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Original Research Article

The Effect of *Crocus Sativus* (Saffron) on the Liver Histology in Diabetic Rats Induced by a High-Fat High-Glucose Diet and Low Dose of Streptozotocin

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ABSTRACT

Diabetes mellitus (DM) contributes to hepatic dysfunction through oxidative stress, inflammation, and insulin resistance. To address its prevalence, *Crocus sativus* L. (saffron) stigma contains antioxidant and hepatoprotective compounds. Therefore, this study aims to examine the effect of saffron ethanol extract on liver function and histological features in rats with DM. A total of 17 male Wistar rats were divided into 4 groups, namely K– (non-diabetic control, n=4), Kd (diabetic control, n=5), P1 (diabetic+saffron 20 mg/kgBW, n=4), and P2 (diabetic+saffron 60 mg/kgBW, n=4). From weeks 1-12, K– received a standard diet, while Kd, P1, and P2 received a high-fat high-glucose diet (HFHGD). Diabetes was then induced at week 4 in Kd, P1, and P2 through intraperitoneal administration of streptozotocin (35 mg/kgBW). K– and Kd received distilled water, while P1 and P2 were treated with saffron ethanol extract from weeks 9-12. Flavonoids, alkaloids, tannins, and saponins were identified through phytochemical analysis. At week 12, the animals were sedated for blood collection and then euthanized for liver sampling. Liver weight, serum levels of aspartate aminotransferase, alanine aminotransferase, and cholesterol were assessed. Liver histology was assessed semiquantitatively. Kd group showed significantly increased liver weight ($p=0.002$) and cholesterol ($p<0.001$) compared to K–. In addition, P2 showed reduced cholesterol ($p<0.01$vs Kd) and histological improvements. Aspartate and alanine aminotransferase levels decreased nonsignificantly ($p>0.05$). These findings suggest that saffron stigma extract can exert notable hepatoprotective effects in rats induced with DM, particularly in cholesterol reduction and histological improvements.

Keywords: *Crocus sativus*, streptozotocin, diabetes, liver histology, cholesterol, alanine aminotransferase, aspartate aminotransferase, high-fat diet, high-glucose diet

Introduction

Diabetes mellitus (DM) is a long-term metabolic disease marked by consistently elevated blood glucose levels, which occur due to impaired insulin action and/or dysfunction in pancreatic β-cell activity. The prevalence of DM in Indonesia is rapidly rising, with projections exceeding 21 million cases by 2030[1]. Among its complications, liver dysfunction is increasingly recognized due to the liver's central role in maintaining glucose and lipid homeostasis. Hepatic insulin resistance in DM interferes with metabolic control, enhancing gluconeogenic activity and fat deposition, which in turn triggers steatosis and liver inflammation[2,3]. Inflammatory cytokines play a crucial role in worsening the disease by promoting oxidative damage and initiating liver cell apoptosis through the Nuclear Factor (NF)-κB signalling mechanism[4]. Increases in hepatic enzymes, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are widely recognized as indicative biochemical markers of hepatic tissue damage[5].

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According to previous studies, *Crocus sativus L* (saffron) is a species classified under the Iridaceae family. It is a long-lived flowering species that develops from a corm beneath the soil and yields purple-colored blooms. The reddish-orange stigmas of these flowers are carefully harvested and dried to produce saffron. In terms of distribution, saffron is not naturally found in Indonesia but can be cultivated in highland areas with cool climates. Globally, the primary producers include Iran, which dominates the market, followed by India (particularly Kashmir), Spain, Greece, Morocco, Italy, and China[6]. Saffron, a medicinal plant, has shown promising antioxidant, antihyperglycemic, and hepatoprotective properties. The stigma has been reported to reduce serum glucose levels, improve insulin sensitivity, inhibit lipogenesis, and suppress inflammatory responses in diabetic rodent models[7,8]. Studies have indicated its ability to suppress the synthesis of major inflammatory mediators, including Necrosis Factor (TNF)-α and Interleukin (IL)-6, which play a central role in the progression of long-term inflammatory disorders, including DM[9]. Saffron extract also exerts a protective effect against liver damage induced by a high-fat diet in rats, indicated by decreased expression of TNF-α and enhanced liver histological features[10]. Although various conventional drugs are currently used for DM management, there is growing scientific interest in evaluating plant-derived compounds due to their potential multifunctional properties. These findings suggest the potential role of saffron as an adjunctive complementary therapy to standard antidiabetic treatments. A recent meta-analysis of clinical trials found that saffron supplementation significantly improved glycemic and metabolic parameters without adverse effects on liver function[11].

