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The antidiabetic effects of hesperidin and piperine in streptozotocin-induced diabetic rats

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Abstract

Objective: Diabetes mellitus is a metabolic and chronic condition defined by increased blood glucose concentrations. The present research aimed to examine and compare the antidiabetic activity of hesperidin and piperine, alone and in combination, in rats with diabetes.

Materials and Methods: In this research, streptozotocin (STZ, 60 mg/kg, intraperitoneally) was employed to cause type 2 diabetes in male rats. Following the induction of diabetes, the positive control group was treated with metformin (500 mg/kg), the normal control group was administered with normal saline, and the remaining groups received oral both compounds hesperidin and piperine (0.25 and 0.5 mg/kg), alone and in combination, for 4 weeks. Body weight alterations and several biochemical markers were evaluated.

Results: The administration of metformin and two doses of hesperidin and piperine (0.25 and 0.5 mg/kg) significantly enhanced the body weight and reduced blood sugar, triglyceride (TG), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels compared to the diabetic control animals. Moreover, treatment with metformin and different doses of hesperidin and piperine decreased the serum levels of liver and kidney markers (hepatic enzymes) and diminished the adverse impacts on their activities.

Conclusion: The findings of the current research indicate the potential of hesperidin and piperine for the management of diabetes and its associated health issues.

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Introduction

Diabetes mellitus is a metabolic and chronic condition affecting 171 million individuals globally, and by 2030, this number is expected to increase to 366 million (Li et al. 2018; Sundaram et al.

2019). This disease has three main types, including type 1, type 2, and gestational diabetes. In type 1 diabetes, the degradation of pancreatic β cells leads to loss of insulin production in the body, which can result from an autoimmune disease. The treatment of patients with type 1 diabetes, which is also referred to as insulin-dependent diabetes, requires insulin administration, the use of automated insulin delivery systems, or insulin pump therapy (Eisenbarth 1986; Kaul et al. 2013). On the other hand, in type 2 diabetes, body cells fail to react appropriately to insulin, preventing it from effectively reducing blood sugar levels and resulting in persistent hyperglycemia due to inadequate insulin production or function (Whicher et al. 2020). This condition damages pancreatic β cells, leading to a complete halt in insulin production and it may cause various complications, such as cardiovascular diseases, as well as microvascular conditions including retinopathy (which can lead to blindness), nephropathy (damage to glomeruli and albumin excretion), and neuropathy (reduced pain sensation). Macrovascular complications involve changes in large blood vessels and the aorta. The third type of diabetes, referred to as gestational diabetes, is observed in pregnant women with no history of diabetes who experience elevated blood sugar levels (Wei et al. 2022; Xaverius et al. 2022).

In diabetes, the body's capacity to metabolize and consume glucose decreases, resulting in increased blood sugar concentrations referred to as hyperglycemia (Kakadiya et al. 2010). Diabetic patients often exhibit changes in glucose and lipid metabolism, as well as alterations in liver enzyme levels. Common early symptoms of diabetes include excessive urination and thirst, overeating, weight loss despite enhanced hunger, exhaustion, and impaired eyesight (Kirk et al. 2015). Elevated intracellular glucose levels contribute to the generation of reactive oxygen species (ROS), while diminished function of enzymes with antioxidant activity (e.g. catalase, glutathione peroxidase, and superoxide dismutase) is implicated in oxidative stress development in diabetes (Arcaro et al. 2014). Most oral hypoglycemic drugs currently used to treat diabetes, e.g. sulfonylureas, biguanides, α-glucosidase inhibitors, and thiazolidinediones, lead to various side effects (Sundaram et al. 2019). Nowadays, herbal medications are used as an effective treatment for diabetes (El-Shahawy et al. 2021).

Hesperidin is a flavonoid glycoside naturally present in the fruit and skin of citrus fruits such as oranges, grapefruits, and lemons, and is occasionally referred to as 'vitamin P' (Shehata et al. 2017). This bioactive substance exhibits numerous activities including anti-inflammatory, anti-hyperglycemic, anti-lipid, anti-tumor, analgesic, antifungal, antiviral, antioxidant, immune system-modulating, liver protective, heart protective, and antidepressant properties (Atta et al. 2023; Lee et al. 2024). Furthermore, flavonoids including hesperidin play a crucial role as prognostic factors and predictors of tumors, particularly hepatocellular carcinoma (Atal et al. 2016). Regarding its hypoglycemic effects, hesperidin stimulates hepatic glycolysis, increases glycogen levels, and reduces hepatic gluconeogenesis (Elshazly et al. 2018). Notably, hesperidin is effective in managing complications associated with diabetes such as diabetic nephropathy, neuropathy, cardiomyopathy, and encephalopathy (Li et al. 2019). Its impact on hypoglycemia and blood lipid reduction in both type 1 and type 2 diabetes is partly attributed to strengthening the antioxidant defense mechanism and reducing the generation of pro-inflammatory cytokines (Zhang et al. 2018).

