ORIGINAL ARTICLE

Caraway distillate ameliorate hyperlipidemia and hepatic steatosis in obese rat

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Abstract

Background: *Carum carvi* L. (family; Apiaceae, common name; caraway) is conventionally used for the treatment of flatulence, dyspepsia, fatness, asthma, and amenorrhea in Unani as well as the Ayurvedic System of Indian Medicine. **Aim and Objective:** The present study was planned to scrutinize the effect of caraway distillate (CD) on hyperlipidemia and hepatic steatosis in a rat model of high-fat diet (HFD)-induced obesity. **Materials and Methods:** HFD-fed rats were administered with CD (7.75 ml/kg body weight [BW] twice a day, orally) and CD1 (7.75 ml/kg BW once a day, orally) for 4 weeks. **Results:** After termination of the study, CD and CD1 treatments showed significant ($P < 0.001$) attenuation in cholesterol, triglycerides, low-density lipoprotein cholesterol, leptin levels, and pancreatic lipase activity and significant ($P < 0.001$) boost in high-density lipoprotein cholesterol. Furthermore, CD and CD1 treatment also noticeably attenuated the extent of hepatic steatosis. **Conclusions:** These results suggest that CD has a strong protective effect against hyperlipidemia and hepatic steatosis in HFD-induced obesity on a rat model.

Key words: *Carum carvi*, hyperlipidemia, leptin, steatosis

INTRODUCTION

Hyperlipidemia is a major factor for atherosclerosis and atherosclerosis-related disorder, such as ischemic cerebrovascular disease, coronary heart disease, and peripheral vascular disease.[1] Clinically, it is mainly defined as the raised total cholesterol (TC) and/or low-density lipoprotein cholesterol (LDL-C).[2-4] Lipid disorders can be mainly due to different factors such as environmental factors (diet rich in saturated fat or a sedentary lifestyle), diseases (chronic kidney disease, type 2 diabetes, hypothyroidism, etc.), and even medications (thiazide, β-blockers, progestins, anabolic steroids, etc.).[5,6] A lot of literature evidence is reported in support of the fact that successful management of dyslipidemia trim downs morbidity and mortality from cardiovascular disease. It is for that reason desired to make a comprehensive strategy. It may include various ways to control lipid levels and to also address associated metabolic abnormalities and modifiable risk factors such as obesity, hypertension, diabetes, and cigarette smoking. Lifestyle change can be the important and easiest step to reduce cholesterol levels.[7] Besides this, various drugs are available in the market such as statins, fibrates, and nicotinic acid which are effective for the management of dyslipidemia.[8,9] Treatment of lipid abnormalities is considered a lifelong battle. Moreover, the safety and effectiveness of long-term lipid-lowering treatment are questionable. *Carum carvi* L. (black caraway) is an indigenous herb that belongs to the family *Apiaceae*. Many research data indicated that *C. carvi* fruit possesses

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Received: 10-08-2021
Revised: 26-10-2021
Accepted: 08-11-2021

antioxidant, antibacterial, antidiabetic, diuretic and cholesterol, and triglycerides (TGs) lowering activities.[10-14] Samojlik *et al*. (2010) found that caraway oil possesses strong antioxidant and hepatoprotective activity.[15] In this present article, the study was designed to explore the hypolipidemic and anti-hepatic steatosis property of caraway distillate (CD) using a high-fat diet (HFD) rat model.

MATERIALS AND METHODS

Herb Material, Preparation, and Phytochemical Study of CD

C. carvi was obtained from a raw material drug dealer, New Delhi, India, in December month and was recognized by the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen and recognition certificate reference number NISCAIR/RHMD/Consult/2011-12/1753/53 were achieved. Samples were washed with tap water to eradicate salt and dried in an air dryer at 37°C for 40 h. A dried sample was ground and the coarse powder was stored at −20°C until used. The dried coarse powder of *C. carvi* L. (1000 g) was soaked in purified water and transferred to the distillation plant along with purified water (12 L). This was distilled at 100°C for about 5 and a ½ h and 7.5 L of distillate (CD) was collected.[16] A preliminary phytochemical analysis of CD and its ingredients was done to assess the presence/absence of various groups of phytochemical compounds.