Experimental studies using HFHGD combined with low-dose streptozotocin (STZ) provide a reliable model to mimic type 2 DM and

its hepatic complications[12]. However, dose-dependent effects of saffron on liver function and histopathological outcomes in this model remain underexplored. Considering that hepatic complications, such as non-alcoholic fatty liver disease (NAFLD) are common in DM, the investigation of the plantas a natural adjunct to protect liver function is well-justified. Therefore, this study aims to investigate the potential hepatoprotective effects of saffron ethanol extract in a rat model of metabolic dysfunction induced by an HFHGD diet combined with low-dose streptozotocin (STZ).

Materials and Methods

Animals and Experimental Design

All animal-related experimental procedures in this study were carried out in compliance with established ethical standards and received official approval from the Animal Ethics Committee of the Faculty of Medicine, Hasanuddin University, as documented in approval letter No. 923/UN4.6.4.5.31/PP36/2024. A total of 17 male Wistar rats (*Rattus norvegicus*), aged 8 to 12 weeks and weighing between 150 – 200 grams, were obtained from certified animal breeders located in Makassar, South Sulawesi. Before the initiation of the experimental procedures, the rats underwent a 1-week acclimatization period in a regulated environment, which included individual caging, a 12-hour light as well as dark cycle, and ambient temperatures maintained between 26°C and 28°C. The rats were randomly distributed into 4 distinct experimental groups. Groups 1, 3, and 4 included 4 samples each, while Group 2 consisted of 5 samples. Group 1 (negative control; K-) received a standard diet and 1 mL of distilled water, Group 2 (diabetic control; Kd) was fed an HFHGD, administered STZ and 1 mL of distilled water, Group 3 (treatment group 1; P1) received HFHGD + STZ + saffron ethanol extract at 20 mg/kg BW, and Group 4 (treatment group 2; P2) received HFHGD + STZ + saffron ethanol extract at 60 mg/kg BW.

Dietary and Streptozotocin Induction

All experimental groups received their respective dietary treatments starting from week 1 and maintained consistently throughout the 12-week experimental period. The standard diet given to Group 1 was a commercial rodent feed (RatBio, Indonesia) composed of approximately 20% protein, 60% carbohydrates, and 4% fat. Meanwhile, the high-fat diet administered to Groups 2–4 was custom-formulated by UD Bancar Jaya (East Java) using ingredients such as fish oil, coconut oil, corn starch, wheat flour, fishmeal, and soybean flour. According to an analysis conducted at the Animal Feed Chemistry Laboratory, Faculty of Animal Science, Hasanuddin University, the diet contained 11.24% protein, 32.6% carbohydrates, and 45.88% fat. While Group 1 had access to standard drinking water, the other groups received glucose-enriched water (20%)[13].

To induce diabetes, a single intraperitoneal injection of STZ (35 mg/kg BW), freshly prepared in 0.01 M citrate buffer (pH 4.5), was administered at the beginning of week 4. In cases where hyperglycemia was not achieved, a second injection at half the initial dose was given 3 days later. Rats not reaching a Fasting Blood Glucose (FBG) level above 250 mg/dL, measured using an Accu-Chek Instant Glucometer, were excluded from further analysis[13].

Body weight and FBG were monitored weekly throughout the experimental period to evaluate metabolic changes and confirm the establishment of a diabetic state. Body weight measurements were recorded from the acclimatization phase (week 0) until week 12, while FBG levels were measured weekly beginning 1 week after the STZ injection (week 5). This observational monitoring approach was consistent with established diabetic rat models, in which weekly tracking of body weight and glycemic levels was used to verify the progression of hyperglycemia and associated physiological alterations[14].

Saffron ethanol extract

Saffron stigma used in this study was obtained from Herat saffron (Afghanistan Red Gold Saffron Ltd, Afghanistan), with registration number KEMTAN RE PL 36.03-B.III.00-02-000057-03/21, and was accompanied by a Certificate of Analysis (CoA) ISO 3632-1 to ensure its quality and purity. A total of 26 grams of dried saffron stigma threads were added to 500 mL of 96% ethanol and macerated for 3 days in a tightly sealed glass jar at room temperature (approximately 25°C). The mixture was then filtered to obtain the saffron ethanol extract, followed by evaporation at 40°C using a vacuum rotary evaporator for approximately 6 hours. After evaporation, a thick extract was obtained and subsequently placed in a water bath for a day[15].