Piperine is the most abundant alkaloid in black pepper (*Piper nigrum* Linn.), which possesses essential therapeutic properties such as antioxidant, anti-apoptotic, anti-inflammatory, anti-convulsant, anti-mutagenic, anti-

depressant, and immune-modulating effects (Liu et al. 2020; Rauscher et al. 2000). Piperine inhibits pro-oxidant enzymes, leading to decreased blood glucose concentrations and regulation of internal antioxidant enzymes. Additionally, it can prevent cardiac disorders by activating the peroxisome-activated receptor gamma (PPAR-γ) pathway (Wang et al. 2020). Piperine substantially decreases total cholesterol, serum triglyceride (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels. Notably, it significantly contributes to glucose intake by promoting the relocation of glucose transporter 4 (GLUT4) to the plasma membrane through the AMP-activated protein kinase (AMPK) signaling pathway (Maeda et al. 2018). Furthermore, piperine stimulates pancreatic digestive enzymes and reduces lipid peroxidation, while also decreasing the levels of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β in collagen-induced arthritis (Samra et al. 2016).

Numerous investigations have reported that hesperidin alone indicated antihyperglycemic and anti-hyperlipidemia properties but due to problems such as low absorption and solubility, it does not have significant effects. However, piperine exerts a strong bio-enhancing impact on various medications. Several drugs have limited gastrointestinal absorption, which leads to decreased oral bioavailability. Piperine exerts its bio-enhancing effects through increasing solubility, enhancing the blood supply, and modifying epithelial cells to boost permeability (Arcaro et al. 2014; Atal et al. 2016).

Therefore, the aim of this study was to investigate synergistic effects of hesperidin and piperine, that their diverse pharmacological properties make them promising therapeutic candidates for diabetes and its complications. To the best of the authors' knowledge, no prior research has examined the antihyperglycemic activity of hesperidin and piperine in a model of diabetes induced by streptozotocin (STZ). Therefore, this study evaluated the antihyperlipidemic, antihyperglycemic, and hepatoprotective effects of hesperidin and piperine in rats with diabetes.

Materials and Methods

Chemicals and drugs

STZ, hesperidin, and piperine were acquired as active ingredients from Gol Exir Pars Company (Iran). Based on the FT-IR spectra shown in Figure 1, the purity of the hesperidin and piperine compounds is about 95%.

Animals

Sixty-three male Wistar rats (200 ± 30 g) were acquired from animal breeding centers. In order to adapt to the laboratory environment, the rats were kept under standard conditions in the animal house of the university for two weeks (12-hr day and 12-hr night cycle at 22 ± 3°C). Then, they were randomly divided into nine groups of seven.

Animal grouping

Diabetic test group 1: Diabetic + 0.25 mg/kg hesperidin

Diabetic test group 2: Diabetic + 0.25 mg/kg piperine

Diabetic test group 3: Diabetic + 0.5 mg/kg hesperidin

Diabetic test group 4: Diabetic + 0.5 mg/kg piperine

Diabetic test group 5: Diabetic + 0.25 mg/kg hesperidin and 0.25 mg/kg piperine

Diabetic test group 6: Diabetic + 0.5 mg/kg hesperidin and 0.5 mg/kg piperine

Diabetic positive control group: Diabetic + 500 mg/kg metformin

Diabetic control group: daily consumption of normal saline

Normal group: daily consumption of normal saline

The rationale behind selecting specific doses of hesperidin and piperine (e.g. 0.25 mg/kg and 0.5 mg/kg) was based on pilot experiments.

