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Original Article

The effect of *Dracocephalum subcapitatum* hydroalcoholic extract on dexamethasone-induced hyperlipidemic rats

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Abstract

Background and purpose: Recent data show the antihyperlipidemic activities of some plants belonging to the genus *Dracocephalum*. In this study, the effects of hydroalcoholic extract of *D. subcapitatum* (O. Kuntze) Lipsky aerial parts were evaluated in a model of hyperlipidemia induced by dexamethasone.
Experimental approach: The extract was prepared by maceration method and its total phenolic content was determined. Seven groups of 6 Wistar rats were used as follows: group 1 (normal control) received vehicle; group 2 (extract control) treated only with 200 mg/kg *D. subcapitatum*; group 3 (hyperlipidemia control) received dexamethasone (10 mg/kg/day, subcutaneously); group 4 (reference) received dexamethasone and atorvastatin (40 mg/kg, orally), and groups 5-7 (test groups) received dexamethasone and simultaneously treated orally with 50, 100, or 200 mg/kg *D. subcapitatum*. All treatments were done for 1 week. Serum lipid profile, fasting blood glucose, malondialdehyde concentration, and liver histopathology were examined.
Findings/Results: Total phenolic content was 77.34 ± 4.9 mg/g as gallic acid equivalent. Treatment with *D. subcapitatum* (200 mg/kg) meaningfully declined triglycerides, total cholesterol, low-density lipoprotein, very low-density lipoprotein, blood glucose, alanine aminotransferase, aspartate aminotransferase, and malondialdehyde levels, and alleviated hepatic steatosis in dexamethasone-induced dyslipidemic rats.
Conclusion and implications: Findings of the current study suggest that *D. subcapitatum* may be effective in the management of hyperlipidemia. Further studies are necessary to determine the clinical efficacy of this treatment and to understand the underlying mechanisms responsible for its ability to lower lipid levels.

Keywords: Dracocephalum; Hyperlipidemias; Hyperglycemias; Lipid peroxidation; Dexamethasone; Rats.

INTRODUCTION

Hyperlipidemia or disorders of lipid metabolism are generally realized with an increase in the plasma levels of lipids and various lipoproteins (1). Hyperlipidemia is known as an important risk factor for the development and progression of cardiovascular diseases such as atherosclerosis and heart attack (2). Primary hyperlipidemia is rarely caused by genetic abnormalities and usually occurs because of unhealthy diet and inactivity (3). Secondary hyperlipidemia may be caused by diabetes, hormonal changes, excessive alcohol consumption, and adverse effects of medications (4).

Oxidative stress which arises from the high creation of reactive oxygen species (ROS) and diminished efficiency of the antioxidative defense pathway plays a great impact on dyslipidemia and the development of atherogenic particles. Many investigations have documented the significant relationship between plasma levels of oxidative stress markers such as malondialdehyde (MDA) and atherogenic disorders (5).

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Various cholesterol and triglyceride-lowering drugs including statins, fibrates, bile acid sequestrants, and cholesterol absorption inhibitors are currently used for managing hyperlipidemia however there is still no certain cure and the available drugs have many side effects (1).

Medicinal plants have been of interest since ancient times for finding novel effective treatments for many diseases (6). Some medicinal herbs and phytochemicals have been defined for their therapeutic value in hyperlipidemia (7).

The genus *Drcacephalum* is one of the genera in *Lamiaceae* family, with approximately 70 species worldwide, 11 of which are found in Iran. The plants of this genus grow naturally in the temperate areas of the Northern Hemisphere (8,9). There are many phytochemical compounds such as alkaloids, flavonoids, terpenoids, lignans, and coumarins in this genus. Various medicinal uses for the treatment of cardiovascular, respiratory, gastrointestinal, and inflectional diseases have been reported for different *Drcacephalum* plants (10).

