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ORIGINAL ARTICLE

Achillea Fragrantissima Ethanolic Extract Exerts Hypocholesterolemia and Hepatic Antioxidant Effects in High Fat-Cholesterol Diet: An Experimental Study

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ABSTRACT

Background: Hypercholesterolemia and oxidative stress consider the main causes for atherosclerotic cardiovascular diseases, that are one of the major non-communicable diseases responsible for more than a third of deaths in Saudi Arabia. Cholesterol-lowering medications as Atorvastatin® (ATOR) are linked to a variety of side effects. *Achillea fragarntissima* (AF) is a valuable medicinal plant in Saudi Arabia with potent antioxidant activity.

Aim: The current study was performed to determine the efficacy of AF in the treatment of hypercholesterolemia through the antioxidant metabolic pathway.

Methodology: Dried aerial parts of AF were extracted by ethanol (70%). Induction of hypercholesterolemia in rats was induced through feeding a high fat-cholesterol diet (HFCD) for 8 weeks. Rats were assigned to two main groups; control group (Cont, n=10) rats fed a standard diet, and hypercholesterolemic group (HFCD) (n=40) rats fed HFCD. The HFCD group was further assigned after measured lipid profile to confirm the induction of hypercholesterolemia to HFCD; HFCD+AF (hypercholesterolemic rats treated orally with 500 mg/kg AF); HFCD+ ATOR (hypercholesterolemic rats treated orally with 20 mg/kg ATOR, as a reference drug); and HFCD+AF+ATOR (hypercholesterolemic rats treated orally with AF+ ATOR). Different treatments were ingested to rats for 4 weeks.

Results: The results revealed that the HFCD group showed significant hyperlipidemia (elevation of serum TC, TG, LDL-C, and VLDL-C levels concurrent with a reduction in serum HDL-C level); significant disturbance in liver functions (elevation in serum ALT, AST, and ALP enzymes activities); and significant oxidative stress (elevation in hepatic MDA level with a reduction in hepatic SOD activity) compared with the Cont group. Besides, hepatic central vein section showed deposition of large lipid within hepatocytes and abundant focal cell necrosis. Oral treatment with AF, ATOR, and the mixture of the drug and AF produced significant hypocholesterolemia, antioxidant, and improved liver function enzymes, with normalized hepatic central vein tissue compared with the HFCD group. The mixture of AF+ATOR had a superior effect than either treatment alone.

Conclusion: In hypercholesterolemic rats, AF may be used to prevent atherosclerosis through improving lipid profile levels, protecting against hepatic oxidative stress, and ameliorating hepatic functions. Thus highlighting its valuable effects in the treatment of atherosclerotic cardiovascular diseases.

Keywords: *Achillea fragarntissima*, lipid profile, hepatic oxidative stress, hepatic function, hypercholesterolemia.

INTRODUCTION

Cholesterol is a necessary substance that has physiological roles to maintain a healthy body. However, if the level of cholesterol in the blood rises significantly, it is called hypercholesterolemia, it causes a variety of harmful conditions including cardiovascular diseases, atherosclerosis, and nonalcoholic fatty liver disease[1]. Cardiovascular disease is caused by a number of factors, with hypercholesterolemia being one of the most important one [2]. Cardiovascular diseases are one of the main non-communicable diseases to be accountable for 32% of all deaths worldwide in 2019 [3]. In Saudi Arabia, cardiovascular diseases have been responsible for about 42% of deaths[4].

Management of hypercholesterolemia is of a high importance in the prevention of cardiovascular events[5,6]. A number of cholesterol-lowering medications, such as statins, PSCK9 inhibitors, fibrates, and bile acid sequestrants are available to treat hypercholesterolemia. Yet, they have been linked to a variety of side effects [7]. Therefore, there has been a considerable interest in promoting natural remedies that have a positive impact in the management of blood lipid levels while diminishing negative consequences [8,9]. Achillea plant is one of the most well-known genera in the family Asteraceae, with over 115 species[10]. One of the most famous plant of this species is *Achillea fragarntissima* (AF). It is considered one of the valuable plants in Saudi Arabia and is known locally as Qaysūm in Arabic [11,12]. *Achillea fragarntissima* is widely found in desert and has been utilized in traditional Arabian medicine for years to treat respiratory, gastrointestinal, diabetes, and other various diseases[13-15].