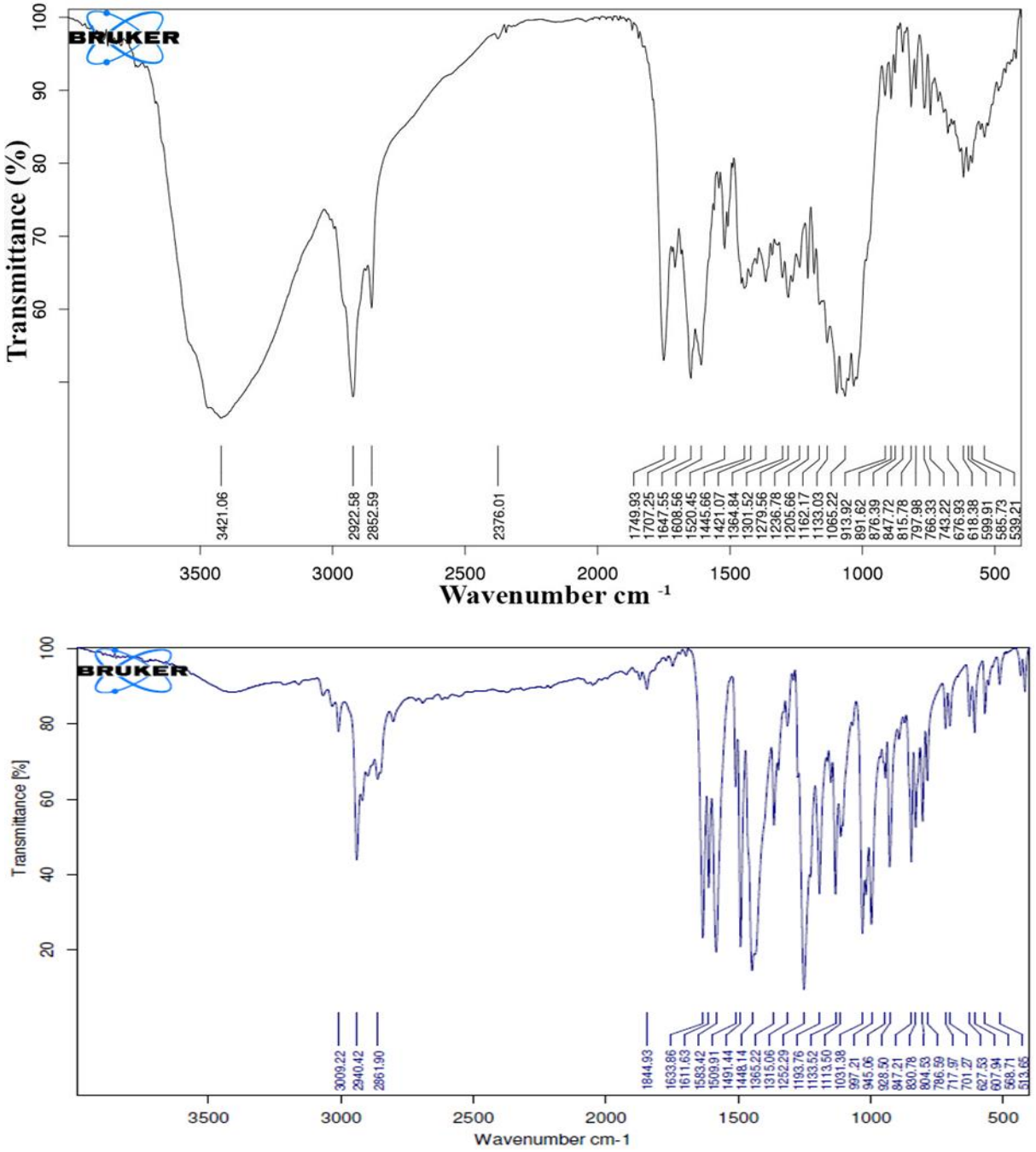

Figure 1. FT-IR spectra of hesperidin (A) and piperine (B)

Induction of diabetes

Initially, eight groups (six experimental groups and two control groups) were treated with STZ (60 mg/kg, intraperitoneally) dissolved in distilled water after 15 hr of fasting. Diabetes was confirmed 72 h after the injection, using the ACCU-CHEK glucometer (Germany) through a wound at the end of the tail. Blood sugar levels more than 350 mg/dl were considered diabetic in this study. To ensure the progression of stable diabetes and its related adverse outcomes, the animals did not undergo any intervention for at least 2 weeks after the initial confirmation of diabetes. After the final confirmation of hyperglycemia in rats, the treatment stage was initiated for the groups (Aramjoo et al., 2022).

Sampling and histological analysis

The treatments were administered once a day orally. The gavage volume was the same for all the groups. On the 30th day, the animals were placed in metabolic cages for 4-hr urine sampling to evaluate total protein levels. On the 31st day, 24 hr after the last gavage and observing a 14-hr fast, a combination of ketamine (10 mg/kg) and xylazine (75 mg/kg) was administered intraperitoneally to induce anesthesia in the animals. Blood samples were gathered from the abdominal aorta, and plasma samples were prepared. Next, the liver of each animal and its bile duct were fixed in 10% formalin buffer fixative for histological examination. Subsequently, the samples underwent dehydration, clarification, and paraffin embedding. Tissue sections with a thickness of 5 µm were prepared from the liver tissue using a microtome machine and underwent hematoxylin and eosin staining.. Pathological findings were recorded in an observation checklist and compared by the researcher. Biochemical tests were performed using an autoanalyzer and relevant kits. The prepared slides were studied under a light microscope by an experienced histologist in a blinded manner to assess inflammation, fat alterations in the sinusoidal gaps, hyperemia, and other possible lesions

Statistical analysis

The obtained data were analyzed by the SPSS software and examined using a one-way ANOVA test. The relevant non-parametric assay was used when the data did not follow a normal distribution. If a statistically significant difference was found, Tukey's post-hoc test was utilized to ascertain the differences between the two groups. In all experiments, a p-value less than 0.05 was regarded as the cutoff point for statistical significance.

