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ORIGINAL ARTICLE

Pharmacological Screening of *Trachyspermum ammi* for Antihyperlipidemic Activity in Triton X-100 Induced Hyperlipidemia Rat Model

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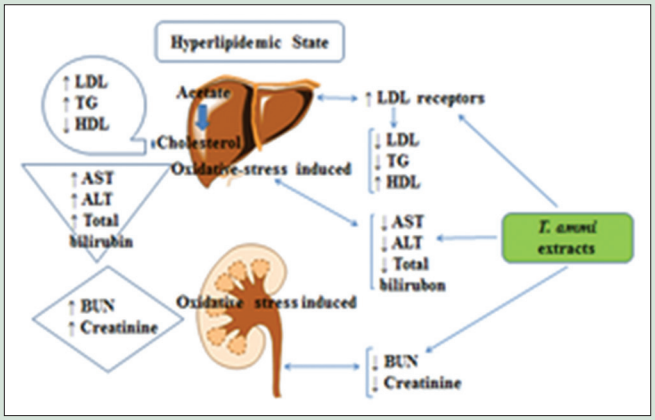
ABSTRACT

Background: Mortality rate is increasing due to cardiovascular problems throughout the world. These cardiac problems are directly associated with dyslipidemia. **Aim:** The aim of this study was to evaluate the antihyperlipidemic effect of aqueous extract and methanol extract of *Trachyspermum ammi* at 1 g/kg, 3 g/kg, and 5 g/kg dose levels in rats. **Materials and Methods:** For this purpose, 45 male albino rats were used and randomly divided into nine equal groups (n = 5). The lipid levels were increased after 24 h of single intraperitoneal injection of Triton X-100 (100 mg/kg) in rats. Aqueous and methanol extracts equivalent to 1 g/kg, 3 g/kg, and 5 g/kg were administered orally to the rats for 21 days. Atorvastatin (10 mg/kg) was used as standard drug. Blood samples were collected at 0, 2nd, 9th, 16th, and 23rd day by a direct cardiac puncture in Vacuette® heparin tubes. Serum was separated and then analyzed for lipid profile, liver function test (LFT), and renal function test (RFT) using standard diagnostic kits. **Results**: Results showed that extracts at 3 g/kg and 5 g/kg decreased the levels of total cholesterol, triglyceride, and low-density lipoprotein and increased high-density lipoprotein concentration in serum. *T. ammi* also decreased LFT and RFT parameters at the end of the study. **Conclusion:** *T. ammi* possessed antioxidant and antihyperlipidemic activities along with hepato- and nephro-protective effects.

Key words: Antihyperlipidemic effect, hepatoprotective, oxidative stress, Trachyspermum ammi

SUMMARY

Aqueous and methanol extracts of *T. ammi* were administered orally at 1-, 3-, and 5 g/kg doses to hyperlipidemic rats (Triton X-100 induced hyperlipidemia) and atorvastatin (10 mg/kg, orally) was used as standard drug. Methanol extract at 5 g/kg showed antihyperlipidemic effect that is identical to that of standard drug.



Abbreviations Used: LDL: Low-density lipoprotein; TC: Total cholesterol; VLDL: Very low-density lipoprotein; HDL: High-density lipoprotein; *T. ammi*: *Trachyspermum ammi*; WHO: World Health Organization; CAD: Coronary artery disease; BHT: Butylated hydroxytoluene; BUN: Blood urea nitrogen; AST: Aspartate transaminase; ALT: Alanine transaminase; IP: Intraperitoneal.

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INTRODUCTION

In the world's developing and under developing countries, mortality rate is increasing due to cardiovascular problems.[1,2] These cardiac problems are directly associated with dyslipidemia, which is the leading cause of atherosclerosis, arteriosclerosis, stroke, obesity, coronary artery diseases, and heart attack.[3-5]

The normal level of cholesterol in plasma can be maintained by biosynthesis of cholesterol, absorption of cholesterol from diet, cholesterol removal from the circulation and elimination of cholesterol through feces and bile.[6] Hyperlipidemia is majorly linked with increased level of low-density lipoprotein (LDL), total cholesterol (TC), very LDL (VLDL), and decreased the level of high-density lipoprotein (HDL) in serum.[7,8] Initially, such abnormal accumulation of lipid in liver causes steatosis or fatty liver. At the chronic stages, destruction of hepatocytes occurs.[9,10]

In general, statins as antihyperlipidemic drugs are most commonly used to decline the increased level of cholesterol and lipid in blood. However, these allopathic medicines have many side effects such as myopathy, hyperuricemia, flushing, hepatotoxicity, dry skin[11-13] and gastric disturbance, abdominal pain, and flatulence.[14,15]

Medicinal plants play an important role in preventing and treating a variety of diseases. Plant and herbal drugs are safer and well tolerated as compared to synthetic drugs.[16] The World Health Organization (WHO) has been estimated that almost eighty percent world's population trusts on the usage of herbal methods for treatment.[17] Therefore, researchers

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are also interested on herbal therapies of cardiovascular complaints having less adverse effects.