For gas chromatography (GC) and GC–mass spectrometry (GCMS) study, the method for sample preparation was discussed in my previous.[16] The chemical constituents were identified using NIST and Wiley libraries. GC and GCMS chromatograms are shown in Figure 1.

Animals, Kits, and Experimental Diets

The procedure employed in this experiment for the use of rats was approved by the Institutional Animal Ethics Committee of Hamdard University (Letter number – 607, June 13, 2011). Wistar Albino male rats, weighing 150– 200 g (8–12 weeks old, 150–200 body weight [BW]), were obtained from the Central Animal House Facility, Hamdard University, New Delhi, India. Before the beginning of the experiment, the rats were accustomed for 1 week to the laboratory circumstances. They were maintained in polycarbonate cages, under restrained temperature (25 ± 2°C) and 12 h light/12 h dark rhythm. The rats were permitted free access to a normal pellet diet (NPD) and water *ad libitum*. TC, TG (Span Diagnostics Ltd., Surat, Gujarat, India), high-density lipoprotein cholesterol (HDL-C) (Reckon Diagnostics Pvt. Ltd., Baroda, Gujarat, India), rat leptin ELISA kit (BioVendor, Czech Republic), QuantiChrom™ Lipase

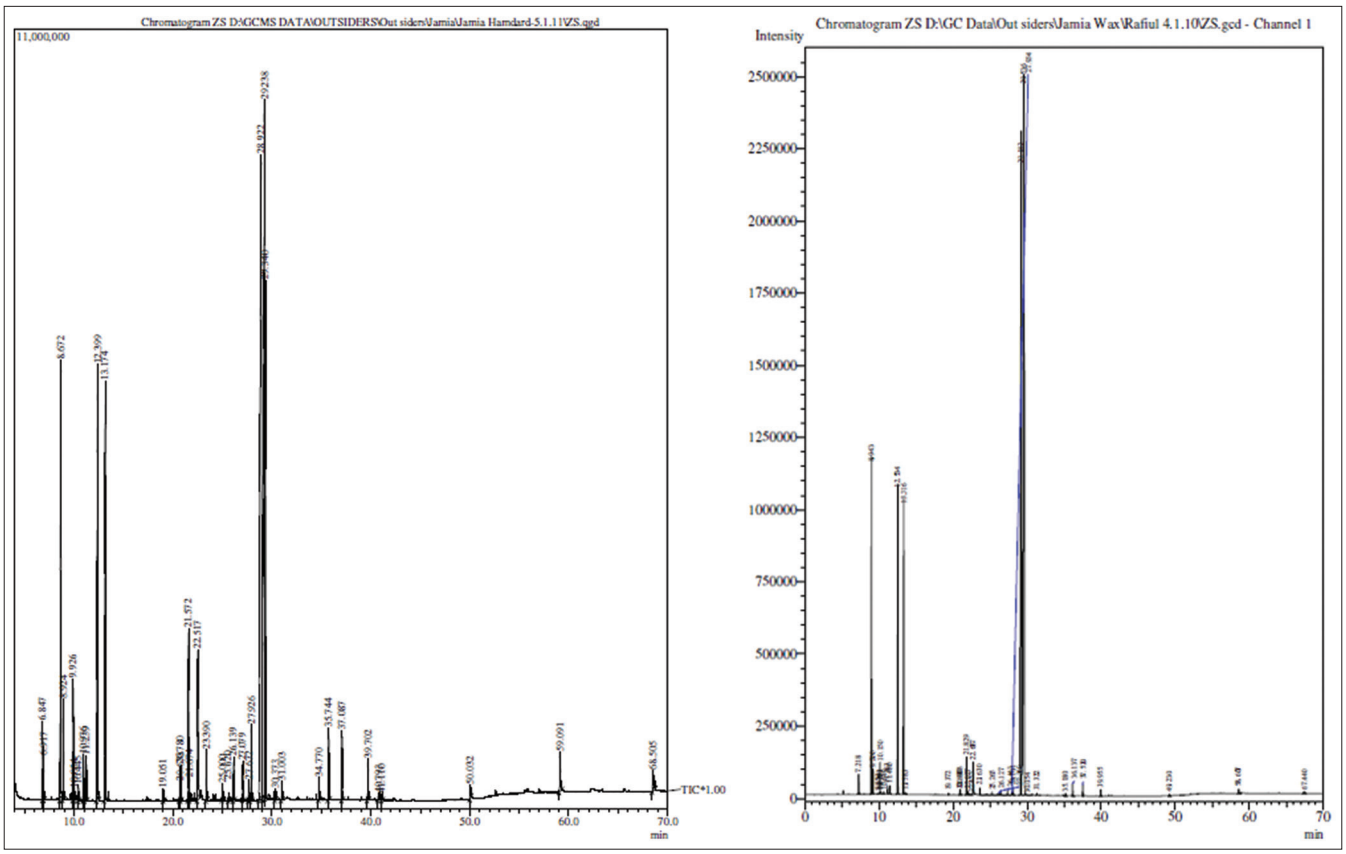

Figure 1: (a) Gas chromatography (GC)–mass spectrometry and (b) GC chromatogram of caraway distillate

Assay Kit (DLPS-100), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (AST; Span Diagnostics Ltd., Surat, Gujarat, India) kit were used in the study. A NPD consisting of 12.5% lipids, 62.3% carbohydrate, and 24.3% protein was purchased from Amrut rat feed, Mfd by Pranav Agro Industries Ltd., Maharashtra, India, while the other groups were fed with HFD. HFD consisting of 60% fat, 20% protein, and 20% carbohydrate (in g/kg) was procured from National Centre for Laboratory Animal Science, National Institute of Nutrition, Hyderabad, India.

Treatments Schedule and Serum Biomarkers Measurements

After 1 week of acclimation, rats were randomly divided into four groups (n = 6): One normal control group, one HFD control group, and the remaining 3–4 as treatment groups. Animals in the normal control group were fed with a NPD while the other groups were fed with HFD throughout the experiment. Treatments were initiated from the 15th day and continued for 4 weeks (i.e., up to 42 days). The treatment groups, HFD + CD and HFD + orlistat, were administered with CD (7.75 mL/kg B.W., twice a day, orally), CD1 (7.75 mL/kg B.W., once a day, orally), and orlistat (30 mg/kg B.W., once a day, orally), respectively, by