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Original Research

Evaluation of antihypercholesterolemic effect using *Memecylon edule* Roxb. ethanolic extract in cholesterol-induced Swiss albino mice

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Abstract

Purpose/Aim: In the present study, we investigate the antihypercholesterolemic effect of the ethanolic extract of *Memecylon edule* in *in vivo*.
Methods: Cholesterol (1%)-induced experimental groups were treated with 100 mg/kg and 200 mg/kg *M. edule* ethanolic extract. The study period of antihypercholesterolemia, the mice body weight, lipid profile, serum enzymes (such as superoxide dismutase, catalase, and glutathione peroxidase), liver marker enzyme, and histopathological study of liver tissues were examined.
Results: The *M. edule*-treated groups have exhibited significant changes in total cholesterol, very-low-density lipoprotein, and low-density lipoprotein, and eventually increased the high-density-lipoprotein activity in serum. Also, it reduced the malondialdehyde level and increased the antioxidant-enzyme activities. The activity is mainly the presence of flavonoids, tannins, saponins, and glycosides in the ethanolic extract of *M. edule*.
Conclusion: The *M. edule* extract contains a different class of secondary metabolites, which reduces the hypercholesterolemic condition in the experimental animal model. The results explored the *M. edule* extract as a potent drug for hypercholesterolemic condition.

Keywords: antihypercholesterolemic; antioxidant; HDL; *Memecylon edule*; phytochemicals

1. Introduction

The plant kingdom provides many compounds with medicinal value to humans and animals that prevent them from having various infectious diseases. The importance of medicinal plants has been reviewed for many years, in addition to traditionally used different medical systems, such as Ayurveda, Unani, and Siddha.[1] Plants are used in different forms, such as health drinks, extracts, taste enhancers, and food additives. Plant-derived metabolites, such as saponins, alkaloids, flavonoids, tannins, essential oils, and phenolics, have many medicinal and pharmacological properties. These bioactive metabolites could control various harmful bacterial and viral diseases with less adverse effects.[2]

Memecylon edule is a small evergreen tree, native to India, but can also be found in China, Thailand, Malaysia, and Indonesia. The tree grows on rocky soils, and dry and wet areas. The *M. edule* leaves and root extract are used for treating dysentery. The *M. edule* root and heartwood decoction is used for the relief of fever and several diseases,

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such as the common cold, measles, and chicken pox.[3] The fruits act as a cooling astringent, and the leaves are used for the treatment of antileucorrhoeic, spasmolytic, and hypoglycemic problems.[4] The *M. edule* leaves also have strong anti-inflammatory and analgesic activities, as reported by Nualkaew et al.[5] The *M. edule* aqueous plant extract contains high amounts of nitrogen, phosphorus, and potassium, which enhance the growth of commercial crops and act as good biofertilizers.[5,6]

Cholesterol is a representative for sterol and a component of all eukaryotic plasma membranes. It is necessary for the growth and viability of higher organisms. However, an increased level of cholesterol is known as hypercholesterolemia. This condition could cause various diseases, such as cardiovascular disease, atherosclerosis, myocardial infarction, and cerebral paralysis.[7] Hypercholesterolemia is one of the most common health problems in Asian and Western countries. Hypercholesterolemia enhances the free-radical generation in various ways and eventually the formation of oxygen free radicals in the human cellular system.[8] The excess free radicals can create various cell dysfunctions, such as cancer, diabetes, cardiovascular diseases, and so on. Plants could act as potent therapeutic agents to control a hypercholesterolemic condition. In this study, we explored the *M. edule* extract as a potential antihypercholestralemic agent in cholesterol-induced Swiss albino mice. Further, we examined the serum and tissue biochemical parameters, and the histopathological examination in treated and control mice.

