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Effects of Saffron (*Crocus sativus L*) Compared to Atorvastatin on the Livers of Hypercholesterolemia Rat Models

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ABSTRACT

Hypercholesterolemia is characterized by elevated blood total cholesterol, and might induce liver inflammation. Atorvastatin as the first-line medication in hypercholesterolemia can cause side effects; Saffron has been reported to have hepatoprotective property. This study compared the anti-inflammatory and hepatoprotective effects of Saffron *vs.* Atorvastatin on the liver of high-fat diet-induced hypercholesterolemia male rats. Thirty-two rats were divided equally into four groups and given a high-fat diet (36 days). The third group was given Saffron (80 mg/kg of body weight/day), and the fourth group was given Atorvastatin (0.2 mg/200 g of body weight/day) from days 16-36. Data were analyzed using SPSS 24.0 with a significance level of $p<0.05$. Total cholesterol (TC) between groups was significantly different at day 15 ($p<0.01$), while at day 37, the Atorvastatin group had a closer TC to the normal diet group than the Saffron group. Expression of Tumor Necrosis Factor-alpha (TNF-α) in the high-fat diet group ($p<0.01$) was significantly higher than that in the Saffron group ($p<0.01$), Atorvastatin group ($p<0.01$) as well as the control ($p<0.01$). The values of total hepatocyte degeneration, the number of inflammatory foci, and the total area width of inflammatory foci in the high-fat diet group were significantly higher than those in the Saffron group ($p<0.01$, $p<0.01$, $p<0.01$, respectively), than those in the Atorvastatin group ($p<0.01$, $p<0.01$, $p<0.01$, respectively) than in the control group ($p<0.01$, $p<0.01$, $p<0.01$, respectively). Saffron exhibited drug-likeness potential as a natural hepatoprotective agent. Further research is needed to understand better the mechanisms underlying Saffron's protective effects.

Keywords: Saffron, Atorvastatin, Non-alcoholic fatty liver disease, Total cholesterol, Tumor necrosis factor-alpha

Introduction

Hypercholesterolemia is a complex condition that can be caused by various etiologies, i.e. high-fat diet, obesity, genetic disorders such as familial hypercholesterolemia (FH), or other diseases like metabolic syndrome.[1–3] Hypercholesterolemia is a lipid metabolism disorder that can cause elevated blood lipid levels due to deficiencies in lipoprotein lipase enzymes, low-density lipoprotein (LDL) receptors, or genetic abnormalities resulting in increased liver cholesterol production.[4–6] In patients with hypercholesterolemia, there is also an increase in free fatty acids (FFA), triglycerides (TG), total cholesterol (TC), and apolipoprotein B (Apo-B) levels in the blood, as well as a decrease in serum high-density lipoprotein cholesterol (HDL-C) level.[3] Elevated TC level in humans if ≥ 200 mg/dL, while in hypercholesterolemia rat models if ≥ 50 mg/dL.[7–9]

According to the World Health Organization (WHO), the global prevalence of elevated total cholesterol among adults is approximately 45%. In Southeast Asia, it is approximately 30%, while in Indonesia, it reached around 35%.[9,10] A significant portion of the Indonesian population has cholesterol levels higher than normal, typically affecting around 39.6% of women and approximately 30.0% of men.[11,12]

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According to The Basic Health Research in Indonesia (Riskesdas, 2018), the prevalence of hypercholesterolemia in Indonesia was approximately 35.9%. This indicates that approximately 1 in 3 adults in Indonesia has a total cholesterol level ≥ 200 mg/dL. In the 25-34 age group, hypercholesterolemia in Indonesia reaches 9.3% and increases with age, reaching 15.5% in the 55-64 age group.[13,14]

One of the most common causes of hypercholesterolemia is poor dietary habits, particularly a high intake of fats. A high-fat diet increases cholesterol levels in the intestines, inducing hypercholesterolemia through inflammatory reactions and oxidative stress, with the release of nuclear factor-kappa β (NF-κβ), nitric oxide (NO), and reactive oxygen species (ROS) that form in the bloodstream and intracellular tissues.[15] The excessive formation of ROS leads to overall hepatocyte damage, as evidenced by changes observed in histopathological examinations, i.e., liver steatosis, infiltration of inflammatory cells, and cell degeneration.[16] This can trigger inflammatory reactions, such as increased pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and fat accumulation in hypercholesterolemia, leading to nonalcoholic fatty liver disease (NAFLD). [17–19]