Extracts of different parts of AF have yielded a variety of constituents to be highly bioactive, including flavonoids (luteolin, vitexin, apigenin, apigeninglucoside, apigenin-neohesperioside, apigenin-rutinoside, chrysophanol D, diosmetin, cirsiliol, and chrysoplenetin), phenolic acids (vanilic, ferulic, protocatechuic, quinic acid, and chlorogenic), lignans (sesamin), alkamides (pellitorin, , anacyclin and 8,9-Z-dehydropellitorin), and terpenic lactones (achillolid A) [16-18].

The plant has been reported to exhibit pharmacological effects such as anti-inflammatory [19], antioxidant [20], antinociceptive [21], antimicrobial [22], antitrypanosomal [23], antidiabetic [24], and antitumor [25]. Due to these beneficial properties, AF can be recognized as a valuable medicinal plants. The current research area of investigating the effect of AF on hypercholesterolemia is lacking. Therefore, this study was conducted to determine the efficacy of AF in the treatment of hypercholesterolemia that could potentially shed the light on the development of a natural medicinal alternative to reduce adverse effect of cholesterol-lowering medications.

MATERIAL AND METHODS

Drugs, chemicals, and kits: Astatin as tablets containing 40 mg Atorvastatin® (Jamjoom Pharmaceutical Company, Jeddah, Saudi Arabian) was purchased from Al Dawaa Pharmacies, Jeddah, Kingdom of Saudi Arabian (KSA). Enzymatic colorimetric kits were provided from Centronic Chemicals Co, Germany. ELISA kits were obtained from Glory Science Co. (Ltd. Del Rio-TX-USA). Bile salt and cholesterol (95%) as white crystalline powder were purchased from Acros Organics Company.

Laboratory animals: Fifty male albino rats (180-210 g) were used in this research. Animals were purchased from KFMRS (King Fahad Medical Research Center), KAU, KSA. Rats were acclimatized under standard laboratory conditions for one week. All rats received care in compliance with the rules of Candian ethics upon approval for KFMRS, KAU, KSA.

***Achillea fragrantissima* (AF) collection:** The aerial parts of AF were collected around Riyadh, KSA. Authentication of the plant

was performed by botanists in the Department of Pharmaceutical and Phytochemistry, Faculty of Pharmacy, KAU, KSA.

Extraction of AF: Powdered dried aerial parts of AF (at 40 °C), after being washed to remove dirt, were ground. Five hundred g of AF powder was extracted by ethanol (70 %). The resulting ethanol extracted was filtrated and evaporated to dryness under reduced pressure using a rotary evaporator (Rota-vapor R-215, Bŭtchi, Switzerland) at less than 35 °C. The dried extract was freeze-dried for 48 h, at –20°C to yield a solid extract.

Each 100 g of AF yield 3.99 g extract, the AF extract was stored at 4 °C until further use. For usage preparation; dried AF extract (100 mg) was diluted with 200 ml distilled water to prepare 0.5 mg/ml solution, which centrifuged to remove any un-dissolved components [25]

Hypercholesterolemia rats model: After the accommodation period, the hypercholesterolemia model was induced as described in previous research [26,27]. At random, the rats (n=50) were assigned to two main groups according to their diets; group one control (Cont, 10 rats); rats fed a basal standard diet and group two (hypercholesterolemic group, 40 rats); rats fed high fat-cholesterol diet (HFCD) [which containing: 2% cholesterol, 1 % bile salt, and 10 % saturated fat] for eight weeks. After 8 weeks blood samples were collected for measuring the lipid profile parameters. Rats with hypercholesterolemia as defined by Kalsoom and Jafari [28] were randomly assigned into four groups (10 each) HFCD; hypercholesterolemic rats fed HFCD and given orally 1 ml of saline daily; HFCD+AF; hypercholesterolemic rats fed HFCD and received concomitant treatment with an oral dose of AF extract (500 mg/kg animal weight (AW)) [24]; HFCD+ ATOR; hypercholesterolemic rats fed HFCD and received concomitant treatment with Atorvastatin (ATOR) as a reference drug (20 mg/kg AW) [29]; and HFCD+ AF+ ATOR; hypercholesterolemic rats fed HFCD and received concomitant treatment with AF extract and ATOR. The rats were given different treatments for four consecutive weeks; rats in the Cont group were orally given saline for 4 weeks.