D. subcapitatum (O. Kuntze) Lipsky is a local wild plant in Northeastern Khorasan in Iran (11). The phylogenetic examination of *Dracocephalum* species displays that *D. subcapitatum* is more closely related to *D. kotschyi* than other species (12). Several flavonoids and terpenoids have been identified in *D. subcapitatum* (11). Although little information is available about the biological effects of *D. subcapitatum*, many pharmacological properties such as antihyperlipidemic, antidiabetic, and antioxidant effects have been recognized for flavonoids and terpenoids as the bioactive substances that are also present in this plant (13,14). The current study was designed to investigate the possible antihyperlipidemic and antioxidant effects of hydroalcoholic extract of *D. subcapitatum* aerial parts in a rat model of hyperlipidemia induced by dexamethasone.

MATERIALS AND METHODS

Plant material and preparation of extract

Aerial parts of *D. subcapitatum* were collected from mountains in Gifan region (North Khorasan Province) in the northeastern part of Iran. After approval of the plant by a botanist, a voucher specimen (SAM4021) was stored in the Herbarium of the Department of Pharmacognosy, Isfahan University of Medical Sciences. The dried and pulverized aerial parts of *D. subcapitatum* (200 g) were soaked with 70% ethanol for preparation of hydroalcoholic extract by maceration technique for 72 h, 4 times (15). Then plant extract was separated using a vacuum system and ethanol was removed by rotary evaporation at 40 °C. The remaining water was separated with a freeze dryer and the obtained dried extract was kept at 4 °C. The yield of *D. subcapitatum* extraction was 35.5% (w/w). For oral administration in rats, the extract powder was suspended in water and administered using an intra-gastric tube.

Determination of total phenolic content

The amount of phenolic compound in *D. subcapitatum* extract was measured by Folin Ciocalto assay. This method is a colorimetric technique based on the reduction of reagent (a mixture of phosphotungstate and phosphomolybdate) in the reaction with phenolic compounds.

Briefly, the standard or extract samples were mixed with sodium bicarbonate (20%) and then with diluted Folin-Ciocalteu reagent. After 2 h of storage at room temperature, the absorbance was read by a spectrophotometer at 765 nm. Quantitation of total phenolic content in the samples was performed using a standard curve obtained from various concentrations of gallic acid (0-500 mg/L). The results were specified in terms of mg of gallic acid equivalents (GAE)/g of dried extract (16).

Animals

Forty-two male Wistar rats (230-250 g) grown in the animal house of the School of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran) were used in this study. Animals were housed under standard laboratory conditions including room temperature of 20-25 ℃ with a standard diet and water and a daily routine with a 12/12-h light/dark cycle. Rats were acclimatized for one week prior to the experimentation. The research procedures were in accordance with international

guidelines for laboratory animal use and care, and ethical approval was obtained from the Institutional Research Ethics Committee of Isfahan University of Medical Sciences (Ethic No. IR.MUI.AEC.1401.018).

Induction of hyperlipidemia

The glucocorticoid model of hyperlipidemia was established by subcutaneous (s.c.) administration of 10 mg/kg dexamethasone (Darou Pakhsh Pharmaceutical Co., Iran) for 7 days in rats (17). The weight of animals was recorded at the start of the experiment and then every other day. At the end of the trial period, all rats were kept fasting overnight and the blood samples were collected from the retro-orbital sinus under anesthesia with ketamine (70 mg/kg)/xylazine (10 mg/kg). The serum samples were used for biochemical assessments. Liver tissue was also isolated from sacrificed rats and immersed in a 10% neutral-buffered formalin solution after weighing. Tissue samples were examined histologically after further processing.

Experimental protocol

Rats were randomly divided into 7 groups, 6 each, as follows: Group one, as the normal control received a daily injection of normal saline (1 mL/kg, s.c.) and oral administration of the vehicle (water); Group two, as the extract control, animals were treated only with *D. subcapitatum* hydroalcoholic extract orally at the dose of 200 mg/kg; Group 3, as the hyperlipidemic control group received dexamethasone (10 mg/kg/day, s.c.); Group 4, as the reference group, atorvastatin (40 mg/kg, orally; Abidi Pharmaceutical Laboratories Co., Iran) was administered simultaneously with dexamethasone (18); Groups 5-7 as the test groups were treated with dexamethasone and simultaneously with 50, 100, or 200 mg/kg of *D. subcapitatum* extract orally (19). All treatments were completed over 7 days.