2. Methods

2.1. Plant and preparation of extract

Leaves of *M. edule* (Melastomataceae) were collected from the shrub forest of Vandalur, Chennai, India. The plant was well cleaned with distilled water, and dried at room temperature for 12/12 light and dark conditions. The dried plant material was ground in an electric grinder and sieved by 0.22 μm mesh size. For 500 mg of the powdered plant sample, 200 mL of 95% ethanol was used for Soxhlet extraction. The extraction was carried out for 8 hours. After the Soxhlet extraction, the excess solvent was evaporated by a rotary evaporator. The obtained crude extract of *M. edule* was kept in a refrigerator in a well-closed container until further use.

2.2. Phytochemical screening

The ethanolic extract of *M. edule* was analyzed for phytochemical constituents, such as alkaloids, flavonoids, saponins, tannins, glycosides, triterpenoids, and essential oils by standard procedures.[9]

2.2.1. Gas-chromatography-and-mass-spectrometry study

The gas chromatography and mass spectrometry (GC–MS) analysis (Agilent 5973, New Jersey, USA) was carried out to identify the phytochemical constituents from the plant extract. The volatile and semivolatile bioactive compounds were identified by GC–MS spectra, and their mass spectra were compared with library reference.

2.3. Animals

Male Swiss albino mice (25–35 g) were purchased from the Department of Zoology, Tamil University, Thanjavur, India. They were housed under standardized conditions (constant room temperature at 25°C with alternating 12-hour periods of light and darkness; humidity 50–60%). The experimental animals were fed on a standard cholesterol-free laboratory diet. All the animal experimentations were premeditated and executed in compliance with the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India, and the Institutional Animal Ethics Committee Guidelines (743/03/ABC/CPSEA dt: 03.03.2003).

2.4. Induction of hypercholesterolemia and treatment protocol

Hypercholesterolemia was induced in the animals by oral feeding of 1% cholesterol for 30 days. All animals had free access to food and water *ad libitum* during the experimental period.

The animals were randomly divided into six groups of six animals in each group. Group 1 (normal, control group) received a standard laboratory diet. Group 2 received plant extract alone (200 mg/kg). Groups 3–6 were administered orally with 1% cholesterol mixed diet for an experimental period. Group 3 served as the cholesterol-induced mice. Group 4 were treated with lovastatin (10 mg/kg) in an aqueous suspension with oral administration. Groups 5 and 6 were orally administered with ethanolic *M. edule* extract (100 mg and 200 mg, respectively). At the end of 30 days, the animals were sacrificed, and blood samples were collected from all groups of mice for biochemical and histological determination.

2.5. Determination of body weight and lipid profiles

The body weights of all mice were recorded throughout the study period. Organ weight was measured at the end of the experiment. Serum levels of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) were measured using diagnostic kits according to the manufacturers' instructions (AGAPPE Diagnostics, Kerala, India, and Ensure Biotech Pvt. Ltd., Hyderabad, India).

2.6. Evaluation of liver marker enzymes

Liver marker enzymes [alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)] were studied according to the procedure given by Ensure Biotech Pvt. Ltd.

2.7. Measurement of antioxidant activity

Serum lipid peroxides[10] and antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase,[11] were determined in both the control and experimental groups of mice.

2.8. Histopathological investigation

At the end of the treatment, the animals were sacrificed under an anesthetic condition, and liver tissue samples were taken for histopathological analysis using hematoxylin-and-eosin staining methods.[12]

2.9. Statistical analysis

Numerical data were obtained from the animal experiments and expressed as mean (± standard error of the mean) standard error. The statistical differences between the high-cholesterol diet, control diet, and standard cholesterol drug with different concentrations of plant extract and the plant extract alone were analyzed by SPSS version 10 software (SPSS Inc., Chicago, IL, USA) hypothesis-testing methods, including the analysis of variance followed by the least-significant-difference test. A p value < 0.05 was considered statistically significant.

3. Results

Table 1 shows that the identified phytochemical constituents from the ethanolic *M. edule* leaves extract. The leaves contains various secondary metabolites such as alkaloids, flavonoids, saponins, tannins and triterpeoids.