Results

Body weight and blood glucose concentrations

The effects of hesperidin and piperine on blood glucose concentrations and body weight were investigated in diabetic animals, and the results are presented in Table 1. The administration of metformin and various dosages of hesperidin and piperine (0.25 and 0.5 mg/kg) led to a significant elevation in the body weight compared to the normal control group. The blood glucose concentrations were significantly higher in the diabetic control group than in the normal group. Treatment with hesperidin (0.5 mg/kg) and piperine (0.25 and 0.5 mg/kg), alone or co-administered, considerably lowered blood glucose concentrations in comparison to the diabetic control group ($p<0.05$). Similarly, the administration of metformin resulted in a considerable drop in blood glucose concentrations in comparison with the diabetic control animals ($p<0.05$). After the 30-day treatment period, hesperidin and piperine at all doses exhibited substantial hypoglycemic effects in STZ-induced diabetic rats, comparable to metformin. Piperine at dose of 0.25 and 0.5 mg/kg and when it was co-administered with hesperidin at 0.25 and 0.5 mg/kg showed higher hypoglycemic effects that were superior to metformin.

Impact of hesperidin and piperine on plasma lipid profile

Table 2 presents the results regarding the impact of hesperidin and piperine on the plasma lipid profile in the different group. Metformin considerably lowered LDL-c and TG and enhanced high-density lipoprotein-cholesterol (HDL-c) compared to the diabetic control group ($p<0.05$). Piperine at doses 0.25 and 0.5 mg/kg, alone or when co-administered, attenuated total cholesterol, LDL-c, and TG content, while raising the plasma levels of HDL compared to the diabetic control group ($p<0.05$ and $p<0.001$).

Table 1. The effect of hesperidin and piperine on body weight and blood glucose concentrations.

Groups	Body weight (g)		Blood glucose levels (mg/dl)	
	Before	After	Before	After
Diabetic + 0.25 mg/kg hesperidin	250.3 ± 7.55	271.69 ± 9.76	350.0 ± 14.34	305.66 ± 17.85
Diabetic + 0.25 mg/kg piperine	228.31 ± 11.4	282.56 ± 7.08	472.66 ± 12.53$^{\#\#\#}$	412.63 ± 4.61$^{\#\#\#}$
Diabetic + 0.5 mg/kg hesperidin	244.12 ± 25.4	261.5 ± 8.93**	375.0 ± 24.66$^{**\#\#}$	332.66 ± 21.67$^{**\#\#}$
Diabetic + 0.5 mg/kg piperine	280.32 ± 4.56	313.21 ± 5.91**	244.33 ± 8.18$^{**\#\#\#}$	162.33 ± 12.53$^{**\#\#\#}$
Diabetic + 0.25 mg/kg hesperidin and 0.25 mg/kg piperine	217.63 ± 6.39	253.21 ± 8.09	445.66 ± 11.00$^{\#\#}$	392.66 ± 6.44$^{\#\#}$
Diabetic + 0.5 mg/kg hesperidin and 0.5 mg/kg piperine	240.15 ± 5.17	264.58 ± 8.56**	293.66 ± 10.53$^{**\#\#\#}$	236.33 ± 14.71$^{**\#\#\#}$
Diabetic + 500 mg/kg metformin	279.8 ± 5.28	286.32 ± 11.6	410.33 ± 23.45$^{**\#\#}$	355.0 ± 4.12$^{**\#\#}$
Diabetic control group	290.14 ± 10.5	279.32 ± 8.37	305.33 ± 10.96	324.33 ± 10.36
Normal group	228.17 ± 7.58	290.14 ± 5.94	86 ± 7.00	79 ± 6.55

$^{**}p < 0.001$ shows significant differences compared to the normal control group. $^{\#\#}$ $p < 0.05$ and $^{\#\#\#}p < 0.001$ against the diabetic control group.

Table 2. The effect of hesperidin and piperine on lipid profile concentrations.

