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Exploring the Potential of Rhubarb Extract in Combating Dyslipidemia, Hepatic Damage, and Oxidative Stress in Hypercholesterolemic Rats

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Abstract

This work intended to determine the therapeutic efficacy of Rhubarb (Rhu) (*Rheum rhabarbarum*, family Polygonaceae)rhizome extract on lipid profile and hepatic functions and explore its mechanism as an antioxidant agent in hypercholesterolemic rats. Hypercholesterolemia was induced by feeding the rats with a high-cholesterol diet (HCD) for 4 weeks. Fifty rats were distributed into 5 groups: 1-control, 2-HCD, 3-HCD+ Atorvastatin (Atorva) (20 mg/kg), 4-HCD+Rhu extract (75 mg/kg), and 5-HCD+Rhu extract (150 mg/kg). Serum lipid profiles [TC, HDLc, LDLc, VLDLc, and TG], hepatic function enzymes biomarkers [ALT, AST, and ALP], and antioxidants markers [MDA, SOD, and CAT] were assessed. Besides, the hepatic histopathological changes were examined in all groups. The results revealed that alkaloids, flavonoids, saponins, terpenes, and anthraquinones were high in Rhu extract. The HCD group showed significantly elevated lipid levels, hepatic dysfunction, and oxidative stress relative to the control group ($p \leq 0.05$). Hepatic tissues had severe changes, increased lipid droplets within many hepatocytes, congestion, and inflammation of hepatic sinusoids. Treatment with Rhu extract at 150 mg/kg significantly corrected and restored these changes. Rhu extract significantly reduced serum lipid profile and alleviated hepatic function. In addition, it significantly restored the antioxidant enzymes activities and reduced lipid peroxidation relative to the HCD group ($p \leq 0.05$). These findings suggest that Rhu extract has markedly hypolipidemic and hepatoprotective effects. Its antioxidant active compounds could explain the underlying mechanics. Therefore, Rhu extract might be developed as a therapeutic agent for managing hypercholesterolemia. Further in-depth investigations of its mechanism are needed.

Keywords: Rhubarb; Rats; Hypercholesterolemia; Hepatoprotective; Antioxidant

1. Introduction

High blood cholesterol level (HC), defined as "abnormally elevated levels of cholesterol in the blood," is a prominent risk factor for cardiovascular diseases (CVDs) globally [1]. Chronic or uncontrolled HC can lead to significant problems such as heart stroke or coronary heart disease [2]. According to the Saudi Health Council National Heart Center, 35 % of Saudi population have hypercholesterolemic, which is significantly higher relative to other countries, placing them at risk of CVDs [3]. From a view of health economics, CVDs entail a burden on healthcare systems directly *via* expenditure and indirectly *via* living years with diseases, low productivity, and premature mortality. The Saudi Vision 2030 recently aims to scale up vitality and longevity *via* decreasing CVDs` economic and clinical burden in a new era of comprehensive healthcare [4]. As a result, keeping cholesterol levels normal is advised to reduce the risk of CVDs.

Many prescription drugs are successful in lowering cholesterol levels. They are, however, pricey and have been linked to serious side effects [5]. Although, adopting a low-saturated-fat diet is an effective dietary strategy for lowering blood cholesterol levels. Nonetheless, these diets are difficult to adopt because of their low palatability and acceptability [6]. According to various sources, medicinal plants serve as the basis for 25–50 % of currently produced drugs used in healthcare [7,8].Because of the increased emphasis on the adverse effects of utilizing synthetic compounds to treat diseases, pharmaceutics is increasingly focused on developing innovative, effective, and less harmful drugs. Natural compounds derived from medicinal plants have greatly aided in the development of medications for various diseases [9].

Rhubarb (Rhu) (*Rheum rhabarbarum*) (Rheum L., Polygonaceae family), one of the most well-known medicinal herbs, is widely used in traditional medicine in Asian countries and is also found in many ancient Greek and Roman treatments. Many

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investigations have shown that Rhu rhizome extract has anticancer [10], antibacterial [11], and anti-inflammatory[12] activities. Furthermore, Rhu has been proven to have hepatoprotective, antiviral, and cholagogue effects [13,14]. It protects against central nervous system diseases [15] and relieves constipation [16]. Over a hundred chemicals have been discovered and extracted from Rhu so far. Anthraquinones, such as rhein, emodin, and aloe-emodin, are among these chemicals [17]. The principal bioactive ingredients, such as physcion, chrysophanol, and their glycosides, have been shown to have antioxidant properties [17].