The GC–MS technique was used for the identification of the most abundant bioactive metabolites in *M. edule* crude extract. The six major compounds identified from the *M. edule* extract are squalene, phthalic acid, hexadecanoic acid, octadecanoic acid, tetratetracontane, and hexadecane (Figure 1, Table 2). The body weight was increased significantly ($p < 0.05$) in cholesterol-induced mice with respect to the control. The cholesterol-induced mice showed a progressive body-weight increase throughout the study period. The body weight of mice treated with *M. edule* leaf extract shows no changes compared to that of cholesterol-induced mice, as indicated in Table 3. The experimental mice organ, such as liver, kidney, and spleen, weights increased in the cholesterol-induced mice, whereas they are normal in the *M. edule* crude-extract-treated mice.

Table 1
Phytochemical constituents of ethanolic extract of *Memecylon edule*.

Phytochemical compounds	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Terpenoids	–
Saponins	+
Glycosides	+
Phlobatannins	–
Oils	+

Table 4 shows the effect of *M. edule* extract on TC, HDL, LDL, and VLDL in the control and experimental mice. In the cholesterol-induced mice, the serum TC, LDL, and VLDL were significantly increased, while decreased HDL levels were calculated. After treatment with *M. edule* extract, the cholesterol-induced mice TC, LDL, and VLDL were significantly reduced, while the HDL level was increased. This result indicates that the *M. edule* extract possesses anti-hyperlipidemic properties. Table 5 illustrates the serum enzymes, such as AST, ALT, and LDH, in the control and experimental animals. The *M. edule* extract-treated group has significantly decreased ($p > 0.05$) serum enzymes AST, ALT, and LDH, as compared to the hypercholesterolemic-induced mice. These significant reductions in serum marker enzymes (AST, ALT, and LDH) are mainly bioactive compounds present in the plant extract.

Table 6 represents the level of lipid peroxide and antioxidant activity in the experimental and control animals. The level of lipid peroxide (malondialdehyde) increased in the cholesterol-induced group, whereas it decreased in the *M. edule* extract-treated group. The antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, are significantly increased in the *M. edule* extract-treated mice. This result clearly indicates that *M. edule* has potent antioxidant properties. Furthermore, there was no significant difference ($p < 0.05$) noticed in the serum lipid and cholesterol profile of the lovastatin-treated mice. Interestingly, the *M. edule* extract-treated groups exhibited a significant activity in both 100 mg/kg and 200 mg/kg concentrations compared with the lovastatin-treated mice. Anandhi et al[13] studied hypercholesterolemic rats treated with lovastatin, which they found to have a significantly lower activity than that in saline-treated rats. Figure 2 shows the histopathological changes in the control and experimental groups of mice. The cholesterol-induced mice have infiltrations of inflammatory cells, necrosis of hepatocytes, vascular and sinusoidal dilatation, disturbed nucleus, and damaged central vein. In the *M. edule* extract-treated mice, the architecture of hepatocytes in the liver has been restored. No significant changes were observed in the *M. edule* alone-treated group of mice.

4. Discussion

The increasing obesity and fatty-liver problems are demanding people to consume chemically synthetic anti-hyperlipidemic drugs that have high side effects. The plant-derived secondary metabolites, such as saponins, sterols, and phenolics, decrease the hyperlipidemia levels, as reported in an earlier work.[14] Plants contain many health-related bioactive compounds, which could effectively control harmful diseases.