Groups	Triglyceride(mg /dl)	Cholesterol(mg/ dl)	Low-density lipoprotein(mg/ dl)	High-density lipoprotein(mg/dl)
Diabetic + 0.25 mg/kg hesperidin	90.66±24.32**	86.00±15.39**	25.78±4.50**	37.32±3.78
Diabetic + 0.25 mg/kg piperine	72.66±19.62$^{**\#\#}$	79.32±2.08$^{**\#\#\#}$	23.64±2.51$^{**\#\#}$	46.66±2.30$^{\#\#\#}$
Diabetic + 0.5 mg/kg hesperidin	80.02±21.7**	81.36±11.37**	26.45±4.04**	41.25±4.04$^{\#\#}$
Diabetic + 0.5 mg/kg piperine	62.66±19.5$^{**\#\#}$	78.63±3.05$^{**\#\#\#}$	21.66±1.15$^{**\#\#}$	48.33±0.57$^{\#\#\#}$
Diabetic + 0.25 mg/kg hesperidin and 0.25 mg/kg piperine	89.21±15.52$^{**\#\#}$	80.33±15.27$^{**\#\#}$	22.96±2.30$^{**\#\#}$	42.01±6.21
Diabetic + 0.5 mg/kg hesperidin and 0.5 mg/kg piperine	74.33±27.3$^{**\#\#}$	74.66±9.50$^{**\#\#}$	21.33±1.73$^{**\#\#}$	42.65±6.11$^{\#\#}$
Diabetic + 500 mg/kg metformin	96.66±4.60**	87.66±17.00$^{**\#\#}$	24.33±1.15**	43.36±5.16$^{\#\#}$
Diabetic control group	116.66±11.37	98.66±8.50	32.02±2.00	35.66±14.29
Normal group	59.00±8.69	73.00±6.24	29.33±1.52	37.82±4.87

$^{**}p < 0.001$ shows significant differences compared to the normal control group. $^{\#\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ against the diabetic control group.

Effect of hesperidin and piperine on serum markers of liver function

Table 3 summarizes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels in diabetic and normal groups. Treatment with piperine (0.25 and 0.5 mg/kg), alone or when co-administered, led to a significant decrease in ALT, AST, and ALP serum levels compared to the diabetic controls ($p<0.05$). Treatment with hesperidin, piperine, and metformin considerably reduced creatinine and blood urea concentrations in diabetic groups compared with the diabetic control group ($p<0.05$).

Liver histology

Figure 2 shows the liver sections of the studied groups. In the diabetic group compared with the control (diabetic control group), decreased acidophilia of peripheral cells in hepatic lobules and increased acidophilia of central cells of lobules were seen (Figure 2H and I). Also, increases of inflammatory cells, appearance of degenerative variations, and necrosis in the liver lobules were observed in diabetic control groups. In diabetic control samples, in addition to the findings of hepatocyte cell compression, sinusoid dilation and loss of hepatic cord order were observed. In the diabetic group treated with hesperidin and piperine, vascular destruction of liver cells was observed but it was very small compared to the diabetic control and metformin group (Figure 2 A-F). However, observing the liver tissue in the diabetic positive control group receiving metformin under a light microscope demonstrated moderate and diffuse degeneration and necrosis of hepatocytes (Figure 2G).

Table 3. The effect of hesperidin and piperine on serum markers of liver function.

Groups	Aspartate aminotransferase	Alanine aminotransferase	Alkaline phosphatase	Creatinine	Urea
Diabetic + 0.25 mg/kg hesperidin	139.0 ± 6.51	123.33 ± 5.80	1023.2 ± 3.30	0.75 ± 0.89	68.9 ± 1.74
Diabetic + 0.25 mg/kg piperine	113.1 ± 3.96$^{**\#\#}$	112.67 ± 24.5$^{\#\#}$	948.8±5.67**	0.66 ± 2.45$^{\#\#}$	54.66 ± 7.54
Diabetic + 0.5 mg/kg hesperidin	148.33 ± 24.8	129.0 ± 30.5	1011.4 ± 4.15**	0.70 ± 0.03	66.0 ± 13.4
Diabetic + 0.5 mg/kg piperine	108.2 ± 6.11$^{**\#\#}$	88.0 ± 18.68$^{**\#\#}$	793.1 ± 9.98$^{**\#\#}$	0.61 ± 0.07$^{**\#\#}$	35.3 ± 4.93$^{\#\#}$
Diabetic + 0.25 mg/kg hesperidin and 0.25 mg/kg piperine	129.4 ± 18.44	108.8 ± 14.5$^{\#\#}$	979.9 ± 14.5$^{\#\#}$	0.68 ± 0.24	49.6 ± 6.02
Diabetic + 0.5 mg/kg hesperidin and 0.5 mg/kg piperine	119.67 ± 9.29$^{**\#\#}$	95.74 ± 10.9$^{**\#\#}$	940.6 ± 5.72$^{**\#\#}$	0.63 ± 0.08$^{\#\#}$	45.3±14.7**
Diabetic + 500 mg/kg metformin	111.67 ± 15.56**	86.66 ± 17.2$^{**\#\#}$	1010.2 ± 4.98**	0.69 ± 0.16**	46.66 ± 13.6$^{**\#\#}$
Diabetic control group	146.6 ± 7.14	138.6 ± 17.47	1063.02 ± 5.11	0.78 ± 0.29	69.2 ± 9.16
Normal group	141.7 ± 2.33	110.4 ± 3.24	477.0 ± 11.3	0.73 ± 0.87	55.6 ± 18.6

$^{**}p<0.001$ shows significant differences compared to the normal control group. $^{\#\#}p<0.05$ against the diabetic control group.