Trachyspermum ammi is an annual ayurvedic herbaceous plant, belongs to the important medicinal family, Umbelliferae or *Apiaceae*.[18] It is well known by several common names such as ajwain/omum, carom seed, and bishop's weed.[19,20] Al-Yunan or Kammun, agyptischer, Xi Ye Cao Guo Qin, ajowan, adiowan, hounastan, ajwan.[21,22] Ajwain is an indigenous plant of Egypt. It grows widely in soil having a great amount of salts such as Southwest Asia and Mediterranean Sea.[23] Ajwain is cultivated and widely distributed in Pakistan, India, Iraq, Afghanistan, and Iran.[24] It is reported that *T. ammi* seeds have anti-platelet,[25] analgesic,[26] antihypertensive,[27] antibacterial,[28] antifungal,[29] antioxidant,[30] insecticidal,[31] anthelmintic,[32] anti-inflammatory,[33] diuretic and anti-lithiasis,[34] detoxification,[35] antiviral,[36] spermicidal,[37] antitussive and bronchodilatory,[38] antiulcer,[39] antispasmodic,[40] digestive stimulant,[41] hepatoprotective,[42] estrogenic,[43] and nematicidal activities.[44] As compared to other effects, the antihyperlipidemic effect of *T. ammi* is still not well explored and well documented.

Latest studies have revealed that ingestion of vegetable oil containing polyunsaturated fatty acids (such as omega-3 and omega-6) decreases plasma level of triacylglycerol and cholesterol and inversely linked to the occurrence of heart diseases.[45]

Phytochemical studies revealed that *T. ammi* contains a significant amount of polyunsaturated fatty acids as well as a good source of dietary fibers. Hence, there is a great need to well explore the antihyperlipidemic activity of *T. ammi*.

To keep in view the medicinal value of *T. ammi* as described in literature review, the present study was carried out to evaluate the antihyperlipidemic effect of aqueous extract and methanol extract of *T. ammi* at 1 g/kg, 3 g/kg, and 5 g/kg dose levels in rats.

MATERIALS AND METHODS

Collection of plant material

T. ammi seeds are widely distributed in Pakistan. They have pale brown color and oval shape. They are bitter and pungent in taste. They are also used as a flavor, spice, and preservative. Seeds of *T. ammi* were purchased from local market. Identification and authentication were done by a Botanist from Department of Botany, Government College University Faisalabad. Seeds were washed and dried under shade. After drying, they were ground to fine powder.

Preparation of extracts

The extract was prepared by cold extraction method. The fine powder (2 kg) was soaked separately with distilled water (6 L) and 98% methanol (6 L) in a sealed close container for 2 weeks with occasional stirring and shaking. The extract was filtered first using muslin cloth and then with Whatman filter paper No. 1. Excess of solvent was evaporated by rotary evaporator at 40°C to get semisolid texture of extract.

Approval by Animal Ethical Committee

This experiment was conducted after the approval from Animal Ethical Committee of Faculty of Pharmaceutical Science, Government College University Faisalabad.

Qualitative phytochemical analysis

A qualitative phytochemical analysis of extracts was carried out to identify the presence of different phytochemicals such as tannin, saponin, alkaloid, steroids, flavonoid, carbohydrates, proteins, phenols, and terpenoids using standard biochemical techniques.[46,47]

Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl method

Liyana-Pathirana and Shahidi[48] was used to analyze the antioxidant activity of extracts. Briefly, it is described as 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution (0.1 mM) and different concentrations of samples solutions (0.1–1.1 mg/mL) in methanol were prepared. Butylated hydroxytoluene (BHT) was dissolved in methanol using as a positive control or reference standard. Test samples (1 mL) and BHT (1 mL) were mixed with DPPH solution (1 mL) separately in test tubes, and these test tubes were kept at 28°C ± 2°C (room temperature) for 30 min. Similarly, blank was prepared, it contained 1 mL of methanol instead of reference standard or sample. After an incubation period at room temperature, absorbance was measured against blank at 517 nm. Each sample was tested in triplicate. Percentage scavenging effect was calculated with following formula:

Scavenging activity (%) = $(A_{blank} - A_{sample}/A_{blank}) \times 100$

Where a blank was optical density (OD) of control.

A sample was OD of test sample.