oral gavage. Normal control group received 0.5% carboxymethylcellulose (CMC) sodium aqueous solution. Orlistat drug was suspended in 0.5% CMC sodium aqueous solution for animal treatment. CD and CD1 were also dissolved in a 0.5% CMC sodium aqueous solution to form a suitable dose. During treatment, the test groups were continued to feed with HFD. The BW was recorded daily during the feeding period and finally, the average value of these parameters was calculated after 6 weeks. At the end of the treatment period, rats were anesthetized with ether, following a 12 h period of fasting. Blood was drawn from the retro-orbital plexus into centrifuged tubes, and the serum was obtained by centrifuging the blood at 4000 rpm for 10 min. The livers were removed from rats, rinsed with phosphate-buffered saline, and then weighed. The liver samples were stored in 10% formalin solution at room temperature for histopathology study. The serum samples were stored at −70°C until they were analyzed. Serums TC, TG, HDL- C, AST, and ALT, in serum, were measured with commercial kits. Evaluations of serum LDL-C were measured using Friedewald's equation.[17] Leptin and pancreatic lipase activity in serum was determined with commercial kits. Liver samples were used for histopathology, hepatic lipids estimation, and thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) assay. A portion of the liver sample was minced and homogenized (10% w/v) for the determination of TBARS, GSH, SOD, and CAT activities.[18,19]

All other chemicals used were of analytical grade. Double-distilled water was used for all biochemical assays.

Histopathological Analysis

The considered rats were sacrificed and four rat liver tissue samples were collected, fixed in 10% formalin buffered solution, cut into 5 μm sections, and stained with hematoxylin/eosin. The sections of liver tissues were studied to find out the level of tissue injure or steatosis by HFD. Steatosis was determined based on the percentage of lipid droplets in stained sections as follows: Score 0, no steatosis; score 1, up to 33% steatosis; score 2, 33–66% steatosis; and score 3, >66% steatosis.[20]

Statistical Analysis

Data analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, California, USA). All data were expressed as mean ± SEM. Groups of data were compared with the analysis of variance (ANOVA) followed by Dennett's t-test to identify significance among groups. The value was considered statistically significant when $P < 1/20$ and highly significant when $P < 1/1000$.