Phytochemical Screening of Saffron Ethanol Extract

Qualitative phytochemical screening of the saffron ethanol extract was conducted to detect major groups of secondary metabolites, including flavonoids, alkaloids, tannins, and saponins, using standard methods as described by Harborne[16].

Treatment Protocol

Saffron ethanol extract administration was initiated at week 9, corresponding to 4 weeks after STZ induction. This timing was selected to ensure adequate stabilization of hyperglycemia and to allow the diabetic state to become well-established before treatment[14]. Saffron ethanol extract was administered orally once daily, where P1 received 20 mg/kg BW and P2 received 60 mg/kg BW. The selection of saffron doses (20 mg/kg BW and 60 mg/kg BW) was based on previous studies that reported no hepatotoxic effects at these dose levels in diabetic rat models[17]. In addition, a dose of 40 mg/kg BW has been previously shown to produce histological changes in hepatic tissue[15]. Therefore, in the present study, 2 doses, 1 below and 1above the 40 mg/kg BW reference, were selected to explore a possible dose–response relationship on liver histopathology in diabetic rats. The K- and Kd groups were administered 1 mL of distilled water via oral gavage, following the same procedure as used for the P1 and P2 groups.

Blood collection

Blood samples were collected at the end of week 12, following a 12-hour fasting period after the final day of dietary and saffron ethanol extract administration. After being fasted for 12 hours overnight, all animals were sedated using a combination of ketamine and xylazine. Biochemical assessments were performed on blood samples taken through retro-orbital collection. Centrifugation at 3000 rpm for 10 minutes was performed on the collected blood to separate the serum. The resulting supernatant was carefully transferred into labelled microtubes and stored at −80°C until it was ready for further examination[18].

Biochemical analysis

Serum levels of AST, ALT, and total cholesterol were analyzed using the fully automated Thermo Scientific Indiko system (Thermo Fisher Scientific Inc., USA).

Histological examination

Before the histological examination, rats were anaesthetized using ketamine and xylazine, followed by cervical dislocation to confirm death. A surgical dissection was then performed to collect liver tissue samples, which were sectioned and processed using standard histological methods. The samples underwent fixation in formalin, followed by paraffin block preparation and staining with hematoxylin-eosin (H&E) for histological assessment. A pathologist from Hasanuddin University Hospital, who was blinded to the treatment groups, conducted the histopathological assessment to eliminate bias. The scale bar for microscopic analysis was calibrated using the ImageJ software. Histological scoring of liver tissue was in line with established criteria adapted from previous studies, as outlined in Table 1[19]:

Statistical analysis

Statistical evaluation was conducted using SPSS version 24, and the data were considered normally distributed and variances homogeneous when Shapiro-Wilk and Levene's tests produced p-values ≥ 0.05. If both criteria were met, One-Way ANOVA ($\alpha = 0.05$) was applied, and in cases where the criteria were not met, the Kruskal-Wallis test was used. Group comparisons after the main analysis were carried out using Tukey's test.

Table 1: Scoring Liver Histology

Assessment	Scoring
Steatosis	0: Involvement less than 5% of hepatocytes
	1: Between 5% and 33% involvement
	2: Involvement of more than 33% up to 66%
	3: Greater than 66% of hepatocytes affected
Lobular	0: No inflammatory cell aggregates
Inflammation	1: Less than two inflammatory foci per field
	2: 2 to 4 inflammatory foci per field
	3: More than four inflammatory foci per field
Ballooning	0: No ballooned cells detected
Hepatocytes	1: Occasional or mild ballooning present
	2: Multiple cells or marked ballooning observed

Results and Discussion

Phytochemical Composition of Saffron Ethanol Extract

The results from the phytochemical screening confirmed that the saffron ethanol extract contained various groups of secondary metabolites, including flavonoids, alkaloids, tannins, and saponins (Table 2).