Experimental animals

Forty-five male albino rats of 80–130 g were used in the present study. They were purchased from the Institute of National Health – Islamabad, Pakistan and kept at the animal house of Faculty of Pharmaceutical Science, Government College University Faisalabad, at a temperature of 25°C ± 1°C and humidity (65%–70%) conditions with 12 h light-dark cycle. Animals were placed in cages 7 days before study for acclimatization and randomly divided into nine equal groups ($n = 5$). Water and chow were provided *ad libitum*.

Induction of hyperlipidemia

Rats were starved for 18 h, and then, hyperlipidemia was induced by single intraperitoneal (IP) injection of 100 mg/kg Triton X-100 prepared in normal saline. After 24 h, serum triglyceride and cholesterol levels were checked and rats with serum TC levels more than 130 mg/dL were considered hyperlipidemic. Treatment was started orally after 24 h of Triton X-100 injection (when hyperlipidemia was induced) for 21 days.[49]

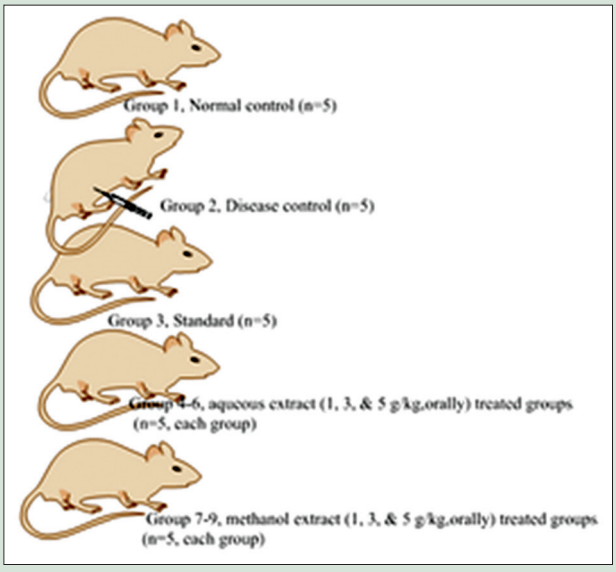

Figure 1: Experimental design

Experimental design

Each group contained 5 rats. After inducing hyperlipidemia, Triton X-100 was injected IP (100 mg/kg) daily during study before administering the extracts/standard.

Group 1: Served as normal control while Group 2: Served as disease control, which received 100 mg/kg Triton X-100 IP injection daily during the study. Group 3: Standard group receiving Triton X-100 + 10 mg/kg atorvastatin daily and Groups 4–9 were treatment groups; Group 4 was given Triton X-100 + aqueous extract at 1 g/kg daily; Group 5 was administered Triton X-100 + 3 g/kg aqueous extract daily; Group 6 receiving Triton X-100 + aqueous extract (5 g/kg) daily; Group 7 was administered Triton X-100 + 1 g/kg methanol extract daily; Group 8 was given Triton X-100 + methanol extract at 3 g/kg daily; and Group 9 received Triton X-100 + methanol extract (5 g/kg) daily.

T. ammi extracts declined the high level of cholesterol caused by Triton X-100.

Collection of blood samples

Blood samples (2.5 mL/sample) were collected on the days 0, 2nd, 9th, 16th, 23rd in Vacuette® heparin tubes by direct cardiac puncture under mild chloroform anesthesia. These collected samples were centrifuged for 10 min at 4000 rpm to get the serum. Then serum was examined for the assessment of biochemical parameters such as triglycerides, TC, LDL, VLDL and HDL, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, creatinine, albumin, and blood urea nitrogen (BUN).

Statistical analysis

Data were expressed as mean ± standard deviation. One way analysis of variance followed by *post hoc* "Tukey" test was applied using IBM SPSS software, version 20.0 to calculate the significance level. $P < 0.05$ was considered statistically significant.

RESULTS

Qualitative phytochemical analysis

A qualitative phytochemical analysis of aqueous and methanol extracts showed that tannins, terpenoids, alkaloids flavonoids, steroids, phenols, and carbohydrates were present in both extracts. Saponins were absent in aqueous extract, but they were present in methanol extract, whereas amino acids were absent in both aqueous and methanol extracts [Table 1].

Table 1: Phytochemical analysis of aqueous and methanol extracts of *Trachyspermum ammi*

Phytoconstituents	Aqueous extract	Methanol extract
Tannin	+ve	+ve
Flavonoid	+ve	+ve
Steroid	+ve	+ve
Terpenoid	+ve	+ve
Saponin	−ve	+ve
Alkaloid	+ve	+ve
Carbohydrates	+ve	+ve
Amino acids	−ve	−ve
Phenols	+ve	+ve

+ve: Present; −ve: Absent

Antioxidant activity

Aqueous and methanol extracts showed significant ($P < 0.05$) dose-dependent % scavenging effect as compared to standard (BHT). At maximum concentration (1.1 mg/mL) BHT, aqueous and methanol extracts exhibited % scavenging effect of 99.33%, 87.47%, and 95.87% respectively as presented in Table 2.