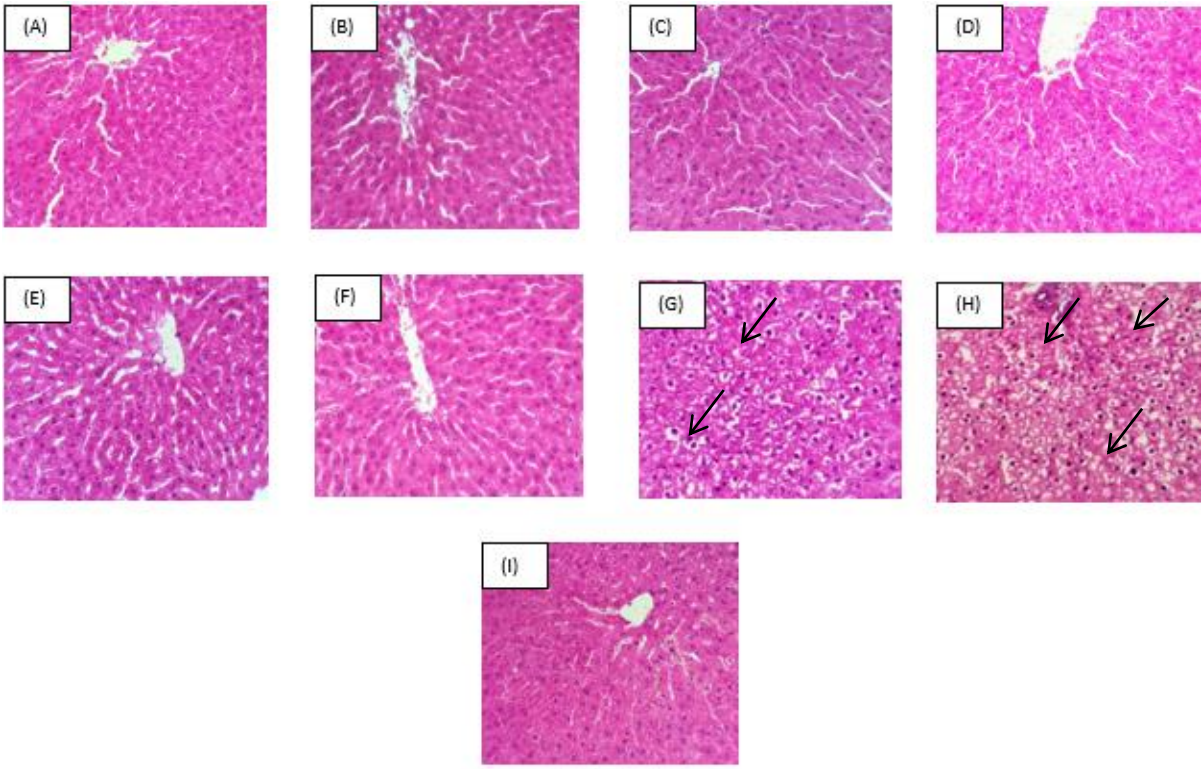

Figure 2. Microscopic images of rat liver tissue. (A) Diabetic + 0.25 mg/kg hesperidin, (B) diabetic + 0.25 mg/kg piperine, (C) diabetic + 0.5 mg/kg hesperidin, (D) diabetic + 0.5 mg/kg piperine, (E) diabetic + 0.25 mg/kg hesperidin and 0.25 mg/kg piperine, (F) diabetic + 0.5 mg/kg hesperidin and 0.5 mg/kg piperine, (G) diabetic + 500 mg/kg metformin, (H) diabetic control group, and (I) normal group

Discussion

The elevation in blood glucose concentrations, degradation of β cells, reduction of glucose uses in tissues, and increase in hepatic glycogenogenesis and glycogenolysis are among the most crucial consequences of diabetes induced by STZ (Al-Ishaq et al. 2019; Sharabi et al. 2015; Zhen et al. 2025). The present study investigated and compared the antihyperlipidemic, antihyperglycemic, and hepatoprotective activities of hesperidin and piperine in rats with diabetes. In this research, rats with STZ-induced diabetes received piperine alone or in combination with hesperidin (0.25 and 0.5 mg/kg) for 30 days. Hesperidin (0.25 and 0.5 mg/kg) reduced blood glucose concentrations in diabetic groups; however, its effect was comparatively weaker than that of piperine.