Table 2: Qualitative Phytochemical Test of Saffron Ethanol Extract

Phytochemical constituents	**Result**
Flavonoid	+
Tannin	+
Alkaloid	+
Saponin	+

Note: + (present), - (absent).

Liver Weight

Liver weight in the Kd group was approximately 1.6-fold greater compared to the K− group, showing a significant difference based on statistical analysis ($p<0.05$) (Table 3). The following data also indicated that administration of saffron ethanol extract 20 mg/kg BW (P1) and 60 mg/kg BW (P2) for 4 weeks could contribute to a reduction in liver weight. However, the change was not statistically significant.

Table 3: Liver weight among experimental groups following saffron ethanol extract intervention

Variable	Group (Mean ± SD) K- n = 4	Kd n = 5	P1 n = 4	P2 n = 4	p
Liver Weight (g)	8.22 ± 0.756	13.354 ± 0.583[a]	12.447 ± 0.2568	11.82 ± 0.1029	0.002*

*Kruskal Wallis test is significant if $p<0.05$. [a]$p<0.05$ compared to K- group.K- (standard diet); Kd (HFHGD and low-dose STZ); P1 (HFHGD and low-dose STZ with saffron ethanol extract 20 mg/kg BW); P2 (HFHGD and low-dose STZ with saffron ethanol extract 60 mg/kg BW).

The diabetic group (Kd) demonstrated a statistically significant elevation in liver weight relative to the control group (K−), with a p-value of 0.002, suggesting hepatic hypertrophy driven by metabolic stress. The progressive fat deposition in hepatocytes contributes to hepatomegaly, as evidenced by increased liver weight in diabetic models. This enlargement of the liver reflects steatosis and inflammatory cell infiltration, which are hallmarks of NAFLD progression associated with type 2 DM[20]. The finding was consistent with the pathophysiology of NAFLD and non-alcoholic steatohepatitis (NASH), where lipid accumulation, hepatocyte swelling, and inflammatory infiltration are typical features[18,21]. The elevated liver weight found in the diabetic groups is consistent with earlier findings from studies employing comparable models involving high-fat diets and STZ induction. Liver hypertrophy has been associated with hepatic steatosis and inflammation, reflecting pathological adaptations to metabolic dysfunction[22].

The administration of saffron ethanol extract at 20 mg/kg BW (P1) and 60 mg/kg BW (P2) resulted in a reduction in liver weight, but not statistically significant. This indicated a possible protective role of saffron against hepatic enlargement. The protective effect is likely associated with the bioactive compounds found in the plant. Flavonoids in the ethanol extract have been reported to inhibit hepatic gluconeogenesis and lipogenesis by downregulating key regulatory genes, such as *SREBP-1c*, *G6Pase*, and *PEPCK*, which may alleviate the metabolic burden on the liver[23,24]. Moreover, flavonoids enhance insulin sensitivity and fatty acid oxidation, improving hepatic lipid handling and may counteract liver enlargement. Alkaloids also exhibit anti-lipogenic effects by modulating lipid metabolic pathways and suppressing inflammatory gene expression[25,26]. These effects help mitigate hepatic lipid accumulation and limit structural changes in liver tissue. Saponins and tannins have been shown to attenuate liver weight and damage by reducing lipid accumulation and promoting hepatic lipid metabolism[27,28].

ALT, AST and Total Cholesterol

The diabetes control group (Kd) exhibited the highest transaminase activity, with mean ALT and AST values of 100.4 ± 22.30 U/L and 190 ± 52.402 U/L, respectively (Table 4). Animals in groups P1 (20 mg/kg BW saffron ethanol extract) and P2 (60 mg/kg BW) showed a downward trend in both liver enzyme levels. These results suggest that saffron administration may attenuate hepatic injury markers compared to the Kd group, but the differences were not statistically significant ($p > 0.05$).

Table 4: ALT, AST and Total Cholesterol among experimental groups following saffron ethanol extract intervention

Variable	Group (Mean ± SD) K- n = 4	Kd n = 5	P1 n = 4	P2 n = 4	p
ALT (U/L)	76.75 ± 13.598	100.40 ± 22.30	76.50 ± 35.865	66 ± 15.121	0.129
AST (U/L)	151.25 ± 25.734	190 ± 52.402	183.75 ± 55.090	161.75 ± 35.612	0.557
Total Cholesterol (mg/dL)	62.75 ± 6.898	82.60 ± 7.300[a]	52.25 ± 5.315[b]	43.50 ± 12.688[a,b]	0.000**

**ANOVA test is significant if $p<0.05$. Turckey HSD: [a]$p<0.05$ compared to K- group, [b]$p<0.01$ compared to Kd group. K- (standard diet); Kd (HFHGD and low-dose STZ); P1 (HFHGD and low-dose STZ with saffron ethanol extract 20 mg/kg BW); P2 (HFHGD and low-dose STZ with saffron ethanol extract 60 mg/kg BW).