Table 2: Antioxidant activity of aqueous and methanol extracts of *Trachyspermum ammi* by 1,1-diphenyl-2-picrylhydrazyl method

Concentrations (mg/mL)	Percentage scavenging effect		
	BHT	Aqueous extract	Methanol extract
0.1	90.07±0.81	60.50±0.46*	78.63±0.55*
0.3	92.23±1.08	72.43±0.45*	82.80±0.46*
0.5	94.73±0.67	78.53±0.45*	88.37±0.25*
0.7	96.80±0.26	81.67±0.42*	91.39±0.47*
0.9	97.67±0.42	84.40±0.53*	93.67±0.31*
1.1	99.33±0.42	87.47±0.35*	95.87±0.42*

Values were expressed as mean±SD. *P<0.05 when compared with BHT as standard at different concentrations (0.1–1.1 mg/mL). BHT: Butylated hydroxytoluene; SD: Standard deviation

Antihyperlipidemic effect

Antihyperlipidemic effect of aqueous and methanol extracts at a dose of 1 g/kg, 3 g/kg, and 5 g/kg is given in Tables 3-7, respectively. It is manifested from these tables that both extracts at a dose of 1 g/kg did not show the antihyperlipidemic activity.

Effect on triglyceride levels (mg/dL)

Aqueous extract reduced the serum levels of triglyceride up to 1.54%, 23.50%, and 35.14% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively. Similarly, methanol extract lowered the triglyceride levels to 3.71%, 32.30%, and 38.73% at dose of 1 g/kg, 3 g/kg, and 5 g/kg respectively at 23rd day as shown in Table 3.

Effect on total cholesterol levels (mg/dL)

Aqueous extract declined the serum levels of TC to 1.50%, 26.17%, and 35.18% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively. Similarly, methanol extract dropped the TC levels up to 3.26%, 29.35%, and 36.14% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively at 23rd day [Table 5].

Effect on low-density lipoprotein levels (mg/dL)

Table 6 shows that aqueous extract decreased the serum levels of LDL up to 5.78%, 48.13%, and 72.33% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively. Similarly, methanol extract reduced the LDL levels to 5.66%, 55.56%, and 75.03% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively at 23rd day.

Effect on very low-density lipoprotein levels (mg/dL)

Aqueous extract lowered the serum levels of VLDL to 1.55%, 22.61%, and 35.36% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively. Similarly, methanol extract declined the VLDL up to 3.70%, 32.81%, and 37.50% at dose of 1 g/kg, 3 g/kg, and 5 g/kg respectively at 23rd day as given in Table 7.

Effect on high-density lipoprotein levels (mg/dL)

Table 4 exhibits that aqueous extract raised the serum levels of HDL up to 6.33%, 61.38%, and 79.76% at dose of 1 g/kg, 3 g/kg, and 5 g/kg, respectively. Similarly, methanol extract increased the HDL levels to 9.12%, 67.22%, and 97.97% at dose of 1 g/kg, 3 g/kg, and 5 g/kg respectively at 23rd day.

Methanol extract at 5 g/kg dose showed promising antihyperlipidemic effect that is identical to that of standard atorvastatin therapy.

Effect on liver function test

AST, ALT, and total bilirubin levels got raised in hyperlipidemic state as indicated in disease control group. Aqueous and methanol extracts showed a dose-dependent decrease in liver enzymes (AST, ALT, and total bilirubin) and exhibited liver protected effect as given in Table 8.

Table 3: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on triglyceride levels (mg/dL)