Kanter et al[15] reported that the *Nigella sativa* aqueous extract has effectively reduced the lipid peroxidation and liver-enzyme activity, and enhanced the activity of the antioxidant-

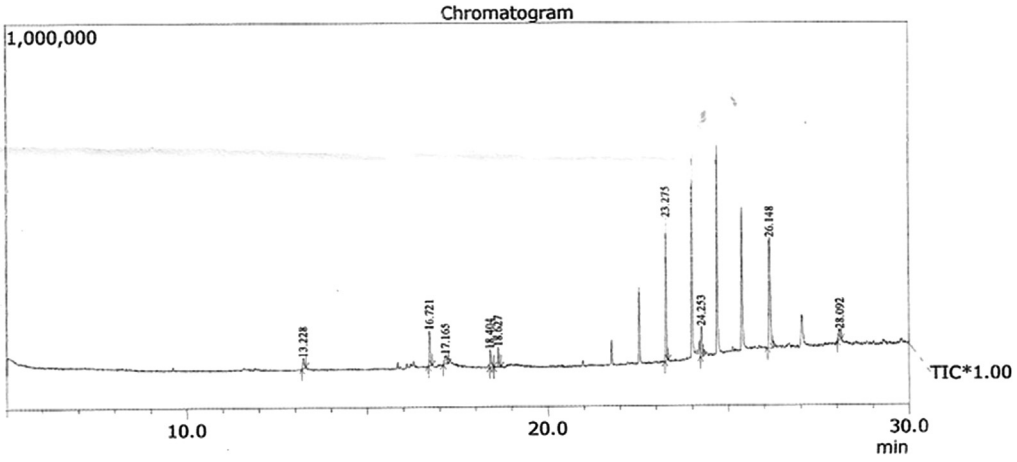

Figure 1. Gas chromatography and mass spectrometry spectrum of ethanolic extract of *Memecylon edule* (TIC – Total ion chromatogram).

Table 2
Bioactive compounds identified from the ethanolic extract of *Memecylon edule* using gas chromatography and mass spectrometry.

Peaks	Retention time (min)	Area	Area (%)	Name of the compounds
1	13.228	95,162	3.64	Phthalic acid
2	16.721	220,993	8.45	Palmitic acid, methyl ester
3	17.165	124,019	5.13	Hexadecanoic acid
4	18.404	133,708	5.11	9-Octadecenoic acid, methyl ester
5	18.627	112,297	4.30	Octadecanoic acid, methyl ester
6	23.275	698,062	26.70	Tetratetracontane
7	24.253	185,806	7.11	Squalene
8	26.168	921,514	35.25	Hexatriacontane
9	29.092	112,728	4.31	Hexadecane

defense system in carbon-tetrachloride-treated rats. Venkadeswaran et al[16] studied *Piper betle* extract or eugenol, which may significantly lower the mean serum levels of TC, triglycerides, LDL cholesterol, and VLDL cholesterol in hypercholesterolemic rats. The lipid-lowering effect of the leaves of *P. betle* is mediated through the inhibition of hepatic cholesterol biosynthesis and the reduction of lipid absorption in the intestine. Wu et al[17] conducted an investigation on the antihypercholesterolemic activity of jatrorrhizine extracted from r*hizoma coptidis*. It showed a potential mechanism on regulating the cholesterol metabolism and lipid-lowering effect in treated mice. Also, the jatrorrhizine exhibited significant decreases in TC, triglycerides, and LDL levels. Besides, the water-soluble extract of a traditional Chinese black tea reduced the blood-cholesterol levels in rats. The study revealed that the black-tea-extract intake elicited a significant antihypercholesterolemic effect in treated animal groups.[18]

Cholesterol is an important building block in cell membranes. It is a main precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D.[19] The abnormal level of cholesterol concentration in the blood is affected by cholesterol synthesis in the liver.[20] However, the high-cholesterol concentration in the blood may increase the risk of atherosclerosis and cardiovascular diseases. In this study, we found that the body weight of mice was significantly decreased in the *M. edule* extract-administered group as compared with the cholesterol-induced mice. Also, the serum profile confirmed that the plant extract possesses an antihypercholesterol activity.

By contrast, the HDL facilitates the translocation of cholesterol from the peripheral tissues to the liver for catabolism. The high-cholesterol-diet (1%) mice had higher ($p < 0.05$) serum cholesterol concentration compared with the mice fed with a normal diet. The plant extract exhibited a significant activity in

Table 3
Effect of *Memecylon edule* extract on body and organ weight in the control and experimental animals.