RESULTS

Phytochemical Analysis of CD

The quantitative phytochemical screening of CD showed the presence of volatile oil (phenolic and non-phenolic terpenoids) while tannins, steroids, flavonoids, and saponins were absent. CD is a distillate product that is why it contains only volatile compounds. The volatile compounds in CD are shown in Table 1.

Chemical Analysis of CD by GC and GCMS

The phytochemical analysis of the CD was investigated by GC and GCMS. 4-ethyl-3-nonen-5-yne (38.93 %), cumin aldehyde (32.17 %), γ–terpinene (8.44%), and β-pinene (7.00%) were found as major components. It is shown in Table 1.

Effect of CD on BW

HFD-fed rats showed a significant ($P < 0.001$) increase in BW as compared to NPD-fed rats. CD, CD1, and orlistat treatment showed significant ($P < 0.001$) diminution in BW as compared to HFD-fed rats [Table 2].

Effect of CD on Lipid Parameters

HFD-fed rats showed significant ($P < 0.001$) increases in serum TC, TG, and LDL-C levels and a significant ($P < 0.001$) decrease in HDL-C as compared to NPD-fed rats [Table 3]. CD and CD1 treatments significantly ($P < 0.001$) decreased serum TG, TC, and LDL-C and significantly

Table 1: Results of chemical composition of CD

Compounds	Area%
α-Pinene	0.4984
α-Thujene	0.4984
β-Pinene	7.0027
β-Phellandrene	0.0213
Myrcene	0.0241
-2-Methyl-5-(1-methylethyl)-1,3-cyclohexadiene	0.5719
(+)-4-Carene	0.0338
Limonene	0.1542
p-Cineole	0.2426
ð-Terpinene	8.4403
Cymene	7.2303
4-Thujanol	0.0334
Linalool	0.0678
1-(3-Isopropenyl-2,2-dimethyl-cyclopropyl)-2-methyl-propan-1-one	0.1285
Phellandral	0.9471
4-Terpineol	0.1644
L-trans-pinocarveol	0.0854
O-Menth-8-ene	0.0594
β-Fenchol	0.1041
Camphor	0.0594
Geraniol acetate	0.1846
Cuminaldehyde	32.117
4-Ethyl-3-nonen-5-yne	38.923
1-Phenylpropanol	0.1075
p-Cymen-8-ol	0.0526
Trans-p-Menth-2-en-7-ol	0.0526
Cis-farnesol	0.0526
1,4-p-Menthadien-7-ol	0.0743
Cuminol	0.3135
Thymol	0.3182
m-Cumenol	0.1949
4-(1-Methylethylidene) cyclohexanol	0.0605
Tetradecanoic acid	0.1740
Pentadecanoic acid	0.1156

CD: Caraway distillate

($P < 0.001$) increased HDL-C as compared to the HFD group. Orlistat treatment significantly ($P < 0.001$) decreased serum TG, TC, and LDL-C and significantly ($P < 0.001$) increased HDL-C against the HFD group.

Effect of CD on Serum Leptin and Pancreatic Lipase Activity

HFD-fed rats demonstrated significant ($P < 0.001$) increases in leptin and pancreatic lipase activity against NPD-fed rats [Table 4]. However, CD, CD1, and orlistat treatment showed a significant ($P < 0.001$) decrease in leptin and pancreatic lipase activity against the HFD group.

Table 2: Effect of CD and CD1 on BW

Groups	Weight gain during treatment (g)
NPD	71.21±5.12
HFD	127.89±6.02$^{\$\$}$
HFD+CD	87.61±3.44**
HFD+CD1	98.41±5.04**
HFD+orlistat	60.22±5.90**

All values were expressed as mean±SEM for six rats in each group. $^{\$\$}P<0.001$ as compared to control group. $^{**}P<0.001$ as compared to HFD groups, NPD: Normal pellet diet, HFD: High-fat diet, CD: Caraway distillate

Effect of CD on Serum AST and ALT

HFD-fed rats showed significant ($P < 0.001$) augmentation in AST and ALT as compared to the control group [Table 5]. CD and CD1 treatment showed a significant ($P < 0.001$) decrease in serum AST and ALT, as compared to the HFD group. Orlistat treatment significantly ($P < 0.001$) decreased in AST and ALT as compared to the HFD group.