Groups	Induction period		Treatment days		
	0 day	2nd day	9th day	16th day	23rd day
Normal control	103.43±0.40	103.30±0.36	100.63±0.60*	102.67±0.61*	101.00±0.20*
Disease control	100.40±0.40	153.57±0.45	166.13±0.32	173.30±0.36	186.27±0.25
Standard	105.37±0.47	161.47±0.42	137.33±0.42* (↓14.95%)	111.10±0.36* (↓31.19%)	91.30±0.36* (↓43.46%)
Aqueous extract (1 g/kg)	107.47±0.45	164.30±0.36	163.93±0.66* (↓0.23%)	162.01±0.25* (↓1.39%)	161.77±0.50* (↓1.54%)
Aqueous extract (3 g/kg)	107.76±0.25	157.03±0.25	140.63±0.40* (↓10.44%)	129.03±0.35* (↓17.83%)	120.13±0.42* (↓23.50%)
Aqueous extract (5 g/kg)	106.30±0.36	159.37±0.40	138.80±0.60* (↓12.91%)	123.50±0.46* (↓22.51%)	103.37±0.47* (↓35.14%)
Methanol extract (1 g/kg)	104.23±0.32	166.30±0.36	162.02±0.25* (↓2.57%)	161.96±0.40* (↓2.61%)	160.13±0.32* (↓3.71%)
Methanol extract (3 g/kg)	102.46±0.35	169.03±0.25	150.27±0.31* (↓11.10%)	131.70±0.36* (↓22.08%)	114.43±0.45* (↓32.30%)
Methanol extract (5g/kg)	101.43±0.45	157.30±0.44	133.40±0.36* (↓15.19%)	111.23±0.45* (↓29.29%)	96.37±0.47* (↓38.73%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↓: Percentage decrease when compared with 2nd day. SD: Standard deviation

Table 4: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on high-density lipoprotein levels (mg/dL)

Groups	Induction period		Treatment days		
	0 day	2nd day	9th day	16th day	23rd day
Normal control	42.72±0.02	43.20±0.20	42.77±0.75*	42.77±0.60*	43.70±0.56*
Disease control	40.43±0.93	25.13±1.21	21.77±0.55	19.07±0.60	13.80±0.66
Standard	43.10±0.66	26.43±0.51	34.27±0.31* (↑29.66%)	41.03±0.75* (↑55.24%)	54.87±0.60* (↑107.60%)
Aqueous extract (1 g/kg)	41.70±0.36	20.70±0.46	21.01±0.82 (↑1.50%)	21.67±0.52* (↑4.69%)	22.01±0.32* (↑6.33%)
Aqueous extract (3 g/kg)	40.88±0.35	22.37±0.85	27.63±0.50* (↑23.51%)	32.13±0.93* (↑43.63%)	36.10±0.36* (↑61.38%)
Aqueous extract (5 g/kg)	41.67±0.50	26.63±0.40	33.43±0.45* (↑25.54%)	39.43±0.45* (↑48.07%)	47.87±0.65* (↑79.76%)
Methanol extract (1 g/kg)	42.50±0.92	21.17±0.65	22.12±0.54 (↑4.49%)	22.85±0.61* (↑7.94%)	23.10±0.52* (↑9.12%)
Methanol extract (3 g/kg)	40.97±0.93	24.80±0.36	31.50±0.46* (↑27.02%)	36.37±0.31* (↑46.65%)	41.47±0.45* (↑67.22%)
Methanol extract (5 g/kg)	41.33±1.01	25.07±0.97	32.60±0.53* (↑30.04%)	38.70±0.46* (↑54.37%)	49.63±0.55* (↑97.97%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↑: Percentage increase when compared with 2nd day. SD: Standard deviation

Table 5: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on total cholesterol levels (mg/dL)

Groups	Induction period			Treatment days	
	0 day	2nd day	9th day	16th day	23rd day
Normal control	85.23±0.25	85.23±0.25	84.23±0.25*	83.30±0.26*	85.20±0.20*
Disease control	78.17±0.15	131.50±0.50	134.47±0.15	148.10±0.10	154.20±0.26
Standard	84.10±0.10	149.10±0.10	122.10±0.10* (↓18.11%)	107.10±0.10* (↓28.17%)	90.23±0.15* (↓39.48%)
Aqueous extract (1 g/kg)	84.23±0.25	145.05±0.21	144.32±0.23* (↓0.50%)	143.00±0.22* (↓1.41%)	142.88±0.31* (↓1.50%)
Aqueous extract (3 g/kg)	83.10±0.10	147.10±0.10	132.10±0.10* (↓10.20%)	119.10±0.10* (↓19.04%)	108.60±0.10* (↓26.17%)
Aqueous extract (5 g/kg)	85.63±0.15	150.10±0.10	123.10±0.10* (↓17.99%)	105.70±0.10* (↓29.58%)	97.30±0.10* (↓35.18%)
Methanol extract (1 g/kg)	83.50±0.10	146.20±0.32	143.12±0.33* (↓2.11%)	142.96±0.15* (↓2.22%)	141.43±0.23* (↓3.26%)
Methanol extract (3 g/kg)	82.10±0.10	143.10±0.10	128.10±0.10* (↓10.48%)	116.70±0.10* (↓18.45%)	101.10±0.10* (↓29.35%)
Methanol extract (5 g/kg)	80.10±0.10	141.10±0.10	119.30±0.10* (↓15.45%)	103.60±0.10* (↓26.58%)	90.10±0.10* (↓36.14%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↓: Percentage reduction when compared with 2nd day. SD: Standard deviation