Samples collection: After the end of the experiment (12 weeks), blood samples were individually withdrawn, then separated serum samples were kept at -80°C for further use in biochemical analysis. Liver samples were collected and preserved either frozen for determination of oxidative stress or in 10% buffered formalin for histopathology.

Determination of serum lipid parameters and liver functions: Serum levels of total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were assessed as the enzymatic colorimetric kits' manufacturer instructions. While very low-density lipoprotein cholesterol (VLDL-C) and atherogenic index (AI) [Log 10 (TG/HDL-C)] were calculated (Oršoli´c et al., 2019). Liver enzymes activities (AST, ALT, and ALP) were assessed as enzymatic colorimetric kits' manufacturer instructions.

Determination of hepatic antioxidant status: The homogenized liver samples were used to assess superoxide dismutase (SOD) activity and the malondialdehyde (MDA) content as ELISA kits' manufacturer instructions.

Histopathological examinations (hematoxylin and eosin (H & E) stain): Liver samples from each group were preserved in neutral buffered formalin (10 %), trimmed, dehydrated in alcohol, cleared in xylene, stained with H&E, then examined under a light microscope (Olympus BX51, USA) for any pathological change.

Statistical analysis: The recorded results were analyzed using SPSS version 25. The results were presented as mean ± SD. The LSD test was used to compare between the different rats groups (P- value of ≤ 0.05 was considered significant).

RESULTS

Impact of *Achillea fragrantissima* extract (AF), Atorvastatin (ATOR), and their mixture on lipid profile in HFCD-fed rats: Table (1) demonstrated the effect of AF, ATOR and their mixture on TC and TG in HFCD-fed rats. Feeding HFCD caused significant increases in both TC and TG (111.95 ± 3.95 and 85.32 ± 1.9, respectively) as compared with the Control (Cont) group (102.18 ± 1.56 and 56.43 ± 1.5, respectively). Oral administration of AF, ATOR and the mixture of the drug and AF produced significant reduction in serum TC and TG levels (104.66 ± 4.43, 101.32 ± 5.45 and 101.02 ±4.34 and 61.85 ± 1.4, 57.31 ± 1.1 and 54.50 ± 1.2, respectively) ($p<0.05$) in comparison to the HFCD group.

Table 1. Hypocholesterolemic effects of AF, ATOR and their mixture on TC and TG in HFCD-fed rats

Groups	TC (mg/dL)	TG (mg/dL)
Cont	102.18 ± 1.56	56.43 ± 1.5
HFCD	111.95 ± 3.95 #	85.32 ± 1.9 #
HFCD + AF (500 mg/kg)	104.66± 4.43 ^	61.85 ± 1.4 ^
HFCD + ATOR (20 mg/kg)	101.32 ± 5.45 ^	57.31 ± 1.1^
HFCD + AF + ATOR	101.02 ± 4.34 ^	54.50 ± 1.2 ^

Data represent mean ± SD. #represents significant relative to control rats, ^ represents significant relative to HFCD group. p ≤ 0.05.

TC: Total cholesterol; TG: Triglyceride.

As depicted in Table 2 which showed the effect of AF, ATOR and their mixture on HDL-C, LDL-C, VLDL-C and AI in HFCD- fed male rats. Serum levels LDL-C and VLDL-C were significantly increased whereas serum level of HDL-C was significantly decreased relative to the Cont group. Oral treatment with AF, ATOR and their mixture caused a significant increase in HDL-C (51.95 ± 1.3, 51.10 ± 2.1, and 52.90 ± 2.4, respectively) associated with significant decreases in serum levels of LDL-C and VLDL-C (39.34 ± 1.8, 38.76 ± 1.2, 37.22 ± 1.6 for LDL- C, 12.37± 1.5 , 11.46 ± 2.8 and 10.90 ± 1.2 for VLDL-C, respectively) as compared with the HFCD group (34.73 ± 2.1, 60.16 ± 1.4 and 17.06 ± 3.7) for HDL-C, LDL-C, and VLDL-C levels, respectively.

Concerning the atherogenic index (AI) in different groups, HFCD exerted a significant increase in AI relative to the Cont group. On the other hand, oral treatment with AF, ATOR and their mixture produced a significant decrease in AI relative to the HFCD group (Table 2).