Effect of Antioxidant Levels

HFD-fed rats showed a significant ($P < 0.001$) decrease in serum SOD and CAT and significant ($P < 0.001$) augmentation in malondialdehyde (MDA) as compared to the control group [Table 6]. In contrast, CD and CD1 treatment significantly ($P < 0.001$) augmented serum SOD and CAT level and significantly ($P < 0.001$) reduced MDA level while orlistat treatment significantly ($P < 0.001$) increased SOD and CAT and significantly ($P < 0.001$) decreased in MDA.

Histopathological Studies of Liver

Our histological examination of the liver demonstrated the inflammation or steatosis in liver tissues of the HFD group as a comparison to the control group [Figure 2]. CD, CD1, or orlistat treatment noticeably attenuated the extent of steatosis or inflammation in liver tissues as a comparison to the HFD group.

DISCUSSION

Diet rich in fats (HFD) given to rats for 7 weeks induced significant weight gain along with derangement of lipid profile. TC, TG, and LDL levels were increased significantly while that of HDL fell significantly. Administration of CD, CD1, or orlistat for 28 days remarkably lowered BW compared with that of the HFD group.

Table 3: Effect of CD and CD1 on lipid levels

Groups	TG level (mg/dl)	TC level (mg/dl)	HDL-C level (mg/dl)	LDL-C (mg/dl)
Control	47.60±2.51	61.80±2.18	34.18±0.89	19.956±0.821
HFD	107.11±4.37$^{\$\$}$	98.50±2.88$^{\$\$}$	24.07±2.59$^{\$\$}$	52.89±3.821$^{\$\$}$
HFD+CD	44.20±2.96**	73.89±1.61**	28.55±2.34**	28.51±1.45**
HFD+CD1	74.22±4.86**	83.89±5.61**	27.35±4.34**	38.51±1.45**
HFD+O	43.20±3.361**	73.08±1.14**	28.66±1.09**	24.9±1.81**

All values were expressed as mean±SEM for six rats in each group. ***P*<0.001 as compared to with HFD group. $^{\$\$}$*P*<0.001 as compared to control group. HFD: High-fat diet, CD: Caraway distillate, HDL-C: High-density lipoprotein cholesterol, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, TG: Triglyceride

Table 4: Effect of CD and CD1 on serum pancreatic lipase activity and leptin levels

Groups	Pancreatic lipase activity (U/L)	Leptin level (ng/ml)
Control	165.03±13.55	1.14±0.122
HFD	559.18±36.02$^{\$\$}$	5.045±0.25$^{\$\$}$
HFD+CD	521.38±5.54	1.21±0.10**
HFD+CD1	531.48±5.54	2.20±0.19**
HFD+orlistat	160.02±7.18**	1.98±0.11**

All values were expressed as mean±SEM for six rats in each group. ***P*<0.001 as compared with HFD group. $^{\$\$}$*P*<0.001 as compared with control group. HFD: High-fat diet, CD: Caraway distillate

Table 5: Effect of CD and CD1 on serum AST and ALT levels

Groups	AST level (IU/L)	ALT level (IU/L)
Control	62.44±1.60	44.46±2.92
HFD	87.75±1.77$^{\$\$}$	67.09±3.39$^{\$\$}$
HFD+CD	65.89±2.07**	46.61±1.74**
HFD+CD1	75.49±3.07**	56.41±2.34**
HFD+orlistat	64.60±1.39**	45.22±2.38**

All values were expressed as mean±SEM for six rats in each group. ***P*<0.001 as compared with HFD group. $^{\$\$}$*P*<0.001 as compared with control group. HFD: High-fat diet, CD: Caraway distillate, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