Piperine is an alkaloid extracted from the fruits and roots of *Piper nigrum* and *Piper longum*, and it has inherent therapeutic properties including antioxidant, anti-apoptotic, anti-inflammatory, anti-convulsant, anti-mutagenic, anti-depressant, anti-inflammatory, immune-modulating, and hepatoprotective effects, as well as the ability to reduce body fat accumulation (Liu et al. 2020). Moreover, piperine decreased the blood sugar level and regulated internal antioxidant enzymes through the inhibition of pro-oxidant enzymes. On the other hand, hesperidin, which has a flavonoid structure, reduced blood glucose levels and oxidative stress while increasing serum insulin concentrations. One of the mechanisms for these effects might be modifications of the function of several crucial enzymes involved in glucose metabolism (Miyake et al. 1998; Sharma et al. 2008)

Weight loss and destruction of muscle proteins are other critical consequences of diabetes induced by STZ, which result from the impairment of carbohydrate metabolism. Treatment with hesperidin and piperine (0.25 and 0.5 mg/kg) prevented a considerable reduction in body weight. However, piperine alone or when co-administered with hesperidin (0.25 and 0.5 mg/kg) exhibited a stronger effect. The main reasons for this effect include elevated renal excretion of lipoproteins, improved insulin sensitivity, secretion and function of lipoprotein lipase, and increased cellular fat absorption (Estakhr and Javdan 2011). The antidiabetic and antioxidant properties of piperine (20 or 40 mg/kg) and curcumin (90 mg/kg) in yogurt, either individually or in combination, were investigated in rats with STZ-induced diabetes. Administering both piperine (20 mg/kg) and curcumin in yogurt did not influence the antioxidant and antidiabetic effects of curcumin. However, the higher dose of piperine (40 mg/kg) led to beneficial effects on blood glucose concentrations in a rat model of diabetes (Arcaro et al. 2014). In another research, the bio-enhancing impact of piperine and metformin on decreasing blood glucose concentrations was evaluated in mice with diabetes caused by alloxan. The combination of piperine (10 mg/kg) and metformin (250 mg/kg) resulted in a more significant reduction in blood glucose concentrations compared to metformin alone, which is consistent with the findings of the current study (Atal et al. 2016). Liu et al. examined the impact of piperine on insulin resistance and inflammation in adipose tissue of mice with obesity. Their study revealed that piperine alleviated diabetes associated with obesity by inhibiting M1 macrophage polarization in adipose tissues and exerting anti-inflammatory effects (Liu et al. 2020). In an investigation conducted by Akiyama et al., hesperidin (10 g/kg) exhibited hypolipidemic and hypoglycemic properties in rats with STZ-mediated marginal type 1 diabetes by changing the function of enzymes involved in glucose regulation and normalizing the lipid and adiponectin content (Akiyama et al. 2009). One study investigated the protective effects of hesperidin at varying dosages (25, 50, and 100 mg/kg body weight) on fasting plasma glucose, hemoglobin, and insulin levels. Rats treated with hesperidin exhibited a considerable dose-related decline in plasma glucose levels compared to the diabetic control group. Moreover, the treated group demonstrated a simultaneous decrease in glycosylated hemoglobin and an increase in hemoglobin and insulin concentrations. In addition, hesperidin administration prevented weight loss and enhanced glycogen levels in the hepatic tissue of rats with diabetes by restoring the functions of glycogen phosphorylase and glycogen synthase (Sundaram et al. 2019).

Diabetes is related to changes in the lipid profile, which are linked to cardiovascular illnesses (Basa and Garber 2001). The administration of metformin along with piperine and hesperidin, alone and when co-administered, significantly lowered TG, TC, and LDL levels, while significantly enhancing HDL-c levels.

Among these compounds, piperine alone and when co-administered with hesperidin exhibited the strongest effects, which can be associated with the suppression of enzymes involved in cholesterol biogenesis, the promotion of cholesterol hydroxylase, and the prevention of intestinal intake of TG.