This research aimed to assess if Rhu extract had hypocholesterolemic, hepatoprotective, and antioxidant effects in hypercholesterolemic rats.

2.Materials and Methods

2.1. Herb material, chemicals, and drug

Rhubarb (Rhu) rhizome was obtained from Abazeer organic food stories in Jeddah, Saudi Arabia (SA). All chemicals were purchased from Scientific Fisher (Germany) and were of high analytical grade. Lorvast® Atorvastatin 20 mg (Tabuk Pharmaceutical Manufacturing Company, Tabuk, SA) was obtained from Al-Dawaa Pharmacies, Jeddah, SA.

2.2. Rats

From King Fahd Medical Research Center (KFMRC), KAU, 50 male albino rats weighing 170 -190 g were provided. The experimental investigation in KFMRC, KAU, SA fulfilled Canadian ethical standards. This study protocol was approved by The Research Ethics Committee Unit, Faculty of Medicine - KAU (Reference No 731-22).

2.3. Rhubarb extract preparation

Fifty g of crushed Rhu rhizome was extracted twice in 750 mL of 60% ethanol over 2 hours under reflux at 60°C. Filtrates were combined and evaporated to dryness using a rotatory evaporator under a vacuum at 60 °C. Until it was used, the extract was kept at -20°C [18].

2.4. Phytochemical screening of Rhu extract

Qualitative phytochemical analysis of various phytocompounds was performed using standard procedures. Qualitative analysis of alkaloids was conducted using Wagner's test; 50 mg of the Rhu extract was dissolved in diluted HCl, then by gentle addition of Wagner's reagent (few drops) along the test tube side, the formation of reddish-brown precipitates indicates the presence of alkaloids [19]. Qualitative analysis of steroids was conducted by dissolving 50 mg of the Rhu extract in 2 ml of each concentrated H_2SO_4 and chloroform, the formation of red colour in the lower layer indicates the presence of steroids [20]. Qualitative analysis of tannins was conducted by dissolving 50 mg of the Rhu extract in distilled water pre-adding $FeCl_3$ (a few drops), the presence of green precipitates indicates the presence of tannins [21]. Flavonoids were screened using an alkaline reagent test, 50 mg of the Rhu extract was mixed with a dilute ammonia solution (5 ml) and filtered, then added H_2SO_4 concentrated, the formation of a yellow colour indicates the presence of flavonoids [22]. Saponins screening was conducted using the Froth test; 50 mg of the Rhu extract was dissolved in distilled water and made up the volume to 20 ml with distilled water, then shake the mixture vigorously for 15 minutes, the formation of stable foam indicates the presence of saponins [19]. Phenols screening was conducted using a Ferric chloride test; 50 mg of the Rhu extract was dissolved in 5 ml distilled water, then 5 % ferric chloride (a few drops) was added, the formation of a bluish-black colour indicates the presence of phenols [19]. Terpenoids screening was conducted using Liebermann-Burchard test; 50 mg of the Rhu extract was added to acetic anhydride solution (a few drops) then H_2SO_4 concentrated (a few drops) was carefully added along the walls of the test tube, the formation of red-brown ring indicates the presence of terpenoids [23]. Screening of terpenes was conducted by adding 3-4 drops of copper acetate solution to 50 mg of the extract, the formation of emeraldgreen colour indicates the presence of terpenes [24]. Qualitative analysis of glycosides was conducted using Salkowski's test; 2 mg of the Rhu extract was diluted in 2 ml chloroform, then a few drops of the concentrated$H_2SO_4$was added, the formation of a reddish-brown colour indicates the presence of glycosides [20]. Qualitative analysis of anthraquinones was conducted using an ammonium hydroxide test; 10 mg of the extract was dissolved in isopropyl alcohol, then added ammonium hydroxide solution, and the formation of red colour indicates the presence of anthraquinones [25].

2.5. Preparation of basal and hypercholesterolemic diet

The standard nutritionally balanced diet (AIN-93M) was prepared [26]. A hypercholesterolemic diet (HCD), including bile salts (0.2%) and cholesterol (1%), was prepared using the procedure of [27].