Biochemical analysis

The serum content of blood glucose and lipid profile including triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol, and also liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using biochemical colorimetric kits produced by Pars Azmoon Company (Tehran, Iran). The level of very low-density lipoprotein (VLDL)-cholesterol was calculated by dividing the triglyceride level by 5 (20).

For the determination of lipid peroxidation, the serum content of MDA was assessed using a standard kit based on thiobarbituric acid reactive substances test. The absorbance of the final-colored complex was measured spectrophotometrically at 532 nm. The standard curve was drawn using different concentrations of MDA tetrabutyl ammonium.

Histopathological analysis

After fixation in formalin solution, the liver tissue specimens were processed through sequential stages including dehydrating in ascending grades of alcohol and then clearing using xylene, embedding in paraffin, sectioning at 5-µm thickness, deparaffinization, rehydrating, and finally staining with hematoxylin and eosin (H&E), mounting onto glass slides, and cover-slipping for microscopic observations regarding histopathological changes.

Statistical analysis

Data were reported as mean ± SEM and subjected to one-way analysis of variance (ANOVA) followed by Tukey post-hoc test on SPSS 25.0. The *P*-values < 0.05 were considered statistically significant.

RESULTS

Total phenolic content

The hydroalcoholic extract of *D. subcapitatum* aerial parts was evaluated for the total phenolic content. The calibration curve was drawn by linear regression for various concentrations of gallic acid. The regression equation was $y = 0.0023x + 0.0804$, in which x is the concentration of gallic acid in the sample (mg/L) with the correlation co-factor $R^2 = 0.994$. The total phenolic content of *D. subcapitatum* extract was assessed as 77.34 ± 4.9 mg GAE per gram of the dried plant extract.

Table 1. The effect of hydroalcoholic extract of *Dracocephalum subcapitatum* on serum biochemical parameters in dexamethasone-induced hyperlipidemic rats. Values are mean ± SEM, n = 6. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ indicate significant differences compared to the normal control; and $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus DEX control.

Groups	TG (mg/dL)	TC (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	FBS (mg/dL)	AST (IU/L)	ALT (IU/L)
Normal control	80.5 ± 11.3	86.4 ± 7.5	11.2 ± 0.9	16.1 ± 5.2	58.8 ± 5.4	81.2 ± 5.1	143.3 ± 8.2	90.9 ± 9.2
DSE control (200 mg/kg)	94.0 ± 13.2	74.2 ± 5.0	10.0 ± 1.1	18.8 ± 2.1	67.0 ± 3.1	78.3 ± 4.0	168.0 ± 6.3	109.2 ± 10.1
DEX control (10 mg/kg)	271.1 ± 25.3$^{\#\#\#}$	128.1 ± 12.3$^{\#}$	17.2 ± 1.3$^{\#}$	54.2 ± 2.2$^{\#\#\#}$	52.1 ± 1.7$^{\#}$	134.3 ± 3.7$^{\#}$	241.2 ± 21.2$^{\#\#\#}$	216.1 ± 25.0$^{\#\#}$
DEX + ATOR (40 mg/kg)	88.2 ± 15.1***	85.3 ± 3.8*	12.5 ± 1.2	17.6 ± 2.4***	48.5 ± 3.0	86.5 ± 2.1***	173.1 ± 8.0*	78.5 ± 8.2***
DEX + DSE (50 mg/kg)	310.3 ± 8.9	98.1 ± 5.4	12.0 ± 1.4	62.0 ± 3.7	42.1 ± 2.3	91.5 ± 3.0***	240.1 ± 15.5	247.0 ± 24.1
DEX + DSE (100 mg/kg)	189.0 ± 20.4*	95.3 ± 6.9	13.2 ± 1.5	37.8 ± 4.1*	57.2 ± 4.3	90.1 ± 6.2***	159.6 ± 15.4**	160.1 ± 20.2
DEX + DSE (200 mg/kg)	145.5 ± 18.6***	70.4 ± 9.9***	8.1 ± 0.4***	29.1 ± 3.0***	59.5 ± 4.1	94.5 ± 5.1***	163.3 ± 14.1**	143.3 ± 17.3*

ALT, Alanine aminotransferase; AST, aspartate aminotransferase; ATOR, atorvastatin; DEX, dexamethasone; DSE, *Dracocephalum subcapitatum* extract; FBS, fasting blood sugar; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoprotein-cholesterol.