In the present investigation, it was found that CD and CD1 could remarkably ameliorate the increases in plasma lipid profile, AST, ALT, leptin, and pancreatic lipase activity and enhance the decreased level of HDL-C during an obese state. We found that HFD for 6 weeks produced a significant augment in the serum TC, TG, and LDL-C levels in HFD-induced obesity in rats. Serum HDL-C levels were reduced in the HFD group rats as compared to the normal healthy control group rats. Our present study results are supported by the work of Lavie and Milani, 2002, which indicated that HFD adversely affects plasma lipids, especially by increasing TC and decreasing the levels of HDL-C.[21,22] The HFD might lead to an augment in the synthesis of phospholipids and cholesterol esters in rats.[23] CD and CD1 treatment significantly lowered the raised TC in HFD-induced obese rats. A substantial reduction of TC in serum by CD and CD1 could be attributed to a diminution in the activities of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase. It is a rate-limiting enzyme in cholesterol biosynthesis.[24] CD and CD1 treatment remarkably ameliorated the raised LDL-C and enhanced the decreased levels of HDL-C in obese rats. Furthermore, a substantial diminution in LDL-C and TC level in serum could be achieved by decreased production of TC by liver tissue and/or efficient removal of the LDL-C by various tissues without subsequent renewal. Significantly elevated levels of total TG in obese rats were remarkably ameliorated by the administration of CD and CD1. Diminutions in TG in serum could be attributed to the inhibition of lipid absorption in the gastrointestinal tract, such as gastrointestinal lipase inhibition.[25] Pancreatic lipase is the main enzyme for fat absorption that hydrolysis TGs in the gastrointestinal tract. Pancreatic lipase inhibitor which assists to limit intestinal fat absorption at the initial stage has been proved as a helpful medication for the treatment of hyperlipidemia and has a great promise as an anti-hepatic steatosis agent. CD significantly inhibits the pancreatic lipase activity on obese rats and thus it prevents lipid absorption that hydrolysis TGs in the gastrointestinal tract. Thus, this result suggested that CD and CD1 would be helpful in the prevention of hepatic steatosis complications through improving hyperlipidemia. Consumption of HFD for 6 weeks produced a significant increase in the leptin in HFD-induced obese rats. Leptin alters the release of several neuropeptides after binding to its receptors (leptin receptor [LepR]), especially neuropeptide Y (NYP) from the hypothalamus and in the hypothalamus leptin inhibits the expression of NPY.[26] NYP acts as a transmitter in the nervous system which is important due to its appetite-stimulating effect.[27] CD was the increased plasma leptin level, resulting in elicits the food intake and enhanced energy expenditure. Liver steatosis is a well-known pathology in severely hyperlipidemic patients. It may progress in some patients to steatohepatitis and cryptogenic cirrhosis.[28] In many obese people, an increase in serum and hepatic TG levels causes hepatic steatosis.[29] CD and CD1 treatment decreased serum lipid levels in HFD-induced obese rats and protect liver steatosis by regulating the hepatic TG levels and excess pronounce changes in the hepatic cells. The elevated levels of liver enzyme (AST and ALT) and hepatic fat deposition in obese rats were lowered considerably by therapy of CD and CD1.

Table 6: Effects of CD and CD1on serum antioxidant levels

Parameters	NPD	HFD	HFD+CD	HFD+CD1	HFD+orlistat
MDA (μM)	4.51±0.05	8.43±0.16$^{\$\$}$	5.32±0.17**	6.02±0.13**	5.05±0.26**
SOD (%)	94.02±1.61	74.97±1.24$^{\$\$}$	90.12±3.32**	83.13±2.12**	93.33±8.12**
CAT (U/ml)	43.33±8.12	31.32±0.59$^{\$\$}$	39.13±0.73**	38.19±0.13**	41.42±0.36**

All values were expressed as mean±SEM for six rats in each group. $^{\$\$}P<0.001$ as compared to control groups, $^{**}P<0.001$ as compared to HFD groups, NPD: Normal pellet diet, HFD: High-fat diet, CD: Caraway distillate, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase

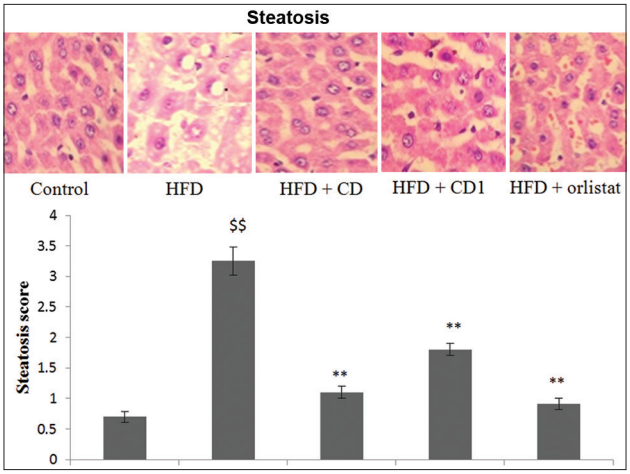

Figure 2: Effect of caraway distillate on liver histology and pathology score with reference to steatosis