Groups	Body weight (g)	Liver (g)	Kidney (g)	Spleen (g)
Control	28.7 ± 0.40	4.91 ± 0.01	0.87 ± 0.12	0.40 ± 0.02
M. edule alone	29.5 ± 0.02	5.10 ± 0.21	1.25 ± 0.04	0.62 ± 0.02
Cholesterol induced (1%)	34.3 ± 0.20[a]	7.34 ± 0.15[a]	1.85 ± 0.20[a]	0.45 ± 0.04[a]
Lovastatin (10 mg/kg)	29.8 ± 0.25[b]	5.10 ± 0.010[b]	0.90 ± 0.01[b]	0.44 ± 0.02[b]
Cholesterol + *M. edule* 100 mg	36.2 ± 0.10[b]	5.20 ± 0.30[b]	1.17 ± 0.03[b]	1.20 ± 0.01[b]
Cholesterol + *M. edule* 200 mg	34.5 ± 0.011[b]	5.15 ± 0.35[b]	1.12 ± 0.04[b]	1.17 ± 0.02[b]

Data are presented as mean ± standard error of the mean for six mice.
[a] p value < 0.05 compared with the control-group mice.
[b] p value < 0.05 compared with the cholesterol-induced-group mice.

Table 4
Effect of *Memecylon edule* extract on serum lipid profiles of the control and experimental animals.

Groups	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)
Control	193.56 ± 0.006	71.05 ± 0.02	20.4 ± 0.07	52.05 ± 0.013
M. edule alone	187.13 ± 0.014	64.85 ± 0.01	27.6 ± 0.01	54.65 ± 0.010
Cholesterol induced (1%)	275.72 ± 0.012[a]	103.04 ± 0.04[a]	12.4 ± 0.05[a]	112.44 ± 0.012[a]
Lovastatin (10 mg/kg)	202.32 ± 0.014[b]	72.54 ± 0.02[b]	20.75 ± 0.03[b]	70.67 ± 0.010[b]
Cholesterol + *M. edule* 100 mg	194.15 ± 0.019[b]	61.06 ± 0.01[b]	19.6 ± 0.03[b]	78.44 ± 0.011[b]
Cholesterol + *M. edule* 200 mg	188.10 ± 0.012[b]	54.15 ± 0.02[b]	21.2 ± 0.01[b]	75.75 ± 0.013[b]

Data are presented as mean ± standard error of the mean for six mice.
HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.
[a] p value < 0.05 compared with the control-group mice.
[b] p value < 0.05 compared with the cholesterol-induced-group mice.

Table 5
Effect of *Memecylon edule* extract on serum markers.

Groups	AST (U/L)	ALT (U/L)	LDH (U/L)
Control	11.35 ± 0.010	13.06 ± 0.009	1923.56 ± 0.013
M. edule alone	11.64 ± 0.011	13.15 ± 0.010	1763.34 ± 0.013
Cholesterol induced (1%)	78.25 ± 0.008[a]	65.95 ± 0.007[a]	2244.40 ± 0.011[a]
Lovastatin (10 mg/kg)	11.68 ± 0.004[b]	16.34 ± 0.006[b]	2055.72 ± 0.010[b]
Cholesterol + *M. edule* 100 mg	11.45 ± 0.010[b]	13.25 ± 0.009[b]	2083.94 ± 0.014[b]
Cholesterol + *M. edule* 200 mg	10.44 ± 0.012[b]	11.14 ± 0.013[b]	2086.94 ± 0.007[b]

Data are presented as mean ± standard error of the mean for six mice.
ALT = alanine transaminase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase.
[a] A p value < 0.05 compared with the control-group mice.
[b] A p value < 0.05 compared with the cholesterol-induced-group mice.