2.6. Experimental protocol

Five groups of ten rats each were classified randomly after a week of acclimatization at a temperature of 24±2 °C and a humidity of 60±10% during a 12:12 h dark/light cycle. The initial group was fed a standard diet and considered a control group. While groups 2-5 were fed HCD after 4 weeks, blood samples from these groups (2–5) were taken to confirm the induction of hypercholesteremia in rats (total cholesterol (TC) ≥ 200 mg/dl ~ ≥ 5.17mmol/L) [28]. The scheme for groups' design was as follows:

Control: Rats were fed a standard diet and administered orally distilled water.
HCD: Rats were fed HCD and administrated orally distilled water.
HCD+ Atrova (20 mg/kg): Rats were fed HCD and administered orally Atorva (20 mg/kg) [29].
HCD+ Rhu (75 mg/kg): Rats were fed HCD and administered orally Rhu extract (75 mg/kg) [30].
HCD+ Rhu (150 mg/kg): Rats were fed HCD and administered orally Rhu extract (150 mg/kg).
After four weeks of treatments, all the rats were fasted for 12 hours on the final day. The rats were anesthetized with ether and the blood samples were collected by the heart puncture technique for biochemical analysis. The hepatic samples were excised immediately and fixed in formalin (10 %) for the histological investigation.

2.7. Measurement of lipid profile indices

Serum lipid profiles [TC, high-density lipoprotein cholesterol (HDLc), and triglycerides (TG)] were determined following the routine procedure as per the manufacturer's instructions in the enzymatic colorimetric kits (Abcam, USA). At the same time, low-density lipoprotein cholesterol (LDLc) and very low-density lipoprotein cholesterol (VLDLc) were calculated.

2.8. Measurement ofhepatic function enzymes

Serum activities of hepatic functions (alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) were measured following the instructions of colorimetric standard kits (Roche Cobas Diagnostic, USA).

2.9. Histopathological investigation

After the standard procedure, the fixed hepatic sections were stained with Hematoxylin-eosin staining (H-E stain) for the histological examination. The slides were examined under a light microscope to detect tissue pathological changes.

2.10. Measurements of antioxidants and lipid peroxidation

The serum activities of antioxidant enzymes [catalase (CAT) and superoxide dismutase (SOD)], as well as lipid peroxidation (MDA) level, were measured according to the instruction's procedure in ELSA LSBio kits (LifeSpan Biosciences, USA).

2.11. Statistical analysis

The SPSS version 27 was used to analyze thedata. Results were presented as the mean ± SD. A one-way ANOVA test was used to evaluate the variance between all groups, followed by Tukey-Kramer test. P-values ≤ 0.05 were considered significant.

3. Results

3.1. Phytochemical screening of Rhu extract

Table (1) presents the phytochemical screening of different phytoconstituents of Rhu extract. Alkaloids, flavonoids, saponins, terpenes, and anthraquinones were high in Rhu extract. Tannins, phenols, terpenoids, and glycosides are moderately present in Rhu extract. Meanwhile, the steroid compound was found in trace amounts in Rhu extract.

Table 1. Phytochemical screening of Rhu extract

Phytoconstituents	Test results
Alkaloids	+++
Steroids	+
Tannins	++
Flavonoids	+++
Saponins	+++
Phenols	++
Terpenoids	++
Terpenes	+++
Glycosides	++
Anthraquinones	+++

+ Indicates trace amount, ++ moderately present, and +++ highly present.

3.2. Impact of Rhu extract on lipid profile indices in hypercholesterolemic rats

Figure 1 illustrates the effect of Rhu extract on lipid profile levels in male rats with hypercholesterolemia. The HCD resulted in significantly higher TC, TG, LDLc, and VLDLc levels relative to the control group ($p \leq 0.05$). The groups of rats that ingested HCD with either Atrova (20 mg/kg), Rhu extract (75 mg/kg), or Rhu extract (150 mg/kg) demonstrated significant reductions in TC, TG, LDLc, and VLDLc levels compared to the HCD group ($p \leq 0.05$).On the other hand, the HCD group showed a significant decline in HDLc levels relative to the control group ($p \leq 0.05$). The groups of rats that ingested HCD with either Atrova (20 mg/kg), Rhu extract (75 mg/kg), or Rhu extract (150 mg/kg) exhibited a significant increase in HDLc levels compared to the HCD group ($p \leq 0.05$).Significant changes ($p \leq 0.05$) existed between the high dose

(150 mg/kg) and low dose (75 mg/kg) of Rhu extract in all tested lipid profile biomarkers, where the high dose was more effective as hypolipidemic than the low dose.