The decrease in serum ALT and AST levels in saffron-treated groups further supports the hepatoprotective effects, but was notstatistically significant ($p > 0.05$). Elevated transaminases in the diabetic group reflect hepatocyte injury due to oxidative stress, inflammation, and mitochondrial dysfunction caused by chronic hyperglycemia[29,30]. In diabetic conditions, particularly those induced by high-fat diets and STZ, liver function tends to be compromised due to chronic hyperglycemia and insulin resistance. These metabolic disturbances lead to increased fat accumulation in hepatocytes, mitochondrial dysfunction, and oxidative stress. Consequently, hepatocellular injury occurs, marked by the leakage of intracellular enzymes such as ALT into the bloodstream. Elevated ALT levels are considered a sensitive biomarker of hepatic injury in diabetic states, reflecting underlying steatosis, inflammation, or hepatocellular damage[19,31]. Increased AST and ALT levels in diabetic rats, as demonstrated in this study, are consistent with earlier experimental findings that link hyperglycemia

and oxidative stress to hepatocellular damage. The enzymes are commonly used as indicators of liver injury in diabetic models[22].

Flavonoids present in saffron aid in alleviating liver inflammation and preventing hepatocyte injury by downregulating the TLR4/MyD88/NF-κB signalling pathway, thereby reducing transaminase leakage into the bloodstream. By modulating this pro-inflammatory signalling cascade, saffron flavonoids may contribute to the preservation of hepatic integrity[32]. Alkaloids and tannins in the plant also help stabilize hepatocyte membranes and enhance cellular repair mechanisms[33,34].

The comparison revealed a statistically significant difference in cholesterol levels between the 4 treatment groups ($p < 0.05$), with the diabetic control group (Kd) showing the highest concentration (82.6 ± 7.3 mg/dL), as presented in Table 4.Total cholesterol was selected as the primary lipid indicator due to its central involvement in lipid metabolic dysfunction and intrahepatic fat accumulation, which occurs alongside insulin resistance and NAFLD progression. Previous findings have indicated that elevated cholesterol levels contribute not only to steatosis but also to hepatocellular injury, thereby serving as a reliable surrogate marker when full lipid profiling is not feasible[35]. In this study, total cholesterol concentrations were elevated in the diabetic group relative to the control group. Consistent with previous studies, the current findings confirm that induction of type 2 diabetes using a high-fat diet along with STZ injection leads to a notable increase in total cholesterol[36]. Elevated total cholesterol concentrations observed in diabetic groups are consistent with previous findings reporting dysregulated lipid metabolism and increased endogenous cholesterol production in diabetic states. Similar patterns of hypercholesterolemia have been reported following high-fat diet feeding combined with STZ induction[22].

These results also show that administration of saffron ethanol extract 20 mg/kg BW (P1) and 60 mg/kg BW (P2) significantly reduced total cholesterol levels compared to the K- and Kd groups, with a stronger lowering effect seen in the P2 group. Total serum cholesterol levels were notably lower in the saffron-treated groups, with the most pronounced effect observed in P2 ($p < 0.01$ vs. Kd). These findings align with earlier studies suggesting that hyperglycemia and high-fat diets can upregulate3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) *reductase* and suppress *lipoprotein lipase* activity, thereby promoting hepatic cholesterol synthesis[37,38]. Flavonoids found in saffron are known to inhibit HMG-CoA reductase, a key enzyme involved in controlling cholesterol biosynthesis. This inhibition contributes to reduced endogenous cholesterol formation and upregulation of LDL receptors, thereby promoting improved lipid metabolism[39,40]. Alkaloids influence cholesterol balance in part by interacting with the gut microbiome and modulating liver gene expression related to lipid metabolism and transport[41]. Tannins inhibit cholesterol absorption and biosynthesis, while saponins bind bile acids and enhance cholesterol excretion[42,43]. The synergistic actions of these phytochemicals contribute to the observed decrease in serum total cholesterol levels.