serum HDL cholesterol levels. The ethanolic extract of *M. edule* contains rich amounts of bioactive constituents, such as saponins, phenolics, flavonoids, and volatile oils, which are involved in a cholesterol-lowering effect.[21] Previous studies proved that saponin has lowered the serum cholesterol levels in animals, including the human system.[22] Saponins are able to control the cholesterol level by preventing the body-cholesterol reabsorption and by increasing excretion.[15] Therefore, the cholesterol-lowering effect of *M. edule* is due to the presence of saponins in the extract. Also, sterols and stanols play a main role in the cholesterol-lowering activity in animals.[23] Plant sterols, such as campesterol and sitosterol, are structurally similar to cholesterol, and so they may act as alternatives to cholesterol.[24]

Hypercholesterolemia is causing fatty liver and abnormal serum enzymes. Altunkaynak[25] reported that a high-fat diet might enhance the mononuclear cell infiltrations, necrosis, and vascular dilatation, an increase in hepatic connective-tissue density in the rat model. The serum marker enzymes, such as AST, ALT, and LDH, were also elevated in the hypercholesterolemic condition.[26] The *M. edule* extract-treated group's AST, ALT, and LDH enzyme activities were significantly decreased. The ethanolic *M. edule* extract-treated group's lipid peroxide and antioxidant activities were regulated to the normal condition. Also, the *M. edule*-treated mice showed well-preserved architecture of the liver and other functional organs. It is evidence that the *M. edule* extract protects the liver from cholesterol problems in Swiss albino mice.

In the present study, we demonstrated that the *M. edule* extract controlled the serum lipids and liver marker enzymes in the cholesterol-induced mice. The *M. edule* extract contains potent bioactive metabolites, which could control the serum lipid profiles, liver marker enzymes, and lipid peroxide, and eventually enhance the HDL activities in the treated groups. The results indicate that the ethanolic extract of *M. edule* possesses a positive effect in the management of serum lipid parameters, and eventually reduces the serum and tissue cholesterol levels. The revelation of the serum parameters is helpful for the assessment of the antihypercholesterolemic profile.

Table 6
Effect of *Memecylon edule* extract on malondialdehyde and antioxidant enzymes.

Groups	MDA (nmol/mL)	SOD (U/mg)	CAT (mU/L)	GSH Px (U/mg)
Control	11.56 ± 0.04	573.56 ± 0.07	12.23 ± 0.01	19.35 ± 0.012
M. edule alone	14.44 ± 0.06	666.43 ± 0.09	13.04 ± 0.02	17.51 ± 0.002
Cholesterol induced (1%)	23.39 ± 0.03[a]	415.11 ± 0.03[a]	9.15 ± 0.07[a]	10.30 ± 0.009[a]
Lovastatin (10 mg/kg)	10.54 ± 0.02[b]	609.15 ± 0.04[b]	10.56 ± 0.05[b]	12.89 ± 0.003[b]
Cholesterol + *M. edule* 100 mg	12.46 ± 0.05[b]	614.72 ± 0.08[b]	12.44 ± 0.06[b]	14.46 ± 0.001[b]
Cholesterol + *M. edule* 200 mg	11.79 ± 0.04[b]	627.32 ± 0.05[b]	12.83 ± 0.01[b]	16.53 ± 0.003[b]

Data are presented as mean ± standard error of the mean for six mice.
CAT = catalase; GSH Px = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase.
[a] p value < 0.05 compared with the control-group mice.
[b] p value < 0.05 compared with the cholesterol-induced-group mice.