Effect of D. subcapitatum on biochemical parameters

As shown in Table 1, exposure of animals to dexamethasone (10 mg/kg) led to a significant upsurge in blood levels of triglyceride, total cholesterol, LDL, VLDL, and fasting blood sugar, and a notable reduction in HDL in comparison with the normal control group. Dexamethasone also resulted in a large elevation in serum activities of liver enzymes including AST and ALT. Administration of atorvastatin as a standard antihyperlipidemic agent significantly decreased serum content of triglycerides (67.4%), total cholesterol (33.4%), VLDL (67.5%), fasting blood glucose (35.6%), AST (28.2%) and ALT (63.7%) compared to the dyslipidemic control group (dexamethasone-administered control group) without any significant effect on HDL level.

Treatment of rats with hydroalcoholic extract of *D. subcapitatum* at all administered doses significantly decreased fasting blood glucose. The high dose of *D. subcapitatum* extract resulted in a significant reduction of 46.32% in triglycerides, 45.04% in total cholesterol, 52.90% in LDL, 46.31% in VLDL, and 29.63% in fasting blood glucose levels, and 32.29% in AST and 33.69% in ALT activities in hyperlipidemic animals compared to the control group received dexamethasone. Though, no meaningful result was observed on HDL value (Table 1).

Assessment of lipid peroxidation displayed that dexamethasone significantly increased the serum content of MDA compared to the normal control. Treatment with *D. subcapitatum* extract at 200 mg/kg and also with atorvastatin showed an anti-lipid peroxidation effect in hyperlipidemic animals (Fig. 1).

Effect of D. subcapitatum on body and liver weight

Our results exhibited that dexamethasone strictly decreased the rats' body weight during the induction of dyslipidemia. Treatment with atorvastatin and *D. subcapitatum* extract was not able to prevent body weight loss induced by dexamethasone. The relative weight of the liver (liver/body weight ratio) was markedly enlarged in dexamethasone-induced dyslipidemic animals when compared with normal rats. Administration of atorvastatin and *D. subcapitatum* extract at 200 mg/kg prevented liver weight gain induced by dexamethasone (Table 2).

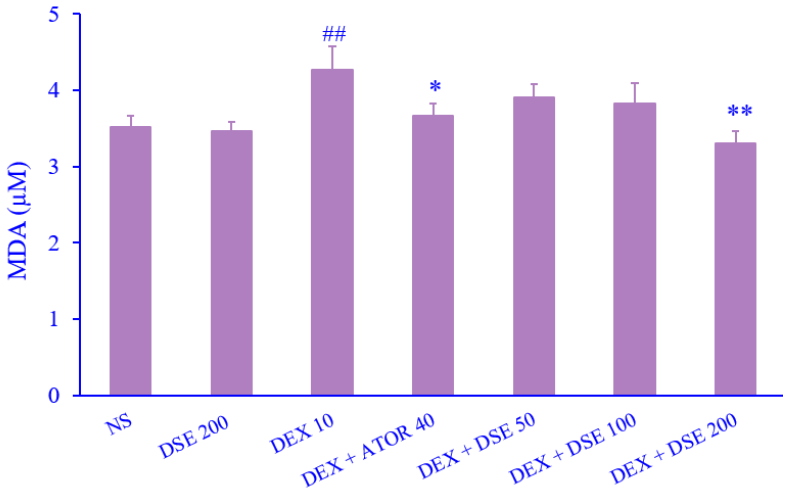

Fig. 1. Effects of *Dracocephalum subcapitatum* extract (50, 100, and 200 mg/kg) and atorvastatin (40 mg/kg) on serum malondialdehyde level in dexamethasone-induced hyperlipidemic rats. Values are mean ± SEM; n = 6. $^{\#\#}P < 01$ indicates a significant difference compared to the normal control; and $^{*}P < 0.05$ and $^{**}P < 0.01$ versus DEX control. ATOR, atorvastatin; DEX, dexamethasone; DSE, *Dracocephalum subcapitatum* extract; MDA, malondialdehyde.