Currently, the first-line treatment for hypercholesterolemia involves Statin medications. One of the most commonly used Statins for hypercholesterolemia is Atorvastatin.[20] This drug's mechanism of action involves inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thereby preventing the conversion of HMG-CoA reductase to mevalonate.[21,22] Clinical studies have shown that Statin treatment has some drawbacks, including treatment-related complications. Prolonged Statin use can increase serum transaminase levels, blood and lymphatic system disorders (anemia), and gastrointestinal disturbances (constipation, stomach pain, bloating,

dyspepsia, diarrhea, nausea, acid regurgitation, and vomiting). Hence, there is a need for alternative treatments with less risk of side effects to be studied, including the phytochemicals that also have indicated therapeutic effects.[23–25]

One plant that has been proposed is Saffron (*Crocus sativus L*), which contains compounds such as *crocin*, *crocetin*, *picocrocin*, and *safranal* that have potential organo-protective effects.[24] The main components of Saffron also have antioxidant effects that inhibit ROS accumulation. Moreover, Saffron possesses anti-inflammatory properties that act on a cytokine called TNF-*α*, thereby reducing the increase in this pro-inflammatory cytokine, which can be proposed to act as a hepatoprotectant.[26] However, whether Saffron extract that is given orally has a drug-likeness comparable to Statins needs to be analyzed. Here, we conducted an *in vivo* study using male rat models of Wistar rats (*Rattus norvegicus*) of hypercholesterolemia to compare the anti-hypercholesterolemia, anti-inflammation, and hepatoprotective effect of Saffron extract *vs.* Atorvastatin.[24,27]

Material and Methods

Animals and treatments

The ethical permission for this study was approved by The Health Research Ethics Commission of Universitas Airlangga: 300/EC/KEPK/FKUA/2023. A total of 32 adult male Wistar rats (*Rattus norvergicus*) aged 2-3 months with weights ranging from 220 to 250 grams[28] were randomly divided equally into 4 groups: (1) standard diet (SD), (2) high-fat diet (HFD), (3) HFD with Saffron extract 80mg/kg of body weight/day, (4) HFD with Atorvastatin 0.2 mg/200 g of body weight/day.[29,30] Each group was placed in 2 containers (53 cm x 39 cm x 17 cm), each holding 4 rats.[31] The rats were housed in a room with a stable temperature of around 22-24°C and a balanced light-dark cycle (12:12 h), with food and water provided *ad libitum*. They were acclimatized for 7 days.[32] All acclimatization and administration processes were performed at The Biochemistry Laboratory, Department of Biochemistry and Physiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. All animals were tested for their TC before the next experimentation (day 1) and were tested again on day 15. The SD group was fed *ad libitum*, a standard diet of HI-PRO-VITE CP594 PT. Charoen Pokphand Indonesia (Latitude: 7° 32' 57.552" S, Longitude: 112° 36' 43.7868" E) with the composition: 13% water, 17.5-19.5% protein, 3% fat, 8% fiber, 7% ash, 0.9% calcium, and 0.9% phosphorus. The HFD groups were fed *ad libitum*, a high-fat diet that contained 3 g beef brain, 5 g egg yolk, 46 g starch, 10 g corn oil, 4 g corn oil, and 0.15 g margarine with lard oil 10 ml/kg body weight/day), for 36 days.[28,33] Saffron extract and Atorvastatin were administered to the rats via intragastric gavage from day 16 to day 36.[34] The Saffron extract used is the stigma part, which contains the active ingredients safranal, crocin, and picocrocin (88.5 mg) and is manufactured by Piping Rock Health Products, LLC 2120 Smithtown Avenue, New York, United States (Latitude: 40° 49' 19.9488" N, Longitude: 73° 7' 47.8236" W). Atorvastatin was used as a generic product of Dexa Medica® OGB brand tablets (Batch No. 352600307 1-OT2- 000569330, Bandung, Indonesia) (Latitude: 7° 29' 11.3028" S, Longitude: 112° 42' 44.6508" E). After the study, the subjects were sacrificed, the blood plasma was collected from each animal for the TC level test, and the liver was harvested for TNF-*α* and histopathology analysis (day 37).