One of the key indicators of diabetes is an elevation in uric acid, urea, and creatinine levels, which are associated with renal dysfunction (Ji et al. 2022). The administration of metformin along with piperine and hesperidin, alone and when co-administered, resulted in a considerable drop in creatinine and urea levels. Due to belonging to the alkaloid and flavonoid groups, piperine and hesperidin have significant antioxidant effects, which inhibit oxidative stress and reduce kidney damage (Rafieian-Kopaei 2013). On the other hand, the administration of metformin along with piperine and hesperidin, alone and when co-administered, reduced AST, ALT, and ALP activities, which contribute to hepatoprotection. This effect might be related to the hypoglycemic properties and the structural nature of these components, as well as their antioxidant activity. One study investigated the influence of piperine on the metabolic conditions of patients with early cirrhosis and non-alcoholic fatty liver disease (NAFLD). The findings revealed that treatment with piperine (5 mg, orally) on a daily basis significantly lowered glucose levels and the function of liver enzymes, and improved dyslipidemia (Nouri-Vaskeh et al. 2024). In another investigation, the antioxidant and antidiabetic properties of piperine (20 and 40 mg/kg) and curcumin (90 mg/kg), individually or combined, were examined in diabetic rats. The concentrations of ALT and AST were lower in the diabetic group receiving 40 mg/kg piperine and curcumin than in the untreated diabetic group (Arcaro et al. 2014). In a study conducted by Rekha et al., diabetic rats receiving hesperidin (100 mg/kg body weight) for 4 weeks had considerably lower total cholesterol, TG, HDL, LDL, VLDL, and blood glucose levels (Rekha et al. 2019). Wang et al. studied the protective properties of hesperidin against hypercholesterolemia in rats with a high-cholesterol diet for 12 weeks. They reported that hesperidin may improve hypercholesterolemia and fatty liver by preventing the production and intake of cholesterol (Wang et al. 2011).

Histopathological evaluation of the liver indicated that piperine and hesperidin, alone or when co-administered, efficiently improved liver damage. However, the administration of piperine, alone or in combination with hesperidin, significantly improved liver conditions in a dose-related manner. In the present work, piperine and hesperidin decreased the function of hepatic enzymes, positively impacted the liver, and prohibited degenerative cellular alterations owing to their antioxidant activity. Consistent with our results, Hanchang et al. indicated that hesperidin, via the regulation of Bcl-xL and Bax, reduced inflammation in the liver of rats with diabetes (Hanchang et al. 2022). In another research, piperine administration decreased histopathological aberrations in diabetic rats, which was mediated through the modulation of caspase-3, Bcl2, and Bax/Bcl2 pathway (Wang et al. 2020). Numerous investigations have reported that piperine exerts a strong bio-enhancing impact on various medications. Several drugs have limited gastrointestinal absorption, which leads to decreased oral bioavailability. Piperine exerts its bio-enhancing effects through increasing solubility, enhancing the blood supply, and modifying epithelial cells to boost permeability (Arcaro et al. 2014; Atal et al. 2016).

Generally, hesperidin has demonstrated potential in managing diabetes by influencing various mechanisms including improving insulin sensitivity, reducing oxidative stress, and modulating glucose metabolism. Specifically, hesperidin can activate the IR/PDK1 signaling pathway, enhance glucose uptake, and inhibit α-glucosidase, an enzyme involved in

carbohydrate digestion but hesperidin could be inserting potential side effects include abdominal pain, diarrhea, and nausea (Mirzaei et al. 2023). On the other hand, piperine may help manage diabetes through various mechanisms including improving insulin sensitivity, regulating blood glucose levels, and potentially reducing inflammation and oxidative stress. Specifically, piperine can influence key transcription factors involved in glucose metabolism, such as HNF-1α and SREBP-1c, and enhance glucose uptake in muscle cells via the CAMKK/AMPK signaling pathway but high doses of piperine may temporarily raise blood glucose levels in the acute setting (Atal et al. 2016).

Potential limitations of this study include animal model limitations, sample size, the absence of mechanisms of action and long-term effects. So, future research should focus on human clinical trials, mechanistic studies, and studies on synergistic effects with other anti-diabetic agents, chronic toxicity, side effect profiles and effects on other metabolic disorders. The future clinical implications of this study can be seen in the form of adjunct therapy for type 2 diabetes, preventive therapy, and alternative drug options, potential for complementary medicine and personalized medicine.

In conclusion, this study provided strong evidence that hesperidin does not show additional antihyperglycemic or anti-hyperlipidemia properties when combined with piperine in STZ-induced diabetic rats. Biotransformation procedures increase drug bioavailability; however, they do not necessarily boost pharmacodynamic properties and may result in unfavorable or even toxic effects, based on the ratio of the medication to the bioenhancer. Furthermore, the suppression of hesperidin effects when combined with a higher concentration of piperine indicates that the biotransformation of hesperidin is essential for its antihyperglycemic and anti-hyperlipidemia properties.

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Conflicts of interest

The authors declared no conflict of interest in this study.

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Ethical Considerations

The experiments were conducted with the approval of the Ethics Committee of Birjand University of Medical Sciences

Code of Ethics

(IR.BUMS.REC.1402.502).

Authors' Contributions

S. Eghbali, M. Malekaneh led the study's conception and design. K. Falahati, Sh. Rajabi and B. Bijari prepared conducted experiments, including data collection, analysis, and interpretation writing drafted the article. All authors reviewed the manuscript critically and approved the final version**.**

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