Accumulation of body fat is correlated with an increase in the lipid peroxidation product, MDA. MDA is a by-product of lipid peroxidation and reflects the degree of oxidation in the body.[30] The MDA level in serum decreased significantly in the group treated with CD, CD1, or orlistat [Table 6]. The reduction in lipid peroxidation could be related to the antioxidant and free radical scavenging properties of CD and CD1. In our present study, CD, CD1, or orlistat treatment showed significantly decreased SOD and CAT (free radicals scavenger) in HFD-fed rats. Thus, this result suggests that the antioxidant activity of CD may, at least partly, contribute to the reduction of adipose tissue.

HFD is known to enhance the synthesis of TG accumulation in the hepatic tissue result in macrovesicular hepatic steatosis. Macrovesicular steatosis results in large fat droplets that replace hepatocyte nuclei, whereas microvascular steatosis results in small fat droplets that accumulate in the hepatocyte cytoplasm. Moreover, CD and CD1 administration noticeably attenuated and prevented the extent of macrovesicular steatosis. GC and GCMS study of CD demonstrated that 4-ethyl-3-nonen-5-yne, cumin aldehyde, γ-terpinene, and β-pinene were as major components. Many data indicate that these components possess strong antioxidant and hypolipidemic potential.[11,31] Thus, this result suggests that the antioxidant activity of CD may, at least partly, contribute to the reduction of serum lipid.

Thus, overall of the results showed that CD has a potential hypolipidemic effect in HFD-induced obese rat model and attenuated the extent of hepatic steatosis. Our results also recommend that the herbal preparations have good quality, therapeutics action, and without any risk of significant unfavorable effect as compared synthetic medicine.

CONCLUSIONS

Overall, it can be concluded from the current study that CD and CD1 ameliorate hyperlipidemia, and hepatic steatosis in the obese rat is mediated through hypolipidemic action, leptin sensitivity, and increased pancreatic lipase inhibition. CD treatment showed a stronger action than CD1. Thus, CD may be a tremendous alternative strategy for developing effective and safe hypolipidemic drugs in the future.

ACKNOWLEDGMENT

The authors are thankful to the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, New Delhi (India), and School of Pharmacy, Al-Karim University, Katihar, Bihar (India), for providing all required facilities for this work.

REFERENCES

1. Kwiterovich PO Jr, Recognition and management of dyslipidemia in children and adolescents. J Clin Endocrinol Metab 2008;93:4200-9.
2. Hegele RA. Plasma lipoproteins: Genetic influences and clinical implications. Nat Rev Genet 2009;10:109-21.
3. Reddy KS, Prabhakaran D, Chaturvedi V, Jeemon P, Thankappan KR, Ramakrishnan L. *et al*. Methods for establishing a surveillance system for cardiovascular diseases in Indian industrial populations. Bull World Health Organ 2006;84:461-9.
4. Enas EA, Chacko V, Pazhoor SG, Chennikkara H, Devarapalli HP. Dyslipidemia in South Asian patients. Curr Atherosc Rep 2007;9:367-74.
5. Mathers CD, Stein C, Ma FD, Rao C, Tomijima IM. Global Burden of Disease 2000. Version 2: Methods and Results; Paper. Geneva: World Health Organization; 2002.