Table 2. Hypocholesterolemic effects of AF, ATOR and their mixture on HDL-C, LDL-C, VLDL-C and AI in HFCD-fed rats

Groups	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	AI
Cont	53.82 ± 1.8	37.07 ± 1.6	11.29 ± 1.9	0.02 ± 0.003
HFCD	34.73 ± 2.1#	60.16 ± 1.4 #	17.06 ± 3.7 #	0.39 ± 0.002#
HFCD + AF (500 mg/kg)	51.95 ± 1.3 ^	39.34 ± 1.8 ^	12.37± 1.5 ^	0.08 ± 0.005^
HFCD + ATOR (20 mg/kg)	51.10 ± 2.1 ^	38.76 ± 1.2^	11.46 ± 2.8 ^	0.05 ± 0.004^
HFCD + AF + ATOR	52.90 ± 2.4 ^	37.22 ± 1.6 ^	10.90 ± 1.2 ^	0.01 ± 0.001^

Data represent mean ± SD. # represents significant relative to control rats, ^ represents significant relative to HFCD group. p ≤ 0.05.

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; AI: Atherogenic index

Impact of AF, ATOR, and their mixture on liver enzymes activities in HFCD-fed rats: Data reported in Figure (1) showed that the HFCD group had significant ($p<0.05$) increases in the serum AST, ALT, and ALP levels (92.45 ± 2.1, 60.57 ± 2.4, and 118.41 ± 1.2, respectively) compared with the Cont (63.62 ±1.8, 37.15 ± 1.6, and 86.59 ± 1.9, respectively). Oral administration of AF, ATOR and the mixture of the drug and AF produced significant decreases in all enzymes liver activities (ALT, AST, and ALP) comparison to the HFCD group. The group treated with the mixture

of the AF and ATOR had a better effect than either AF or ATOR alone.

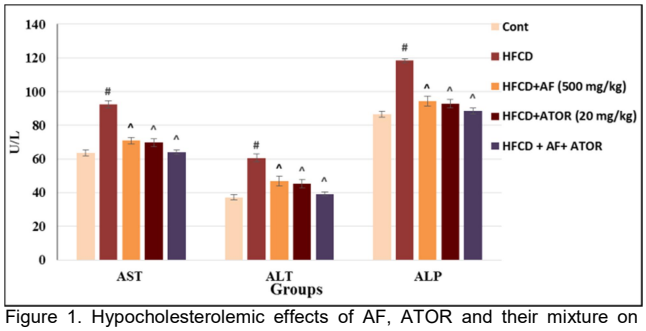

Figure 1. Hypocholesterolemic effects of AF, ATOR and their mixture on AST, ALT and ALP in HFCD-fed rats

Data represent mean ± SD. #represents significant relative to control rats, ^ represents significant relative to HFCD group. p ≤ 0.05.

Impact of AF, ATOR, and their mixture on hepatic malondialdehyde (MDA) in HFCD-fed rats: After four weeks of treatment, the HFCD group had a significant rise ($p<0.05$) in hepatic MDA levels as compared to the Cont group. Treatment with AF or drug induced significant reduction ($p<0.05$) in MDA value in comparison to the HFCD group. The mixture of AF and ATOR had the better effect in comparison to the HFCD in reducing the hepatic MDA level (Figure 2).

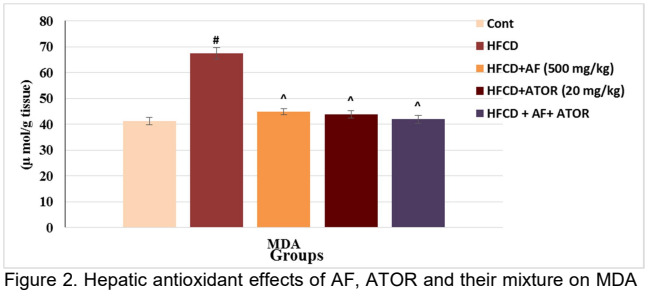

Figure 2. Hepatic antioxidant effects of AF, ATOR and their mixture on MDA in HFCD-fed rats.

Data represent mean ± SD. #represents significant relative to control rats, ^ represents significant relative to HFCD group. p ≤ 0.05.

Impact of AF, ATOR, and their mixture on hepatic antioxidant SOD activity in HFCD-fed rats: The results revealed that the HFCD group recorded significant reduction in hepatic SOD activity ($p<0.05$) when compared to the Cont group after four weeks of treatment. The treatment with AF or drug induced significant improvement ($p<0.05$) in hepatic SOD activity relative to the HFCD group. The mixture of AF and ATOR had the better effect in comparison to the HFCD group in increasing the hepatic SOD activity (Figure 3).

