Karipova A, Baryshnikova I, Zhalmakhanov M, Piven L, Medvedeva I, Sergey Z, Nazgul O. 2022. Pathogenetic mechanisms of relationship of metabolic and morphofunctional disorders of thyroid and adrenal glands in diabetes mellitus and obesity. Open Access Macedonian Journal of Medical Sciences **10**(B), 232–239. https://doi.org/10.3889/oamjms.2022.8151

Zuvairiya U, Jayaraman S, Sankaran K, Veeraraghavan VP, R G. 2024. Studies on the effect of piperine on hepatocyte nuclear factor 1 alpha (HNF-1α) and sterol regulatory element-binding protein 1c (SREBP-1c) levels in high-fat-diet and sucrose-induced type 2 diabetes mellitus rats. Cureus **16**(2), e54061. https://doi.org/10.7759/cureus.54061