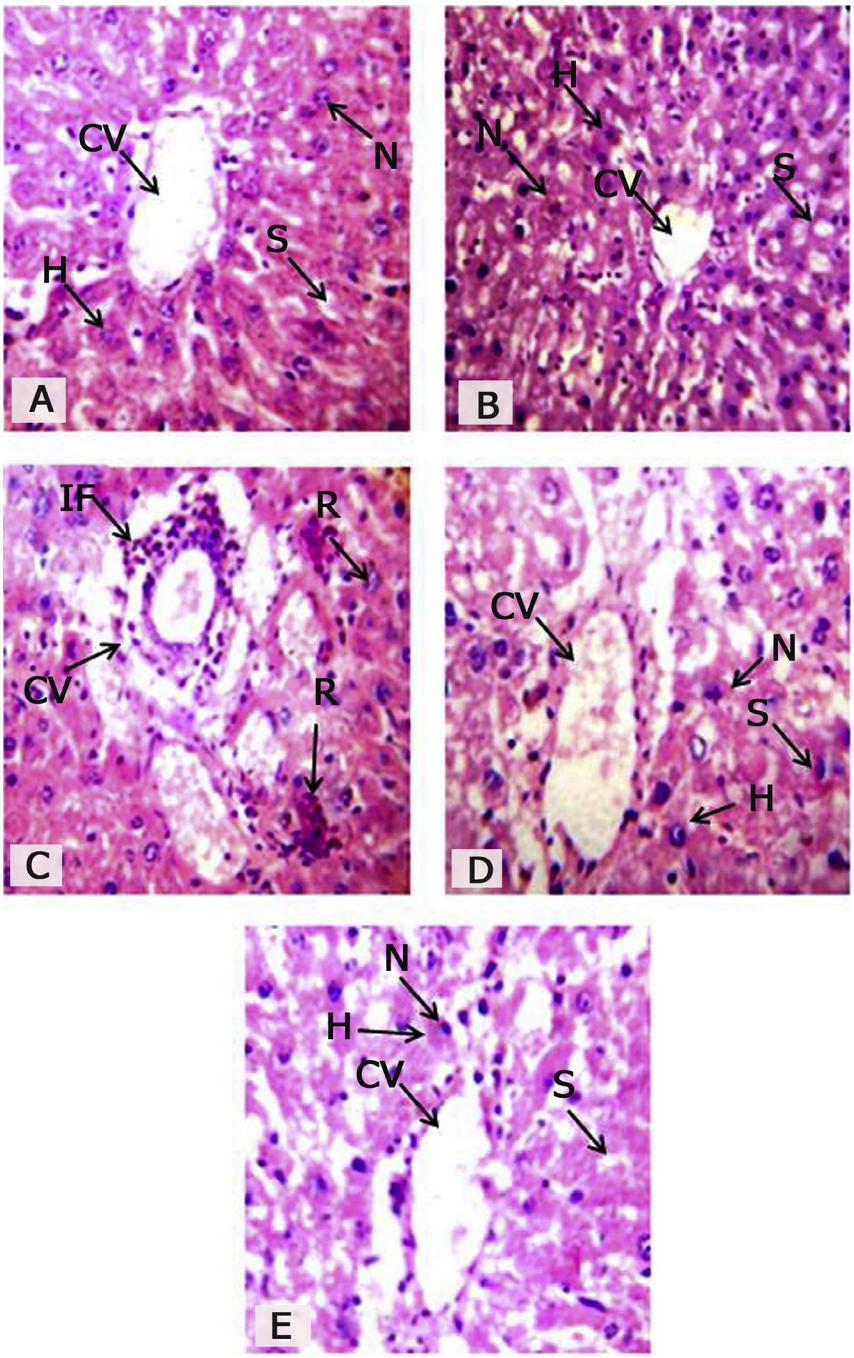

Figure 2. (A) The control group that received a normal diet shows normal central vein (CV), hepatocytes (H), nucleus (N), and sinusoids (S); (B) the plant extract alone shows similar architecture of the control group; (C) the cholesterol-induced mice showed infiltrations of inflammatory cells (IF), damaged central vein (CV), and residual (R); and (D,E) the *Memecylon edule*-treated group shows normal architecture of the liver.

Conflicts of interest

The authors declared no conflicts of interest in the work.

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