Table 2. The effect of hydroalcoholic extract of *Dracocephalum subcapitatum* on body and liver weight in dexamethasone-induced hyperlipidemic rats. Values are mean ± SEM, n = 6. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ indicate significant differences compared to the normal control; $^{*}P < 0.05$ versus DEX control.

Groups	Initial body weight (g)	Final body weight (g)	Body weight changes (%)	Relative liver weight (%)
Normal control	210.4 ± 9.7	216.3 ± 8.5	+2.80 ± 0.13	3.83 ± 0.12
DSE control (200 mg/kg)	190.3 ± 7.5	201.6 ± 5.9	+5.93 ± 0.25$^{\#}$	3.95 ± 0.06
DEX control	230.6 ± 10.2	212.5 ± 6.4	-7.85 ± 0.39$^{\#\#\#}$	5.11 ± 0.18$^{\#\#\#}$
DEX + ATOR (40 mg/kg)	241.5 ± 4.9	223.0 ± 9.5	-7.66 ± 0.94$^{\#\#\#}$	4.10 ± 0.13
DEX + DSE (50 mg/kg)	220.6 ± 9.3	200.7 ± 10.7	-9.02 ± 0.15$^{\#\#\#}$	5.55 ± 0.11$^{\#\#\#}$
DEX + DSE (100 mg/kg)	189.7 ± 10.2	179.9 ± 8.9	-5.16 ± 0.13$^{\#\#\#}$	4.76 ± 0.07$^{\#\#\#}$
DEX + DSE (200 mg/kg)	235.0 ± 9.4	215.6 ± 10.1	-8.25 ± 0.17$^{\#\#\#}$	4.54 ± 0.09$^{\#\#, *}$

ATOR, Atorvastatin; DEX, dexamethasone; DSE, *Dracocephalum subcapitatum* extract.

Effect of D. subcapitatum on liver histopathology

Assessment of liver sections of normal control animals and rats that were treated only with *D. subcapitatum* extract for histopathological alterations showed the typical architecture of the normal hepatocytes (Fig. 2A and B). In dexamethasone-treated animals, there was fatty degeneration, diffused steatosis of hepatic parenchymal cells, and cellular swelling (Fig. 2C). Histopathological examination of liver sections of rats treated with atorvastatin (Fig. 2D) and with *D. subcapitatum* extract at 50, 100, and 200 mg/kg (Fig. 2E-G) showed a great extent of improvement in liver histopathological alterations as decreasing in micro- and macro-vesicular fat accumulation especially at the high dose of extract.