Selection Criteria

Inclusion Criteria

The inclusion criteria for this study were as follows: (1) male Wistar rats (*Rattus norvergicus*), (2) two to three months of age, (3) weights ranging from 220 to 250 grams, and (4) assessed as healthy, with no abnormalities or diseases using Body Condition Scoring (BCS).[35,36]

Exclusion Criteria

The exclusion criteria of this study were rats that died during the study.[37]

Determination of total cholesterol and TNF-*α* expression

Total cholesterol (TC) levels were determined by taking venous blood from the rats' tails and using UV/ Vis Spectrophotometry (UV-1900 spectrophotometer, Shimadzu Pvt. Ltd., Japan).[38,39] Meanwhile, liver organ preparations were cut transversely in the right lobe, stained with immunohistochemistry (TNF-*α* 52b83: sc-52746 Santa Cruz Biotechnology, Inc.; USA), and counterstained using Mayer's Hematoxylin.[40]

Histological analysis

To see the severity of hepatocyte damage in liver tissue using transverse or transverse sections in the right lobe with Hematoxylin-Eosin staining.[41] Histopathological examination was performed under a light microscope (Olympus CX23 light microscope, Olympus Company, Tokyo, Japan) using 200x and 400x magnification with 10 fields of view visual fields/slide/observer. Two independent examiners will make observations in a blinded manner.[42] Hepatocyte degeneration was calculated by counting the number of degenerated nuclei of hepatocytes in one field of view.[43] The inflammatory foci were calculated by counting the number and area in one field of view.[44,45] The ImageJ application calculates hepatocyte degeneration [400x of magnification]), the number of inflammatory foci (numbers per visual field [200x of magnification], and total area width per visual field [200x of magnification]).[46–48]

Statistical analysis

All data were analyzed using SPSS 24.0.[49,50] Before comparing the statistics, we conducted Shapiro-Wilk normality and Levene homogeneity tests. The total cholesterol, TNF-*α* expression level, and histopathology abnormalities between groups were analyzed using one-way analysis of variance (ANOVA). The *post-hoc* was done with the Least Significant Difference (LSD) test. A significance level of $p<0.05$ was used.[51]

Results and Discussion

Total Cholesterol (TC)

Total Cholesterol (TC I) was the first measurement after acclimatization (day 1). The second measurement was carried out after giving a high-fat diet (day 15) called TC II. The third measurement was done after giving Saffron extract or Atorvastatin (day 37), called TC III. Based on the results, the TC I levels of all animals were normal (<50 mg/dL); TC II levels in the group with a high-fat diet (HFD) increased compared to the control group with a standard diet (SD) ($p<0.01$). The TC III levels in the group with the administration of Saffron extract (HFD+Saffron) and the group with the administration of Atorvastatin (HFD+Atorvastatin) had lower TC levels than those who received a high-fat diet only (HFD) ($p<0.01$). Data on TC levels of the animals are described in Table 1.

Table 1: Data analysis of differences in total cholesterol (TC) levels between groups

No.	Groups	Mean±SD (mg/dL)		
		TC I	TC II	TC III
1.	SD	40.43±6.24	45.29±3.63	44.00±4.29
2.	HFD	45.72±5.59	61.00±5.91	69.58±6.43
3.	HFD+Saffron	44.5±5.86	61.88±5.11	55.88±5.51
4.	HFD+Atorvastatin	45.43±7.32	62.72±8.01	53.00±5.13
	One way-ANOVA (*p*)	0.380	0.000*	0.000*

Notes: SD (standard diet); HFD (high-fat diet); HFD+Saffron (high-fat diet+Saffron extract 80 mg/kg of body weight/day); HFD+Atorvastatin (high-fat diet+Atorvastatin 0.2 mg/200 g of body weight/day); TC I (total cholesterol level day 1 after acclimatization), TC II (total cholesterol level day 15 after high-fat diet administration); TC III (total cholesterol level day 37 after Saffron extract or Atorvastatin administration). *Significant with $p<0.05$