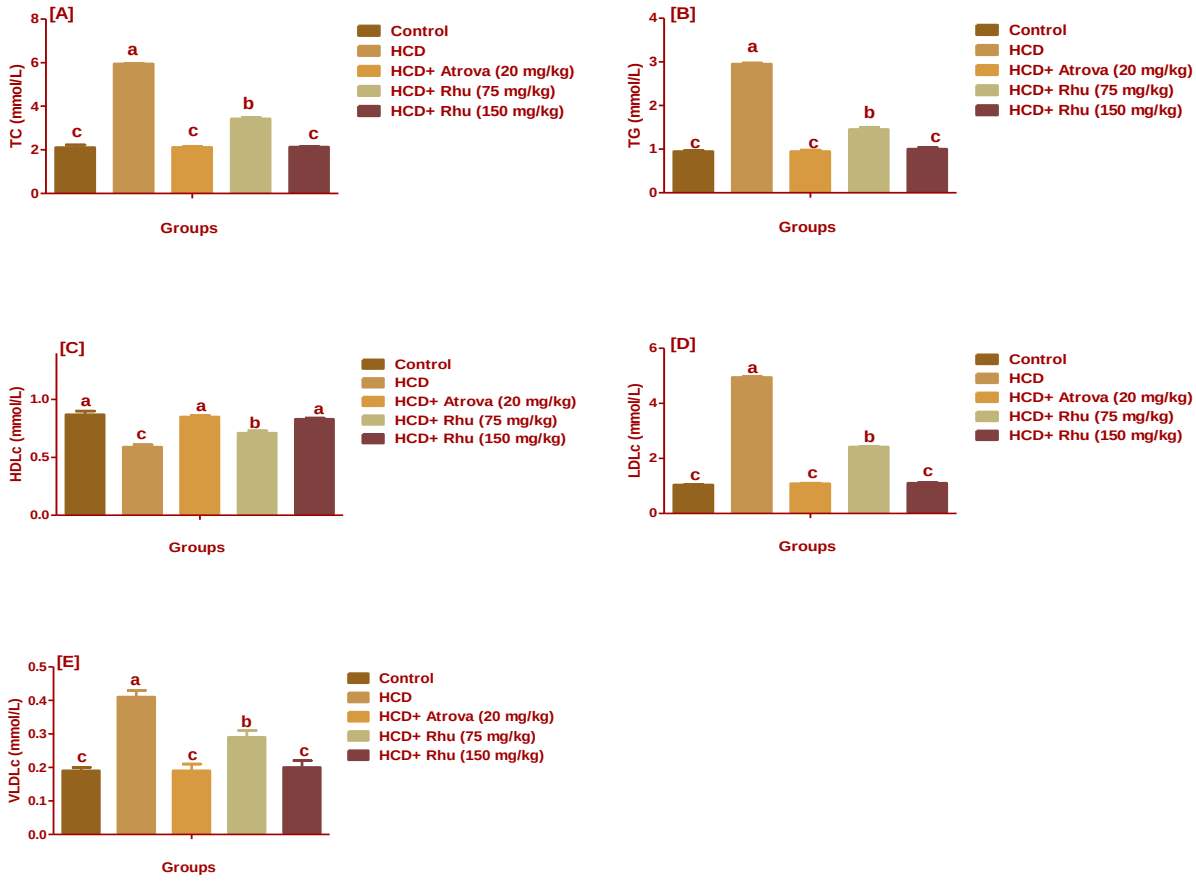

Figure 1. Impact of Rhu extract on lipid profile biomarkers in hypercholesterolemic rats. A: TC; B: TG, C: HDLc, D: LDLc, and E: VLDLc. All values represent the mean ± SD (n = 10). Values with different superscript letters in the same column vary significantly at p≤0.05.