6. World Health Organization. Integrated Management of Cardiovascular Risk: Report of a WHO Meeting; Geneva: World Health Organization; 2002. p. 9-12.
7. Gupta R, Sarna M, Thanvi J, Rastogi P, Kaul V, Gupta P. High prevalence of multiple coronary risk factors in Punjabi Bhatia community: Jaipur Heart Watch-3. Indian Heart J 2004;56:646-52.
8. Indrayan A. Forecasting Vascular Disease Cases and Associated Mortality in India. Reports of the National Commission on Macroeconomics and Health. Ministry of Health and Family Welfare, India; 2005. p. 2.
9. Steinberg D. Low-density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997;272:20963-6.
10. Gorinstein S, Leontowicz H, Leontowicz M, Drzewiecki J, Najman K, Katrich E, *et al*. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. Life Sci 2006;78:655-63.
11. Thippeswamy NB, Akhilender K, Rajeshwara N, Achur N. Antioxidant and antibacterial properties of phenolic extract from *Carum carvi* L. J Pharm Res 2013;7:352-7.
12. Eddouks M, Lemhadri A, Michel JB. Caraway and caper: Potential anti-hyperglycaemic plants in diabetic rats. J Ethnopharmacol 2004;94:143-8.
13. Lahlou S, Tahraoui A, Israili Z, Lyoussi B. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J Ethnopharmacol 2007;110:458-63.
14. Lemhadri L, Hajji MJ, Eddouks M. Cholesterol and triglycerides lowering activities of caraway fruits in normal and streptozotocin-diabetic rats. J Ethnopharmacol 2006;106:321-6.
15. Samojlik I, Lakic N, Mimica-Dukic N, Dakovic- Svajcer K, Bozin B. Antioxidant and hepatoprotective potential of essential oils of coriander (*Coriandrum sativum* L.) and caraway (*Carum carvi* L.) (Apiaceae). J Agric Food Chem 2010;58:8848-53.
16. Haque MR, Ansari SH, Najmi AK. *Cuminum cyminum* L. fruits distillate ameliorates the high fat diet-induced obesity. Pharmacogn Commun 2013;3:49-57.
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
18. Haque MR, Ansari SH. Aromatic aldehyde compound cuminaldehyde protects nonalcoholic fatty liver disease in rats feeding high fat diet. Human Exp Toxicol 2019;38:823-32.
19. Haque MR, Ansari SH. Anti-obesity effect of arq zeera and its main components thymol and cumin aldehyde in high fat diet induced obese rats. Drug Res 2018;68:637-47.
20. Kleiner DE, Brunt EM, VanNatta M, Behling C, Contos MJ, Cummings OW, *et al*. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.
21. Corbett SW, Stern JS, Keesey RE. Energy expenditure in rats with diet-induced obesity. Am J Clin Nutr 1986;44:173-80.
22. Lavie CJ, Milani RV. Obesity and cardiovascular disease: Hippocrates paradox? J Am Coll Cardiol 2003;42:677-9.
23. Han LK, Zheng YN, Xu BJ, Okuda H, Kimura Y. Saponins from platycodi radix ameliorate high fat diet-induced obesity in mice. J Nutr 2002;132:2241-5.
24. Bobek P, Hromadova M, Ozdin L. Effect of oyster mushroom (*Pleurotus ostreatus*) and its ethanolic extract in diet on absorption and turnover of cholesterol in hypercholesterolemic rat. Mol Nutr Res 1996;40:589-91.
25. Zhi J, Melia AT, Guerciolini R, Chung J, Kinberg J, Hauptman JB, *et al*. Retrospective population-based analysis of the dose-response (fecal fat excretion) relationship of orlistat in normal and obese volunteers. Clin Pharmacol Ther 1994;56:82-5.
26. Huang H, Xu Y, Pol DV. Nicotine excites hypothalamic arcuate anorexigenic proopiomelanocortin neurons and orexigenic neuropeptide Y neurons: Similarities and differences. J Neurophysiol 2011;106:1191-202.
27. Wang Y, Kuropatwinski KK, White DW, Hawley TS, Hawley RG, Tartaglia LA, *et al*. Leptin receptor action in hepatic cells. J Biol Chem 1997;272:16216-23.
28. Adams LA, Sanderson S, Lindor KD, Angulo P, The histological course of nonalcoholic fatty liver disease: A longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005;42:132-8.
29. Roden M, Anderwald C, Furnsinn C, Waldhausl W, Lohninger A. Effects of short-term leptin exposure on triglyceride deposition in rat liver. Hepatology 2000;32:1045-9.
30. Murdolo G, Piroddi M, Luchetti F, Tortoioli C, Canonico B, Zerbinati C, *et al*. Oxidative stress and lipid peroxidation by-products at the crossroad between adipose organ dysregulation and obesity linked insulin resistances. Biochimie 2013;95:585-94.
31. Hassan A, Nauman M, Anjum FM, Hussain S, Nadeem M. Comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and Cumin (*Cuminum cyminum*). J Agric Food Chem 2010;58:8231-7.

Source of Support: Nil. **Conflicts of Interest:** None declared.