Measurement of TC II levels was carried out after the administration of a high-fat diet, namely on day 15 and on day 37. The experimental animals that received high-fat diet induction experienced increased TC levels compared to those with a standard diet (TC II). Previous studies have shown that animals induced with a high-fat diet for 15 days have increased TC levels in plasma compared to the control group.[33] In this study, the high-fat diet induction included feeding the experimental animals high fat and combining it with lard. Previous research stated that lard at a dose of 10 ml/kg body weight can increase plasma triglyceride (TG) and total cholesterol levels.[52] Feeding in excessive experimental animals, a high-fat diet significantly increases fat intake, which is then transported to the liver via chylomicrons absorbed from the intestine. The liver responds by increasing the synthesis of TG and endogenous cholesterol. Excess fat is transported to the liver, and TG is stored in hepatocytes.[53] The liver releases TG and cholesterol-rich, very low-density lipoprotein (VLDL) into the blood. In the blood, VLDL is hydrolyzed by lipoprotein lipase into intermediate-density lipoprotein (IDL), which is further converted into low-density lipoprotein (LDL). LDL carries cholesterol to peripheral tissues, so an increase in LDL leads to the accumulation of cholesterol in the blood.[2] This fat accumulation leads to a condition known as liver steatosis, where more than 5-10% of the liver weight is adipocytes. Cell degeneration in the form of steatosis and/ or necrotic hepatocytes is the next stage of nonalcoholic fatty liver disease (NAFLD). Excessive fat accumulation in the liver also triggers oxidative stress and mitochondrial dysfunction. This is caused by excessive production of reactive oxygen species (ROS) during fat metabolism.[54] Oxidative stress damages liver cells, intracellular structures, and steatosis.[27] Oxidative stress-induced damage to hepatocytes triggers an inflammatory response. Immune cells such as macrophages, Kupffer cells, were activated and produced pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1 beta (IL-1β). This chronic inflammation contributes to the development of nonalcoholic steatohepatitis (NASH), which is an advanced form of NAFLD. Chronic inflammation causes activation of liver stellate cells, which leads to excessive extracellular matrix production and tissue fibrosis. Fibrosis can progress to cirrhosis, which is the final stage of NAFLD and is characterized by the destruction of the liver structure and impaired liver function.[55]

Total Cholesterol III levels were measured after the administration of Saffron extract or Atorvastatin, which was carried out on day 37 before the termination of the animals. A significant decrease in total cholesterol levels was observed in animals that received Saffron extract compared to the group induced by a high-fat diet. Previous studies have shown that giving Saffron extract to experimental animals effectively reduces TC, low-density lipoprotein (LDL), and TG levels and increases high-density lipoprotein (HDL) levels. Similarly, previous research reported that Saffron extract can reduce blood fat levels by reducing TC levels.[56,57] The active crocin in Saffron is important in lowering blood lipid levels by reducing oxidative stress and inflammation and inhibiting cholesterol absorption in the intestine. This process may explain the decrease in TC in our study. Two previous studies also reported that Saffron extract can reduce serum TG, TC, and LDL levels while increasing serum HDL levels.[58,59]

In our study, however, the decrease of TC in HFD+Saffron animals was smaller than in the HFD+Atorvastatin group. We observed a significant reduction in total cholesterol levels in the subjects treated with Atorvastatin compared to the group induced with a high-fat diet. A study has shown that Atorvastatin administration can significantly lower total cholesterol (TC), LDL, and triglyceride (TG) levels in rats induced with a high-fat diet.[60] Similarly, this study found that Atorvastatin effectively improved hyperlipidemia in rats by reducing serum TC and LDL levels but not TG levels.[61] Atorvastatin works by inhibiting the HMG-CoA reductase, which reduces cholesterol production in the liver and increases the number of LDL receptors on the surface of liver cells to enhance LDL uptake and catabolism.[62] Atorvastatin also operates through another mechanism that reduces oxidative stress in the liver tissue and lowers the ROS level via the AMP-activated protein kinase (AMPK) pathway.[27]

TNF-α Expression in Liver Tissue

The TNF-α expression in the liver tissue of each group can be seen in Figure 1. The highest levels of TNF-α expression were found in the HFD group (Table 2). To the SD group, the levels of TNF-α expression in the HFD+Atorvastation group were closer than in the HFD+Saffron group.