3.3. Impact of Rhu extract on the hepatic function enzymes in hypercholesterolemic rats

Table 2 reveals that hepatic enzymes (ALT, AST, and ALP) were significantly higher in the HCD group (54.14 ± 2.19, 89.43 ± 3.22, and 118.63 ± 4.68 U/L, respectively) relative to the control rats (35.45 ± 1.15, 41.23 ± 1.25, and 93.14 ± 3.56 U/L, respectively) ($p \leq 0.05$). The groups of rats that ingested HCD with either Atrova (20 mg/kg), Rhu extract (75 mg/kg), or Rhu extract (150 mg/kg) demonstrated significant reductions in serum hepaticenzymes compared to the HCD group ($p \leq 0.05$). The values of hepatic enzymes were closer to the control values, particularly in the Atrova group and the high-dose Rhu extract group. On the other hand, there were significant changes between the hepatoprotective effects of the high and low doses of Rhu extract groups ($p \leq 0.05$).

Table 2. Impact of Rhu extract on hepatic function enzymes in hypercholesterolemic rats

Groups	**ALT** (U/L)	**AST** (U/L)	**ALP** (U/L)
Control	35.45 ± 1.15 ^{c}	41.23 ± 1.25 ^{c}	93.14 ± 3.56 ^{c}
HCD	54.14 ± 2.19 ^{a}	89.43±3.22 ^{a}	118.63 ± 4.68 ^{a}
HCD+ Atrova (20 mg/kg)	37.32 ± 2.55 ^{c}	42.42±2.16 ^{c}	94.32 ± 3.45 ^{c}
HCD+ Rhu (75 mg/kg)	48.18 ±2.231 ^{b}	71.20±3.72 ^{b}	111.33 ± 5.33^{b}
HCD+ Rhu (150 mg/kg)	38.44 ± 2.43 ^{c}	43.07± 1.82 ^{c}	94.01 ± 5.36 ^{c}

All values represent the mean ± SD (n = 10). Values with different superscript letters in the same column vary significantly at p≤0.05.

3.4. Impact of Rhu extract on hepatic histopathological changes in hypercholesterolemic rats

After four weeks of treating hypercholesterolemic rats with either Atrova (20 mg/kg), Rhu extract (75 mg/kg), or Rhu extract (150 mg/kg), histological examinations were conducted on the hepatic tissues of all groups. The control group's hepatic tissues showed normal hepatocytes and blood sinusoids arranged radially centered on the normal central vein (Figure 2A). The hepatic of the HCD group had severe alterations, unclear, vacuolar degeneration of hepatocyte cells, widened sinusoids with rising deposition of lipids cells (Figure 2B&C). Treatment of hypercholesterolemic rats with Atrova (20 mg/kg) showed most hepatocytes apparent normal with a noticeable absence of lipid deposition within the cells, except few cells showed slight hydropic degeneration of hepatocytes and slightly widened sinusoids (Figure 2D). Treatment of hypercholesterolemic rats with Rhu (75 mg/kg) showed lipid droplets within many hepatocytes, sinusoid dilation, and slight vacuolar degeneration of hepatocytes (Figure 2E). While treatment of hypercholesterolemic rats with Rhu (150 mg/kg) showed absence of lipid deposition with the hepatocytes looks similar to the control, expect few sinusoids dilation (Figure 2F).