Liver histology

Kd group exhibited the highest mean steatosis score (1.8 ± 0.583), followed by P1 (1.25 ± 0.75) and P2 (1.0 ± 0.408), while K- group showed no evidence of steatosis (0.000 ± 0.000). Although these values suggest a trend of reduced steatosis in the treatment groups, no statistically significant difference was observed ($p = 0.136$). Meanwhile, lower mean scores in the P1 and P2 groups reflect a downward shift in the distribution of steatosis severity, supporting a potential protective effect of the intervention (Table 5).

The mean score for lobular inflammation was highest in Kd group (2.2 ± 0.583), with significant variation found among all groups ($p = 0.036$). Subsequent pairwise analysis demonstrated that Kd group differed significantly from K−. The lower averages in P1 (1.75) and P2 (1.5) suggest a partial reduction in inflammatory severity, but were not statistically significant (Table 5).

Hepatocyte ballooning had a decreasing pattern across the experimental groups, with Kd showing the highest mean score (1.2 ± 0.374), followed by P1 (1.0 ± 0.000) and P2 (0.75 ± 0.478). K− group consistently remained at 0.000 ± 0.000. Although the difference was not statistically significant ($p = 0.077$). In comparison, the reduced values in P1 and P2 suggest less frequent or milder hepatocellular ballooning following treatment (Table 5).

Table 5: Histological Assessment of the Liver among experimental groups following saffron ethanol extract intervention

Variable	Group (Mean ± SEM)				p
	K- n = 4	Kd n = 5	P1 n = 4	P2 n = 4	
Steatosis	0.000 ± 0.000	1.80 ± 0.583	1.25 ± 0.75	1 ± 0.408	0.136
Lobular Inflammation	0.000 ± 0.000	2.20 ± 0.583[a]	1.75 ± 0.478	1.50 ± 0.288	0.036*
Balloning Hepatocyte	0.000 ± 0.000	1.20 ± 0.374	1 ± 0.000	0.75 ± 0.478	0.077

*Kruskal Wallis test is significant if $p<0.05$. [a]$p<0.05$ compared to K- group.

K- (standard diet); Kd (HFHGD and low-dose STZ); P1 (HFHGD and low-dose STZ with saffron ethanol extract 20 mg/kg BW); P2 (HFHGD and low-dose STZ with saffron ethanol extract 60 mg/kg BW).

Histological analysis reinforced the biochemical findings, where Kd group exhibited the most severe hepatic damage, including steatosis, lobular inflammation, and ballooning (Figure 1).

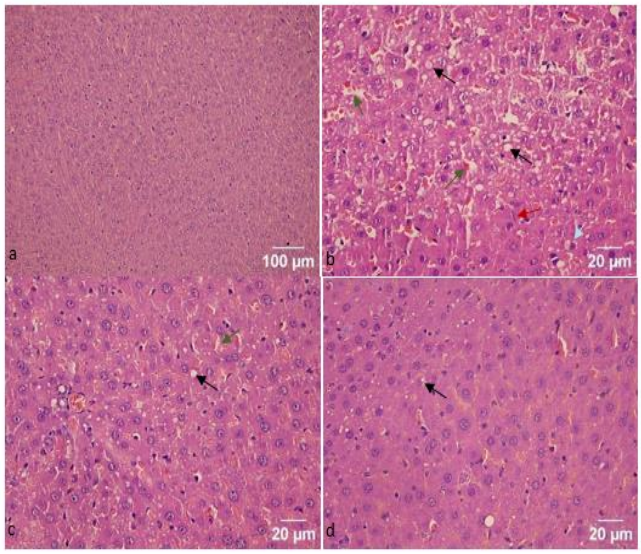

Figure 1: Rat Liver Histology. K- (standard diet); Kd (HFHGD and low-dose STZ); P1 (HFHGD and low-dose STZ with saffron ethanol extract 20 mg/kg BW); P2 (HFHGD and low-dose STZ with saffron ethanol extract 60 mg/kg BW). a: Group K- (100x magnification); b: Group Kd; c: Group P1; and d: Group P2 (400x magnification). The black arrow points to fat infiltration; the red arrow points to inflammatory infiltrate; the blue arrow points to ballooning hepatocytes; the green arrow points to sinusoidal dilatation containing erythrocytes.