Table 2: Data analysis of TNF-α expression in the liver tissue

No.	Groups	Mean±SD (% fraction area)	One way-ANOVA (*p*)
1.	SD	8.02 ± 2.72	0.000*
2.	HFD	58.68 ± 10.42	
3.	HFD+Saffron	25.13 ± 5.02	
4.	HFD+Atorvastatin	11.37 ± 4.91	

Notes: SD (standard diet); HFD (high-fat diet); HFD+Saffron (high-fat diet+Saffron extract 80 mg/kg of body weight/day); HFD+Atorvastatin (high-fat diet+Atorvastatin 0.2 mg/200 g of body weight/day). *Significant with $p<0.05$

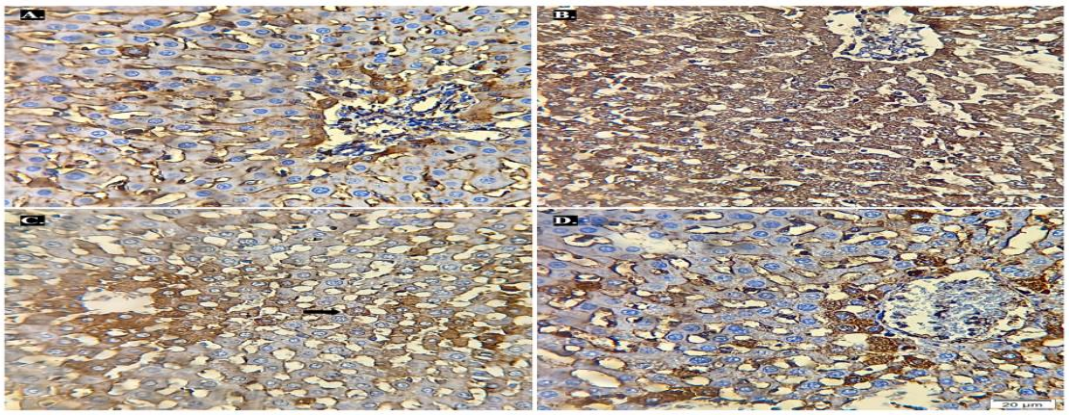

Figure 1: Expression of TNF-*α* in liver tissue. A) SD (standard diet). B) HFD (high-fat diet). C) HFD+Saffron (high-fat diet+Saffron extract 80 mg/kg of body weight/day). D) HFD+Atorvastatin (high-fat diet+Atorvastatin 0.2 mg/200 g of body weight/day). Arrows point to hepatocyte degeneration (200x magnification, scale: 20 µm).

Our study's findings align with previous research indicating that the induction of a high-fat diet can trigger chronic inflammation and increase the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-*α*).[63] A high-fat diet induces increased expression of TNF-*α* in various tissues, including the liver. This mechanism involves the activation of the toll-like receptor 4 (TLR4) and nuclear factor-kappa beta (NF-κβ) signaling pathways, leading to higher production of pro-inflammatory cytokines, including TNF-*α*. Other research has shown that a high-fat diet causes an imbalance in the gut microbiota, subsequently increasing the production of lipopolysaccharides (LPS) and inflammatory cytokines like TNF-*α* and interleukin-1 beta (IL-1β), contributing to liver inflammation, steatosis, and degeneration.[64] Similarly, previous studies have reported that the inflammatory response due to a high-fat diet is triggered by increased TNF-*α* expression in the liver.[65]

Previous research has demonstrated that Saffron extract has hepatoprotective effects against carbon tetrachloride (CCL4) induced liver fibrosis in rats. Saffron administration significantly reduces the expression of pro-inflammatory cytokines, including TNF-*α*, in the liver tissues by mitigating liver inflammation and fibrosis through its anti-inflammatory and antioxidant properties of crocin and picocrocin.[57] A study showed that administering Saffron extract to rats on a high-fat diet significantly reduced TNF-*α* levels in liver, kidney, and lens tissues.[66] However, the exact mechanisms have been debatable, and further investigation is needed to fully understand its drug-likeness potential therapeutic effects in hypercholesterolemia.[58,59]