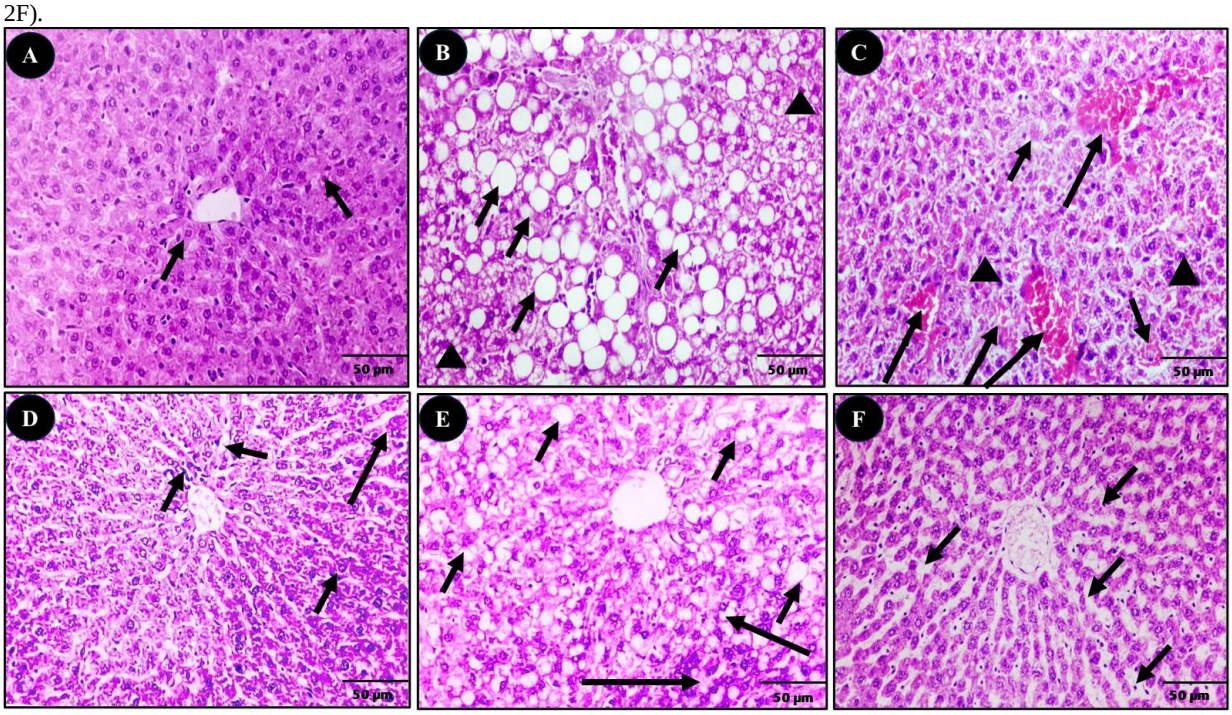

Figure 2.Impact of Rhu extract on hepatic histopathologyin hypercholesterolemic rats (H-E stain x200). Figure (A) represents control group hepatic tissues with normal hepatocytes and blood sinusoids (small arrows) arranged radially centered on the normal central vein. Figures (B & C) represent hepatic of HCD group that showed severe alterations, unclear, vacuolar degeneration of hepatocytes cells (arrowheads), widened sinusoids with rise deposition of lipids cells (small arrows) which disrupt hepatocyte structure. As well as congestion and inflammation of hepatic sinusoids (large arrows). Figure (D) represents hepatic of HCD + Atrova (20 mg/kg) group that showed most hepatocytes apparent normal with a noticeable absence of lipid deposition within the cells, except few cells showed slight hydropic degeneration of hepatocytes (small arrows) and slight widened sinusoids (large arrows). Figure (E) represents hepatic of HCD+ Rhu (75 mg/kg) group that showed lipid droplets within many hepatocytes (small arrows), sinusoid dilation, and slight vacuolar degeneration of hepatocytes (large arrows). Figure (F) represents hepatic of HCD+ Rhu (150 mg/kg) group that showed absence of lipid deposition with the hepatocytes looks similar to the control, expect few sinusoids dilation (small arrows).

3.5. Impact of Rhu extract on serum redox state biomarkers in hypercholesterolemic rats

In hypercholesterolemic rats, after 4 weeks of treatment, serum MDA levels increased, while SOD and CAT activities decreased significantly relative to the control group ($p \leq 0.05$).

The groups of rats that ingested HCD with eitherAtrova (20 mg/kg), Rhu extract (75 mg/kg), and Rhu extract (150 mg/kg) demonstrated a substantial improvement in all serum redox state biomarkers compared with the HCD group ($p \leq 0.05$). In terms of lowering MDA and raising antioxidant enzyme levels, the high dose of Rhu extract outperformed the low dose of Rhu extract (Figure 3).