Table 6: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on low-density lipoprotein levels (mg/dL)

Groups	Induction period		Treatment days		
	0 day	2nd day	9th day	16th day	23rd day
Normal control	22.43±0.40	21.33±0.42	22.30±0.36*	20.40±0.40*	21.33±0.35*
Disease control	18.2±0.26	72.39±0.40	79.23±0.32	94.37±0.35	102.41±0.38
Standard	20.33±0.35	90.76±0.33	60.83±0.21* (↓32.98%)	43.33±0.35* (↓52.26%)	17.69±0.38* (↓80.51%)
Aqueous extract (1 g/kg)	21.47±0.42	94.70±0.44	92.93±0.26* (↓1.87%)	90.01±0.36* (↓4.95%)	89.23±0.31* (↓5.78%)
Aqueous extract (3 g/kg)	20.17±0.31	93.50±0.40	76.65±0.41* (↓18.02%)	60.33±0.42* (↓35.48%)	48.50±0.46* (↓48.13%)
Aqueous extract (5 g/kg)	23.30±0.44	91.80±0.26	62.71±0.41* (↓31.69%)	42.33±0.26* (↓53.89%)	25.40±0.36* (↓72.33%)
Methanol extract (1 g/kg)	20.77±0.32	93.30±0.36	91.37±0.47* (↓2.07%)	90.67±0.20* (↓2.82%)	88.02±0.30* (↓5.66%)
Methanol extract (3 g/kg)	21.70±0.36	84.47±0.35	67.37±0.40* (↓20.24%)	55.51±0.38* (↓34.28)	37.54±0.39* (↓55.56)
Methanol extract (5 g/kg)	19.70±0.30	82.48±0.39	56.60±0.40* (↓31.38%)	36.45±0.35* (↓55.81%)	20.60±0.36* (↓75.03%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↓: Percentage reduction when compared with 2nd day. SD: Standard deviation

Effect on renal function test

Table 9 exhibits the nephroprotective effect of extracts. Both extracts caused a dose-dependent reduction in creatinine, BUN and increase in albumin. There is increase in serum creatinine, BUN and decrease in albumin in hyperlipidemic condition due to oxidative stress which is confirmed by elevated levels of renal function parameters

Table 7: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on very low-density lipoprotein levels (mg/dL)

Groups	Induction period		Treatment days		
	0 day	2nd day	9th day	16th day	23rd day
Normal control	20.6±0.40	20.53±0.40	20.43±0.40*	20.33±0.31*	20.23±0.25*
Disease control	19.97±0.25	30.51±0.46	33.50±0.30	34.47±0.42	37.25±0.25
Standard	21.33±0.35	32.31±0.30	27.40±0.40* (↓15.20%)	22.57±0.31* (↓30.15%)	18.35±0.35* (↓43.21%)
Aqueous extract (1 g/kg)	21.77±0.32	32.86±0.31	32.79±0.20 (↓0.21%)	32.40±0.36* (↓1.40%)	32.35±0.37* (↓1.55%)
Aqueous extract (3 g/kg)	21.45±0.41	31.53±0.35	28.51±0.38* (↓9.58%)	25.40±0.40* (↓19.44%)	24.40±0.36* (↓22.61%)
Aqueous extract (5 g/kg)	21.60±0.36	31.67±0.42	27.35±0.38* (↓13.64%)	24.27±0.32* (↓23.37%)	20.47±0.42* (↓35.36%)
Methanol extract (1 g/kg)	21.00±0.20	33.26±0.42	32.78±0.26 (↓1.44%)	32.39±0.23* (↓2.62%)	32.03±0.32* (↓3.70%)
Methanol extract (3 g/kg)	20.77±0.25	33.43±0.25	30.33±0.35* (↓9.27%)	26.35±0.41* (↓21.18%)	22.46±0.44* (↓32.81%)
Methanol extract (5 g/kg)	21.50±0.30	31.79±0.22	26.27±0.38* (↓17.36%)	22.31±0.30* (↓29.82%)	19.87±0.35* (↓37.50%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↓: Percentage reduction when compared with 2nd day. SD: Standard deviation

Table 8: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on liver function tests