In this study, Atorvastatin administration was found to significantly lower the mean TNF-*α* expression in liver tissue of high-fat diet-induced animal models compared to controls, and the reduction was higher than that in those treated with Saffron extract (Tabel 2). Atorvastatin is widely known for its ability to reduce cholesterol and lipid levels in the blood, primarily through the inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase via various biochemical mechanisms.[20,67] These mechanisms include lipotoxicity, oxidative stress, inflammation, and apoptosis, all of which contribute to the pathogenesis and progression of metabolic syndromes, including hypercholesterolemia.[68] The mechanism of Saffron as anti-hypercholesterolemia was reported in several previous studies, arguably via the work of its substantial components, i.e. anthocyanin, flavonoids, and glycosides. These components' antioxidant and anti-inflammatory properties heightened the LDL oxidation, while the ethanolic extract of Saffron stigma and petals the absorption of dietary fat due to hydrolysis of the lipids. On the other hand, crocin is reported to work as a competitive inhibitor of pancreatic lipase enzyme, hence producing mall-absorption of the lipids. [34,69,70]

Histopathology of The Liver

The hepatocyte degeneration, as indicated by necrotic hepatocyte nuclei in HFD, was the highest. When compared to controls, the necrosis was higher in the HFD+Saffron group than in the HFD+Atorvastatin group (p=0.000). The description of hepatocyte degeneration in each group of experimental animals can be seen in Table 3.

Table 3: Data analysis of histopathology of the liver tissue

No.	Groups	Mean±SD			One way-ANOVA (p)
		Hepatocyte cell degeneration in µm	Number of inflammatory foci in µm	Area of inflammatory foci in µm^2	
1.	SD	4.14±1.78	0.95±0.12	1664.14±559.65	0.000*
2.	HFD	13.07±5.48	3.18±0.73	15275.01±5338.48	
3.	HFD+Saffron	9.97±1.61	2.28±0.29	9746.49±3607.14	
4.	HFD+Atorvastatin	8.05±1.47	1.73±0.40	5778.88±1612.53	

Notes: SD (Standard diet); HFD (High-fat diet); HFD+Saffron (High-fat diet + Saffron extract 80 mg/kg of body weight/day); HFD+Atorvastatin (High-fat diet + Atorvastatin 0.2 mg/200 g of body weight/day). *Significant with p<0.05

The inflammatory foci (both in numbers and area width) decreased after treatment with Saffron extract compared to controls (Figure 2). This is consistent with previous research reports that Saffron extract can significantly dampen hepatocyte cell degeneration, including steatosis and inflammatory foci.[71] Other studies have also suggested that Saffron powder can be used to prevent NAFLD by improving conditions of cell degeneration, including steatosis, hepatocyte ballooning, and inflammatory foci, i.e., by modulating peroxisome proliferator-activated receptor alpha (PPAR*α*) gene expression, which indirect hepatotoxic effect via the decrease of the mitochondrial β-oxidation that impaired the regulation of the uptake and clearance of fatty acids.[55] As demonstrated in previous studies, the mechanism by which Saffron extract reduces fat accumulation in the liver can prevent hepatocyte cell degeneration from progressing to liver steatosis.[56] In our study, however, these decreases were smaller when compared to the HFD+Atorvastatin group (Figure 2).