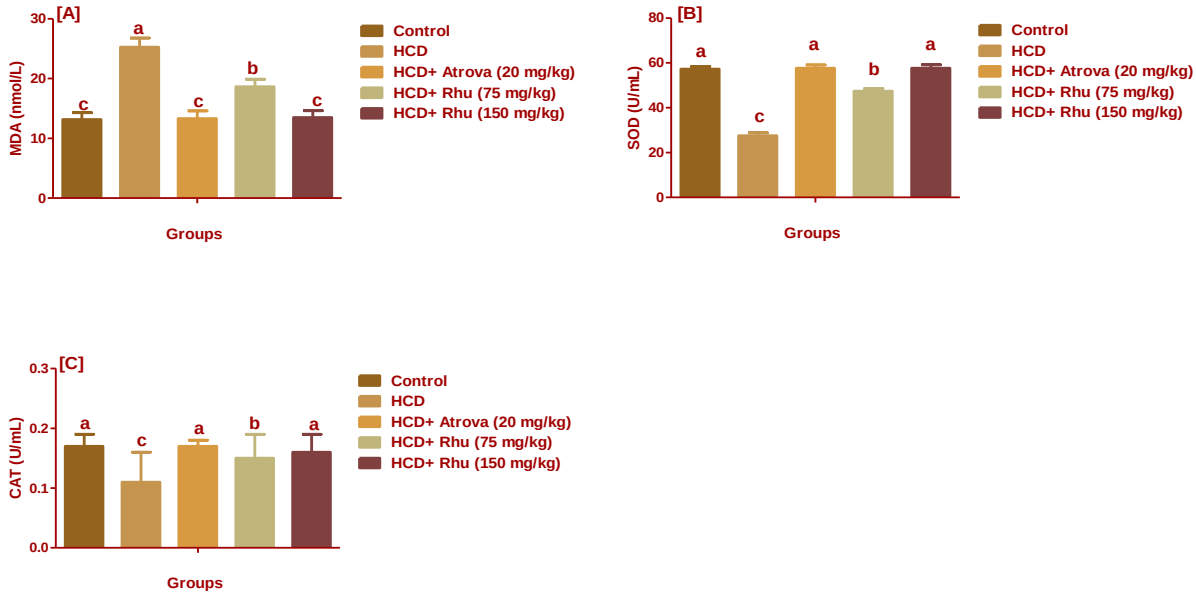

Figure 3.Impact of Rhu extract on serum redox state biomarkers (MDA, SOD, and CAT) levels in hypercholesterolemic rats.A: MDA; B: SOD, C: CAT. All values represent the mean ± SD (n = 10). Values with different superscript letters in the same column vary significantly at $p \leq 0.05$.

4. Discussion

Hyperlipidemia is a metabolic disorder; it is a leading risk cause of coronary heart disease and atherosclerosis. Its incidence has considerably risen due to the significant changes in eating habits and lifestyle [31]. Although cholesterol-lowering drugs are commonly used to cure hyperlipidemia, some cause toxic effects, such as elevation in nerve damage, risk of diabetes, myopathy, and liver transaminases [32, 33]. So, safer natural remedies with fewer side effects are required [34]. Rhubarb (Rhu) extract has antioxidant, hypoglycemic, renal- and hepato-protective, and anti-inflammatory properties [35]. This study aimed to explore the potential therapeutic lipid-lowering and hepatoprotective effects of Rhu extract and explore its mechanism as an antioxidant agent.

The qualification of several bioactive constituents in the Rhu extract revealed that the following five classes, namely alkaloids, flavonoids, saponins, terpenes, and anthraquinones, were detected to be high. Meanwhile, tannins, phenols, terpenoids, and glycosides were detected to be moderate in Rhu extract. The results are consistent with [36-38]. Rhu belongs to plants with higher phenolic and anthraquinones content. The phenols and tannins responsible for several biological activities were identified in Rhu alcoholic extract [39]. Moreover, Khattak et al. [40] found that the Rhu extract contains various active compounds such as alkaloids, phenols, glycosides, flavonoids, terpenoids, and anthraquinones with a wide variety of pharmacological properties.