Groups	AST (IU/L)	ALT (IU/L)	Total - bilirubin (mg/dL)
Normal control	92.37±0.35*	71.27±0.25*	0.52±0.03*
Disease control	147.23±0.25	82.47±0.45	0.64±0.03
Standard	95.33±0.35* (↓35.25%)	66.37±0.47* (↓19.52%)	0.47±0.02* (↓26.56%)
Aqueous extract (1 g/kg)	146.86±0.40 (↓0.25%)	82.00±0.42 (↓0.57%)	0.63±0.02 (↓1.56%)
Aqueous extract (3 g/kg)	129.23±0.25* (↓12.23%)	80.87±0.15* (↓1.94%)	0.56±0.03* (↓12.5%)
Aqueous extract (5 g/kg)	105.33±0.35* (↓28.46%)	76.44±0.44* (↓7.31%)	0.53±0.03* (↓17.19%)
Methanol extract (1 g/kg)	146.01±0.46 (↓0.83%)	81.97±0.32 (↓0.61%)	0.62±0.01 (↓3.13%)
Methanol extract (3 g/kg)	122.37±0.47* (↓16.89%)	75.30±0.41* (↓8.69%)	0.52±0.03* (↓18.75%)
Methanol extract (5 g/kg)	99.33±0.31* (↓32.53%)	72.47±0.45* (↓12.13%)	0.49±0.03* (↓23.44%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↓: Percentage reduction when compared with disease control group. AST: Aspartate transaminase, ALT: Alanine transaminase; SD: Standard deviation

Table 9: Effect of aqueous and methanol extracts of *Trachyspermum ammi* on renal function tests

Groups	Creatinine (mg/dL)	BUN (mg/dL)	Albumin (g/dL)
Normal control	0.62±0.03*	20.40±0.36*	4.26±0.04*
Disease control	0.81±0.03	32.19±0.27	3.74±0.05
Standard	0.62±0.03* (↓23.46%)	19.49±0.48* (↓39.45%)	4.12±0.04* (↓10.16%)
Aqueous extract (1 g/kg)	0.80±0.03 (↓1.23%)	32.00±0.35 (↓0.59%)	3.75±0.02 (↓0.27%)
Aqueous extract (3 g/kg)	0.64±0.04* (↓20.99%)	22.41±0.31* (↓30.38%)	4.06±0.05* (↓8.56%)
Aqueous extract (5 g/kg)	0.62±0.03* (↓23.46%)	19.20±0.20* (↓40.35%)	4.14±0.05* (↓10.70%)
Methanol extract (1 g/kg)	0.80±0.02 (↓1.23%)	31.67±0.42 (↓1.62%)	3.76±0.01 (↓0.54%)
Methanol extract (3 g/kg)	0.63±0.03* (↓22.22%)	21.25±0.28* (↓33.99%)	4.07±0.04* (↓8.82%)
Methanol extract (5 g/kg)	0.61±0.03* (↓24.69%)	17.34±0.39* (↓46.13%)	4.17±0.05* (↓11.50%)

Values were expressed as mean±SD. *P<0.05 when compared with disease control group. ↓: Percentage reduction when compared with disease control group; ↑: Percentage increase when compared with disease control group. BUN: Blood urea nitrogen; SD: Standard deviation

in disease control group as compared to normal control grou$P$ values [Table 9].

DISCUSSION

Hyperlipidemia is a well-considered threat factor for cardiovascular disorders, especially CAD (coronary artery disease). Numerous studies have exhibited a clear-cut relationship between cardiovascular events and high cholesterol levels.[50] Cardiovascular ailments have been thought globally as chief cause of death, almost 16.7 million deaths per year according to WHO. One out of each four middle-aged personnel in Pakistan is suffering from cardiovascular complaints.[51] Various studies exposed that food plays an essential role in the development of atherosclerosis and hyperlipidemia. Several studies on the animals and human have assessed that the saturated fatty acids and cholesterol cause hypercholesterolemia by changing the lipoprotein mechanisms and pattern and elevating TC. In several animal models, cholesterol-rich food has been often used to induce hypercholesterolemia, raise the cholesterol levels, and correlated metabolic changes.[52]

Triton X-100 has been commonly used to induce the acute hyperlipidemia by blocking the removal of triglyceride and cholesterol in various animal models[53,54] particularly the rat models, for the screening of synthetic or natural antihyperlipidemic drugs.[55] The present study indicated that IP injection of Triton X-100 (100 mg/kg) prepared in normal saline was appropriate to induce hyperlipidemia in rats. Similar findings were reported by Jahromi and Ray,[56] Sudha *et al.*,[57] and Gundamaraju *et al.*[58] that Triton X-100 (100 mg/kg) in rats elevated their serum lipid profile parameters. It is well identified that HDL has a defending role in cardiovascular diseases, particularly coronary artery disease.[59] Similarly, high levels of LDL in serum correlate with an increased risk for atherosclerosis development.[60] There was a significant elevation in TC, triglyceride, LDL, and VLDL levels, whereas the level of HDL in all groups was reduced after 24 h of IP injection of Triton X-100 [Tables 3 and 4]. When aqueous and methanol extracts of *T. ammi* were co-supplemented with Triton X-100, the increased levels of triglycerides, TC, VLDL, and LDL declined considerably. These results correlated with the findings of Dhulasavant *et al.*[61] and Iqbal *et al.*[62] who described that aqueous and ethanolic extracts of *Cinnamomum tamala* considerably

reduced the levels of triglyceride, TC, LDL, and VLDL whereas both extracts increased the HDL levels significantly. Martinez *et al.*[63] also stated similar findings that correlate with our results in the present study.