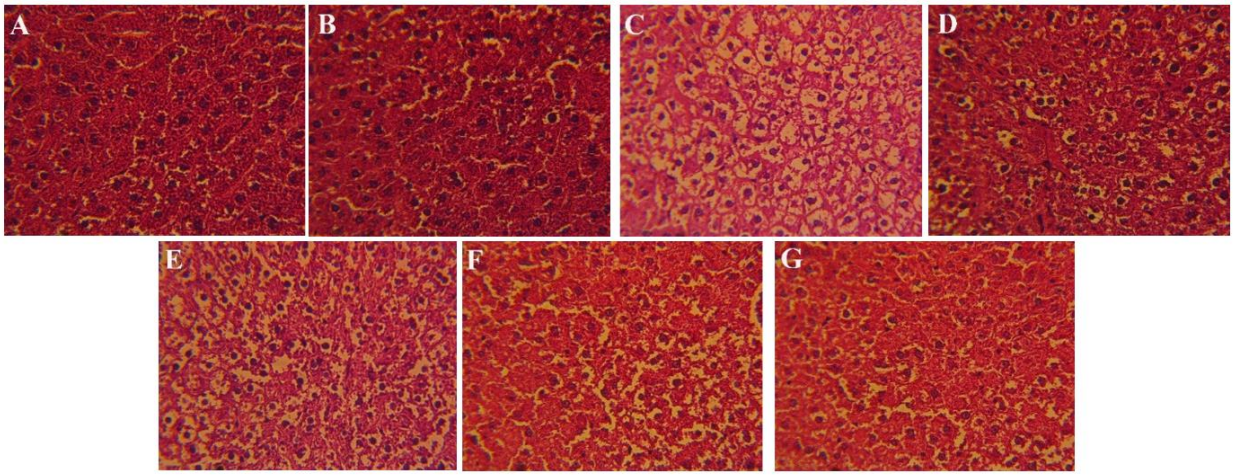

Fig. 2. Representative hematoxylin and eosin histological sections of the liver tissue of (A) normal control group and (B) *Dracocephalum subcapitatum* extract alone-treated group showing normal hepatocytes appearance; (C) dexamethasone-induced hyperlipidemic group indicating diffused steatosis, fatty degeneration, and cellular swelling; (D) atorvastatin-treated group showing mild vesicular steatosis; (E-G) *Dracocephalum subcapitatum* extract-treated groups with doses of 50, 100, and 200 mg/kg showing moderate vesicular steatosis. Magnification: ×400.

DISCUSSION

In this animal model of hyperlipidemia, the effects of hydroalcoholic extract of *D. subcapitatum* aerial parts as a *Drcacephalum* plant with limited pharmacological data were evaluated in rats.

Glucocorticoids-induced dyslipidemia is an animal model in which the administration of dexamethasone as a potent corticosteroid disturbs the fat and glucose metabolism and leads to the metabolic syndrome-like disorder with symptoms of hyperlipidemia and hyperglycemia (21). Dexamethasone augments the serum content of fatty acids and lipids because of the enlarged lipolysis in adipose tissue and more synthesis of fatty acids and VLDL in hepatic tissue. It also declines the clearance of blood lipoproteins by affecting apolipoprotein genes and lipoprotein receptors (22). Decrease in the activity of lecithin cholesterol acetyltransferase and downregulation of LDL receptor contribute to the elevation of total serum cholesterol level during exposure to a high dose of dexamethasone (23,24). Development of fatty liver occurs because of more lipid and fatty acids synthesis and less creation of triacylglycerol *via* the diminished activity of hepatic lipoprotein lipase, activation of adenosine monophosphate-activated protein kinase, and enlarged lipogenesis as a result of upsurges in the activity of crucial lipogenic enzymes including fatty acid synthase, acetyl-coenzyme A carboxylase, and diminution of the fatty acid beta-oxidase activity (25,26). Dexamethasone excess raises liver size with enlarged hepatocytes because of lipid accumulation and motivation of pregnane X receptor/Yes-associated protein (PXR/YAP) without effect on hepatocyte proliferation (27). Moreover, dexamethasone causes liver toxicity due to oxidative damage and enhanced expression of the hepatic enzyme's gene (28).

Insulin resistance and hyperglycemia are other unwanted effects of glucocorticoids due to the hampering uptake of glucose by peripheral tissues and distressing insulin signaling (29).

Oxidative stress also contributes to the pathogenesis of many adverse effects of glucocorticoids during high dosages in long-term use through dysregulation of physiological processes *via* ROS-induced damages (30).

In this investigation, dexamethasone-induced lipid peroxidation, dyslipidemia, and hyperglycemia with significant alterations in the liver architecture and the body and liver weights. Treatment with *D. subcapitatum* aerial parts extract notably improved the serum liver function markers, lipid and glucose profile, and also liver histopathological and weight changes in hyperlipidemic rats at 100 and 200 mg/kg.