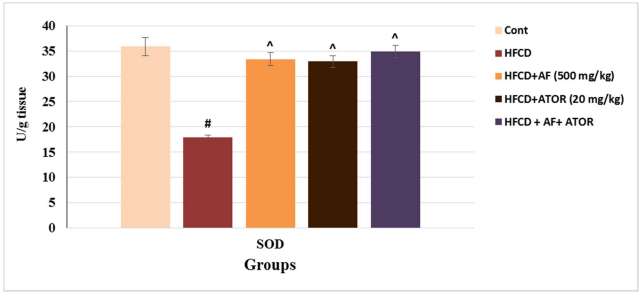

Figure 3. Hepatic antioxidant effects of AF, ATOR and their mixture on hepatic SOD activities in HFCD-fed rats.

Data represent mean ± SD. #represents significant relative to control rats, ^ represents significant relative to HFCD group. p ≤ 0.05.

Impact of AF, ATOR, and their mixture on liver tissue histopathology in HFCD-fed rats: Figure 4.A represented a photomicrograph of a liver segment from the Cont rats, a normal central vein (CV) can be seen, surrounded by normal hepatocytes and blood sinusoids. A liver section from HFCD rats showed many hepatocytes, numerous unstained vacuoles (lipids), and localized cell necrosis (Fig.4.B). The majority of hepatocytes in a liver segment at CV looked normal, with active nuclei and no lipid deposits visible. There is an exception of a few cells in the HFCD + AF animals group that displayed small fat droplets and moderate sinusoid dilatation (Fig.4.C). A liver segment from the HFCD +ATOR rats group showing no fat formation in hepatocytes, with the exception of mildly inflammatory cells near the portal area (Fig.4.D). A liver segment at CV from the HFCD + AF +ATOR rats group showing normal hepatocyte structure with no lipid buildup (Fig.4.E).

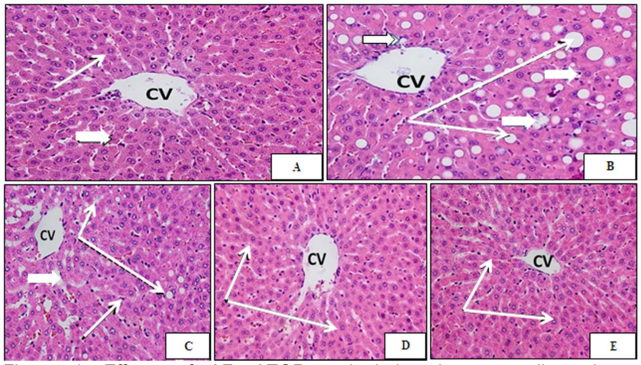

Figure 4. Effects of AF, ATOR and their mixture on liver tissue histopathology in HFCD-fed rats (Bar = 50 µm). Figure (4 A) showing a photomicrograph of a liver segment from the Cont rats, there is a normal central vein (CV) visible, surrounded by normal hepatocytes (big arrows) and blood sinusoids (large arrow). A liver section from HFCD rats showing many hepatocytes, numerous unstained vacuoles (lipids) (big arrow) and localized cell necrosis (arrows) (Fig.4.B). The majority of hepatocytes in a liver segment at CV looked normal, with active nuclei and no lipid deposits visible (big arrows). There is an exception of a few cells in the HFCD + AF group that displayed small fat droplets and moderate sinusoid dilatation. (Fig.4.C). A liver segment from the HFCD +ATOR group showing no fat formation in hepatocytes, with the exception of mildly inflammatory cells near the portal area (big arrows) (Fig.4.D). A liver segment at CV from the HFCD + AF +ATOR rats group showing normal hepatocyte structure with no lipid buildup (big arrows) (Fig.4.E).

DISCUSSION

Hypercholesterolemia is defined as an increase in total cholesterol levels in the blood. Basically, it is a type of "hyperlipidemia" (high lipid level in the blood) and "hyperlipoproteinemia" (high lipoprotein level in the blood) [30]. Hypercholesterolemia has several complications; it induced heart diseases, stroke, peripheral vascular disease [31]. Statins are the most powerful and efficient drugs for treatment of hyperlipidemia [32]. Through antioxidant, anti-inflammatory, and other mechanisms, atorvastatin has been demonstrated to be a highly effective member of the statin family for stabilizing plaque, avoiding strokes and decreasing blood cholesterol [33]. People are becoming more interested in plant-based antioxidants as a result of their significance in maintaining human health. The AF or Qaysoom is used for folk medicine [34]. The AF is rich in phenolic acids, terpenoids, flavonoids, phenolics, and lignans [35]. The goal of this research was to investigate if AF, ATOR, and their combination might be beneficial to treat hypercholesterolemic rats.