In our study, extracts at different doses dropped the triglyceride, TC, LDL, and VLDL levels in the serum of rats and a significant increase was also observed in HDL levels, and it can be suggested that extracts have a direct role in lipids metabolism. These findings correlate with the results of Khan *et al.*[64] who evaluated that cinnamon bark at different doses reduced triglyceride and TC levels and controlled free fatty acids levels in type 2 diabetic patients. Hence, it indicates the efficacy of *T. ammi* in preventing the increased levels of lipid profile in hyperlipidemia.

Biochemical studies disclosed that triglycerides are independently linked to cardiovascular diseases.[65] Most of the antihypercholesterolemic drugs do not diminish the triglycerides levels but the aqueous and methanol extracts of *T. ammi* equivalent to 5 g/kg significantly reduced it by 35.14% and 38.73% respectively at 3 weeks treatment in the current study and a substantial lessening in triglycerides and LDL levels after supplementation of extracts at dose of 3 g/kg and 5 g/kg demonstrated its importance in prevention and management of cardiovascular diseases. These results also correlate with the findings of Zari and Allogmani[66] who stated that *Cinnamomum zeylanicum* oil caused a considerable reduction in triglycerides, TC, LDL, and VLDL levels with a significant increase in HDL level in streptozocin-induced diabetic rats. Hence, it may be considered that the *T. ammi* may be helpful in controlling the metabolism of certain lipoproteins that demonstrating the antihyperlipidemic effect of the *T. ammi*. Similar results also described by Wiesenfeld *et al.*[67] who assessed that flaxseed plays a main role in declining free and ester cholesterol in humans or animal models.

Reactive oxygen species and free radicals are most important causative agents for the development of several diseases such as cardiovascular diseases, cancer and neurodegenerative diseases.[68] Extracts showed a powerful antioxidant activity at 1.1 mg/mL when assessed against DPPH free radical [Table 2]. In this study, the effectiveness of extracts was compared with atorvastatin-a standard lipid-lowering drug. Khanna *et al.*[69] also stated a similar comparison between *Phyllanthus niruri* and standard drug in albino rats. In the present study, treatment with either extracts or atorvastatin improved the lipid profile. Methanolic extract at a dose of 5 g/kg significantly reduced the TC (36.14%), triglyceride (38.73%) and LDL concentration (75.03%) and markedly increased the HDL concentration (97.97%) in serum at treatment day 23rd. *T ammi* extracts treated groups at dose of 3 g/kg and 5 g/kg revealed a considerable antihyperlipidemic effect when compared with untreated control group. This may be occurred due to the existence of some antihyperlipidemic compounds which may act like inhibitor of some enzymes as HMG CoA reductase that inhibits the production of cholesterol in liver or declined the absorption of cholesterol from intestine.[70] Similar results were observed by Farnier and Davignon[71] and Mozaffarian *et al.*[72] who stated that administration of statins such as atorvastatin, lovastatin, and pravastatin decreased the production of cholesterol in liver by inhibiting the HMG-CoA reductase enzyme.

On the basis of these findings, it may safely be said that methanolic extract (5 g/kg) and atorvastatin (10 mg/kg) were equally efficacious in the management of hyperlipidemia. These results recommended that *T. ammi* produced an alteration in hepatic fat content, serum lipids, and showing a protective role on hyperlipidemia with good antioxidant effect. Therefore, *T. ammi*may be one of the potential sources for preventing and management of hyperlipidemia and its complications with less side effects and well tolerance. This data can be supportive in future for the treatment of hyperlipidemia in patients.

CONCLUSION

It was concluded that *T. ammi* possessed good antioxidant potential and significantly lowered the levels of triglyceride, TC, LDL, VLDL, ALT, AST, total bilirubin and increased the levels of HDL, albumin along with decreasing BUN and creatinine. It is suggested that *T. ammi* can act as antihyperlipidemic agent by sharing the mechanism of action of statins along with ameliorating the oxidative stress in vital organs.

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Conflicts of interest

There are no conflicts of interest.

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