There are several reports about the useful effects of some *Drcacephalum* plants in improving hyperglycemia and hyperlipidemia (31-33). *D. kotschyi* is a well-known species in this genus with various therapeutic properties including blood lipid-lowering effect. In a model of high-fat hyperlipidemia, administration of hydroalcoholic extract and polyphenolic fraction of *D. kotschyi* for 14 or 21 days significantly attenuated triglycerides, total cholesterol, and LDL at 80 and 120 mg/kg (30). Aslian *et al.* reported that *D. kotschyi* extract decreased blood levels of triglycerides (54%), total cholesterol (40%), LDL (54%), and blood glucose (25%) and increased HDL (45%) in adipose tissues in diabetic rats (32). In their *in vitro* study, *D. kotschyi* extract augmented expression of some crucial genes involved in lipid metabolism and adipogenesis including peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element-binding type 1 (SREBP-1), forkhead box O-1 (FOXO1), and protein kinase B while suppressed c-Jun N-terminal kinase (JNK) in 3T3-L1 adipocyte cells (32).

In the study conducted by Pouraboli *et al.*, methanol extract of *D. polychaetum* shoot at 300 mg/kg showed hypolipidemic properties through lessening cholesterol and triglycerides levels in diabetic rats. It also diminished blood glucose content (27.1%) at 120 min in the oral glucose tolerance test (33).

Folin Ciocalteu assay revealed total phenolic content as 77.34 ± 4.9 mg GAE in 1 g of the dried extract of *D. subcapitatum* which proposes this plant as a good source of antioxidants. In our study, *D. subcapitatum* extract showed anti-lipid peroxidative activity. Although no previous data is available on the impact of *D. subcapitatum* on oxidative stress, there are many reports of the antioxidant actions of *Drcacephalum* plants through enhancing activities of catalase and superoxide dismutase, scavenging of free radicals, hindering lipid peroxidation, and increasing total antioxidant power due to the various phytochemicals, especially phenolic ingredients (32-34).

The presence of different bioactive constituents in the *D. subcapitatum* extract including flavonoids (as a main group of phenolic compounds) such as luteolin, xanthomicrol, calycopterin, apigenin, and isokaempferide, and some terpenoids like neral, geranial, limonene, ursolic acid, and oleanolic acid may be accountable for the antihyperlipidemic and hypoglycemic actions of this plant (11). The beneficial effects on the serum glucose and lipid profile have been established for many flavonoids and terpenoids. Limonene as a cyclic monoterpene has improved fatty liver, hyperglycemia, and hyperlipidemia profile and reduced the size of white and brown adipocytes in obese mice (35). It has been reported that terpenoids regulate PPARs and subsequently affect lipid and glucose metabolism and energy homeostasis (14). A large number of animal and clinical data support the effectiveness of flavonoids for managing hyperlipidemia, diabetes, and oxidative stress (13). For example, luteolin as a flavonoid which is also found in *D. subcapitatum* can improve hyperglycemia and hyperlipidemia and reduce proinflammatory cytokines and oxidative stress in diabetic rats (36). Apigenin has shown a lipid-lowering effect by reducing triglycerides, total cholesterol, and LDL and increasing HDL in hyperlipidemic mice (37).

CONCLUSION

Regarding our results, hydroalcoholic extract of *D. subcapitatum* aerial parts showed beneficial activities in hyperlipidemia caused by dexamethasone by declining serum lipids, glucose, lipid peroxides and transaminases, and improving liver histopathological changes. Further investigations are required to clarify the mechanisms of antihyperlipidemic action of *D. subcapitatum* and to verify its clinical benefits of using in patients with dyslipidemic disorders.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

L. Safaeian was responsible for the research plan, designing the animal study, supervising the investigation, analyzing the data, and editing the manuscript; Z. Yazdiniapour planned the herbal experiments; P. Karimian conducted the histopathological analysis; S. Hajibagher and Z. Bakhtiari performed the experiments, collected the data, and prepared the draft of the manuscript. All authors read and confirmed the finalized article.

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