The condition of hyperlipidemia is a substantial risk factor for developing atherosclerosis, it is defined as an increase in TC, TG, and LDL levels as well as a reduction in HDL level [36]. The risk of

coronary heart disease (CHD) was also found to be reduced when LDL levels were reduced[37]. Furthermore, higher plasma HDL levels are linked to a lower risk of CHD. HDL is widely believed to lower the risk of atherosclerosis by facilitating reverse cholesterol transference [38].

The current findings revealed that the levels of TG, TC, VLDL, LDL, AI, as well as HDL in the plasma of the HFCD rats differed significantly from those in the Cont rats. The AF, ATOR and their mixture reversed these changes. These results may be due to glycosides, sterols, flavonoids and triterpenes found in AF [39]. Al-Shami et al. (2013) reported that the presence of a number of glucosides, alkaloids, triterpenoids, lignan, sterols, anthraquinones, and lactones might account for AF antihypercholesterolemic effect [40]. Besides, the AF reversed bile acid production, according to the suppression of TG levels. As a result of this extract's large reduction in TG levels, it's important to note that VLDL-C production will be disrupted [41], because VLDL-C is indirectly engaged in LDL-C formation, and so VLDL-C raises plasma LDL-C [42].

It had been observed that plasma TG concentrations are inversely related to HDL-C levels [43], so if AF treatment lowers TG level, the HDL-C level should be rised. These findings suggested that AF could be used as a supplement to help people with hyperlipidemia. The hypolipidemic effect of AF could be explained via enhancing lecithin-cholesterol acyl transferase activity. Endothelial cells need this enzyme to convert free cholesterol to HDL, as well as increase reverse cholesterol transport and inhibit absorption of LDL[44].

Administration of ATOR to HFCD rats resulted in decreased blood TC and LDL levels, as well as a lower atherogenic index, whereas serum HDL level was increased, as reported by Bakker-Arkema et al. [45] and Hunninghake et al. [46]. These findings could be explained by the enzyme HMG-CoA reductase, which catalyzes the alteration of HMG-CoA to mevalonate, a rate-limiting step in the synthesis of endogenous cholesterol, which is inhibited by ATOR. The up-regulation of LDL receptors on the cell membrane is responsible for the enhanced removal of LDL from plasma [45].

This investigation confirmed that the HFCD rats had significantly higher levels of liver enzymes than the Cont rats, but rats treated with AF, ATOR, or their combination had significantly lower levels of liver enzymes. After treatment with AF, there was a substantial drop in liver enzymes, indicating that the Achillea extract is hepatoprotective as proved in Al-Ezzy et al. [47]. The antioxidant components of AF, such as terpenoids, phenolics, phenolic acids, flavonoids, and lignans, are thought to play a role in hepatoprotection role of AF [35]. Flavonoids are antioxidants, free radical scavengers, and anti-lipoperoxidants that protect the liver[48]. The results were confirmed by the histopathological findings in the treated groups.

The etiology of atherosclerosis is heavily influenced by oxidative stress [49]. In the current study, hyperlipidemic rats had a large increase in hepatic MDA content and a considerable decrease in the hepatic antioxidant enzyme SOD content. Increased hepatic MDA causes the production of free radicals, which result in decreased membrane functions eventually leading to macrovascular and microvascular dysfunction [50]. Hyperlipidemic rats had elevated MDA levels and decreased SOD levels, which were effectively reversed by administration of AF, ATOR, or their mixture. This could be owing to the phytochemicals found in AF, such as flavonoids, saponins, and tannins, which have been shown to have antioxidant properties [51]. Antioxidants reduce lipid peroxidation and other free radical-mediated actions by acting as radical scavengers. As a result, they aid in the prevention of a wide range of diseases caused by radical reactions [52].

CONCLUSION

In light of the obtained results, AF showed the ability to lower liver enzyme levels and lipid levels, as well as neutralize the oxidative stress in hypercholesterolemic rats. More research on AF is needed to ensure that it will be effective in treating hypercholesterolemia in humans.

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