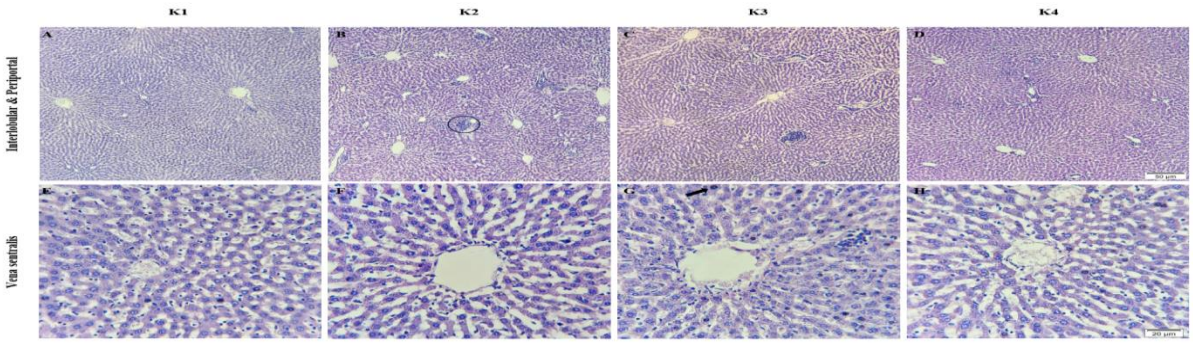

Figure 2: Histopathological features of liver. SD (standard diet); HFD (high-fat diet); HFD+Saffron (high-fat diet+Saffron extract 80 mg/kg of body weight/day); HFD+Atorvastatin (high-fat diet+Atorvastatin 0.2 mg/200 g of body weight/day). A) Normal interlobular & periportal area with microscope magnification 200x, scale: 50 µm. B) Numerous inflammatory foci were observed. C) Fewer inflammatory foci compared to the HFD group. D) Fewer inflammatory foci compared to the HFD+Saffron group. E) Central vein area with normal hepatocytes and microscope magnification 400x, scale: 20 µm. F) Significant hepatocyte cell degeneration was observed. G) Less hepatocyte degeneration compared to the HFD group. H) Less hepatocyte degeneration compared to the HFD+Saffron group. The circles indicate areas of inflammatory focus, and the arrows point to hepatocyte degeneration.

Previous research has revealed that Atorvastatin can significantly lower plasma cholesterol levels and improve inflammatory foci and fibrosis, thereby serving as a therapeutic agent in hypercholesterolemia.[72] The liver is the primary organ where cholesterol synthesis occurs. Cholesterol synthesis has increased significantly in rats treated with Atorvastatin as a compensatory response, although the excess synthesized cholesterol does not accumulate in the plasma as plasma cholesterol levels decrease. Thus, Atorvastatin administration led to increased cholesterol excretion through feces and heightened cholesterol elimination, which reduces cholesterol accumulation in the liver.[22] This is consistent with previous research reporting that Atorvastatin inhibits the HMG-CoA reductase, thereby reducing cholesterol production in the liver. This inhibition is the primary mechanism by which Statin drugs lower blood cholesterol levels. [27,62]
In previous research, induction of a high-fat diet has been shown to cause triglyceride (TG) accumulation within hepatocytes, known as liver steatosis, which is the initial stage of nonalcoholic fatty liver disease (NAFLD).[73] Other studies have also reported that Wistar rats induced with a high-fat diet combined with lard exhibited a 2- to 4-fold increase in TG concentration, which is a trigger for metabolic syndrome.[74] The excessive influx of fatty acids into cells leads to increased mitochondrial stress and the release of reactive oxygen species (ROS), where ROS act as triggers that regulate cell apoptosis, referred to as lipoapoptosis. Sustained lipoapoptosis can result in tissue and organ damage, including the liver.[75] Moreover, a high-fat diet can lead to the infiltration of inflammatory cells within the liver lobules, a sign of liver inflammation. This finding is consistent with previous research that revealed significant increases in the infiltration of inflammatory cells within the liver lobules due to a high-fat diet.[1] Histological examinations showed the presence of macrophages and other inflammatory cells infiltrating liver tissue, indicating a strong inflammatory response due to the high-fat diet.[76]
Although the anti-hypercholesterolemia, anti-inflammatory, and hepatoprotective drug-likeness of Saffron extract in HFD animals in our study were less than that of Atorvastatin, we succeeded in demonstrating that these three effects exist in the Saffron extract and significantly affect liver health. Future studies are called to analyze the component of Saffron that has the most drug-likeness in terms of combating NAFLD and NASH.

Conclusion

In conclusion, prolonged fat accumulation in the liver, induced by a high-fat diet, leads to hepatocyte degeneration and inflammatory foci formation. Both Saffron extract and Atorvastatin have an anti-inflammatory, hepatoprotective effect, although the latter showed a more prominent result.

Conflict of Interest

The authors disclose no conflict of interest.

Authors' Declaration

The authors hereby attest to the originality of the work presented in this article and assume all liability for any claims pertaining to its content.

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