The prolonged ingestion of a high-cholesterol diet (HCD) resulted in significant hyperlipidemia, elevated hepatic enzymes, and oxidative stress in rats. The hepatic tissues of the HCD group had vacuolar degeneration of hepatocyte cells and widened sinusoids with a rise in the deposition of lipid cells, which disrupted hepatocyte structure. These results run in parallel with [41]. Hypercholesterolemic triggers hepatic dysfunctions and over-production of ROS with depleted antioxidant capacity; it is a significant risk factor for fatty liver and chronic liver diseases [42]. Elevation in transaminase liver enzymes due to fed HCD is evidence of hepatic cell injury [43]. This could be explained through the hyperlipidemic elevated free radicals and induced changes in hepatic membrane permeability, which released enzymes into the bloodstream [44]. Hypercholesterolemia is a risk factor for hepatic damage, correlated to elevation in lipid content. Fed animals HCD induced plenty of necrosis with accumulation of lipids around hepatocytes, inflammatory cell infiltration, and fatty changes [45, 46]. Several pieces of evidence documented oxidative damage and inflammation after feeding HCD; rats fed HCD showed changes in physiological antioxidant abilities and affected tissue redox imbalance. Lipid disruption is linked with the unbalanced production of free radicals and endogenous antioxidants [47]. High LDLc or cholesterol forms a vicious cycle, accompanied by vascular endothelial form foam cells. It induces mitochondrial dysfunction, which accelerates ROS and thus converts LDL to oxidized LDL (Ox-LDL), which elevates inflammation and oxidative stress [48].

Treatment of hypercholesterolemic rats with Atrova (20 mg/kg) caused a significant reduction in lipid indices concurrent with a significant increase in HDLc levels relative to the HCD group. In addition, the reduced liver enzymes protect hepatic tissues

and neutralize oxidative stress. The obtained results are consistent with other studies that proved the beneficial impact of Atrova in reducing blood lipid level [49, 50].

In the present study, treatment of hypercholesterolemic rats with Rhu extract (75 and 150 mg/kg) revealed significant hypolipidemic impacts relative to the HCD group. Besides, Rhu extract induced hepatoprotective effects in treated rats. Treatment with Rhu exhibited substantial improvement in all serum redox state biomarkers compared with the HCD group. The high dose was more effective than the low dose in hypolipidemic, hepatoprotective, and antioxidant effects and protecting the hepatic tissues.

The hypolipidemic effects of Rhu were agreed with Gholami et al. [51] and thus could be attributed to its active constituents. Recently, Wu et al. [52] revealed that Rhu extract contains several compounds that can regulate lipid metabolism, reduce lipogenesis, inhibit pancreatic lipase, regulate lipid factor expression, and stimulate lipolysis. In addition, Rhu increases bile production and reduces cholesterol esters in the liver [51]. Rhu phenol and flavonoids exhibit hypolipidemic effects, inhibit cholesterol synthesis, and enhance intestinal cholesterol excretion *via* gene expression [53]. Rhu glycosides showed hypoglycemic and hypolipidemic effects in diabetic mice [54].

The hepatoprotective effect of Rhu extract agrees with Neyrinck et al. [55], who revealed that Rhu extract alleviated hepatic injury and suppressed inflammatory and oxidative stress markers. The phytochemical compounds in Rhu could modify antimicrobial peptide production and induce changes in gut microbiota composition, which explains hepatoprotective effects. Asgharian et al. [56] found that Rhu hydroalcoholic extract at 200 and 400 mg/kg daily for 10 days prevents the harmful impact of lead-induced hepatotoxicity in rats.

The current study revealed that treated hypercholesterolemic rats with Rhu extract showed a significant antioxidant impact. Several investigations documented the antioxidant activity of Rhu extracts [44]. Bdaiwi et al. [57] revealed that Rhu aqueous extract induced significant hypoglycemic, hypolipidemic, and antioxidant effects in mice exposed to oxidative stress. Rhu extract contains potent antioxidant phytochemical compounds, which have redox properties and antagonize the side effects induced by elevated ROS levels in several disorders [52].

5. Conclusion

This study demonstrated the therapeutic effects of Rhu extract in lowering lipid indices. The administration of Rhu extract to hypercholesterolemic rats resulted in hepatoprotective effects and prevented the histopathological alteration in hepatic tissues. The mechanisms may be implemented through the antioxidant pathway. The beneficial therapeutic impacts induced by Rhu extract were dose-dependent. These findings suggest that Rhu extract may be a candidate therapy for hypercholesterolemia. Further in-depth investigations of its mechanism and clinical applications are needed.

6. Conflicts of interest

There are no conflicts to declare.

7. Funding sources

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