Although differences were not always statistically significant, saffron-treated groups showed trends of improved liver architecture, with reduced fat accumulation and inflammatory cell infiltration. Liver inflammation is a hallmark in rodent models induced by a high-fat diet. The chronic intake of lipids promotes excessive fat deposition in hepatocytes, which in turn triggers cellular stress and activates immune

responses. As a result of this process, hepatic macrophages (Kupffer cells) are triggered to produce key pro-inflammatory factors, including TNF-α and IL-6. The subsequent recruitment of inflammatory cells, including neutrophils and monocytes, exacerbates hepatic injury. The inflammatory mechanisms are commonly observed in metabolic disorders, where insulin resistance and hyperglycemia further accelerate hepatic lipid accumulation and immune activation[19].The presence of sinusoidal dilation was also observed as a relevant structural alteration. This dilation reflects pathological changes in hepatic microcirculation and is often linked to mitochondrial dysfunction and hepatocellular stress. Impaired mitochondrial oxidative phosphorylation leads to reduced adenosine triphosphate (ATP) production and ionic imbalance, resulting in hepatocyte swelling and increased intrahepatic pressure, which can secondarily promote sinusoidal expansion[44]. In addition, liver sinusoidal endothelial cells (LSECs), which are critical regulators of hepatic vascular tone and permeability, undergo phenotypic and functional changes during metabolic dysfunction. These alterations include reduced nitric oxide bioavailability and heightened oxidative stress, which disrupts sinusoidal homeostasis and favor capillarization[45]. LSEC dysfunction contributes to vascular remodeling and impaired blood flow, thereby facilitating sinusoidal dilation and erythrocyte congestion[46]. The changes are also closely associated with increased expression of adhesion molecules. In addition, the changes enhances leukocyte infiltration and exacerbate hepatic inflammation in the early stages of NAFLD, potentially driving progression toward NASH[47]. These processes represent an adaptive but pathological response to hepatic injury and circulatory imbalance[46].

Histopathological assessment in this study revealed higher scores of steatosis, lobular inflammation, and hepatocyte ballooning in the diabetic groups than in the controls. The results are consistent withprevious studies showing that high-fat dietary exposure in experimental animals promotes hepatic lipid accumulation and inflammatory responses, as evidenced by increased NAFLD activity scores, including steatosis, inflammatory cell infiltration, and hepatocellular ballooning[36].

Flavonoids and alkaloids present in saffron have demonstrated potent hepatoprotective activities through multiple mechanisms. These compounds attenuate hepatic lipid peroxidation and suppress inflammatory cascades, thereby reducing hepatocellular injury. Flavonoids and alkaloids also contribute to the preservation of mitochondrial integrity, which is critical for maintaining energy homeostasis and limiting oxidative stress within hepatocytes. Moreover, their ability to promote hepatocyte regeneration suggests a reparative role in damaged liver tissue[24,32,33]. In addition, tannins and saponins found in saffronethanol extract have been reported to reduce hepatic steatosis and inflammatory infiltration. These effects are mediated by their antioxidant properties, which scavenge reactive oxygen species (ROS), and their regulatory influence on the expression of pro-inflammatory cytokines[27,48].

This study has several limitations, where the short duration of the intervention may limit the generalization of the results. Molecular analyses such as oxidative stress markers, inflammatory cytokines, and gene expression were not performed, which could have provided deeper insight into the mechanisms of action. In addition, only total cholesterol was measured, while other lipid parameters, such as HDL, LDL, and triglycerides were not assessed due to limited resources. Despite these limitations, the results offer early evidence that saffron ethanol extract may help protect the liver and improve metabolic function in a type 2 DM model.

Conclusion

In conclusion, this study shows that saffron ethanol extract exhibits notable hepatoprotective and hypolipidemic effects in a rat model of metabolic liver injury induced by an HFHGD combined with low-dose STZ. Improvements were observed in liver weight, serum alanine aminotransaminase and aspartate aminotransaminase levels, total cholesterol, and histopathological features, such as steatosis, inflammation, and hepatocyte ballooning. These findings support saffron's potential role in alleviating hepatic alterations associated with diet-induced metabolic impairment. Further studies are recommended to explore the underlying molecular mechanisms.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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