Cucumis melo ssp. Agrestis var. Agrestis Ameliorates High Fat Diet Induced Dyslipidemia in Syrian Golden Hamsters and Inhibits Adipogenesis in 3T3-L1 Adipocytes

Kripa Shankar¹, Sumit K. Singh², Durgesh Kumar³, Salil Varshney¹, Abhishek Gupta¹, Sujith Rajan¹,², Ankit Srivastava¹,³, Muheeb Beg¹, Anurag Kumar Srivastava¹, Sanjeev Kanojiya⁴, Dipak K. Mishra²,⁵, Anil N. Gaikwad¹

¹Division of Pharmacology, CSIR-Central Drug Research Institute, ²Division of Botany, CSIR-Central Drug Research Institute, ³Academy of Scientific and Innovative Research, CSIR-Central Drug Research Institute, ⁴Division of Toxicology, CSIR-Central Drug Research Institute, ⁵Sophisticated Analytical Instrument Facility, CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India

INTRODUCTION

Dyslipidemia is a lipoprotein metabolism disorder that displays increased cholesterol, triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-c) along with decreased high-density lipoprotein-cholesterol (HDL-c) in blood.[1,2] Dyslipidemia is a major risk factor attributed to the development of cardiovascular diseases (CVDs). According to World Health Organization, CVDs are the number one cause of deaths worldwide. Therefore, it is a prime consideration for treatment of dyslipidemia to reduce elevated levels of lipid profile, i.e. TGs, total cholesterol (TC), and LDL-c along with an increase in HDL-c. Treatment options for these disorders include diet interventions, physical exercise, surgery, and medication. The anti-dyslipidemic drugs that are currently available in the market include statins, fibrates, niacin, ezetimibe, and bile acid binding resins.[1,4] However, a small but clinically significant population taking these medications experience adverse effects. Statins are known to increase hepatic enzymes and muscle toxicity.[1,6] Likewise, long-term therapy of fibrate in patients causes supersaturation of gallbladder bile, which increases the incidence of cholesterol gallstones.[7] Exploration of medicinal plants has always been an immense source of

ABSTRACT

Background: Cucumis melo ssp. agrestis var. agrestis (CMA) is a wild variety of C. melo. This study aimed to explore anti-dyslipidemic and anti-adipogenic potential of CMA. Materials and Methods: For initial anti-dyslipidemic and antihyperglycemic potential of CMA fruit extract (CMFE), male Syrian golden hamsters were fed a chow or high-fat diet with or without CMFE (100 mg/kg). Further, we did fractionation of this CMFE into two fractions namely; CMA water fraction (CMWF) and CMA hexane fraction (CMHF). Phytochemical screening was done with liquid chromatography-mass spectrometry LC- (MS)/MS and direct analysis in real time-MS to detect active compounds in the fractions. Further, high-fat diet fed dyslipidemic hamsters were treated with CMWF and CMHF at 50 mg/kg for 7 days. Results: Oral administration of CMFE and both fractions (CMWF and CMHF) reduced the total cholesterol, triglycerides, low-density lipoprotein cholesterol, and very low-density lipoprotein-cholesterol levels in high fat diet-fed dyslipidemic hamsters. CMHF also modulated expression of genes involved in lipogenesis, lipid metabolism, and reverse cholesterol transport. Standard biochemical diagnostic tests suggested that neither of fractions causes any toxicity to hamster liver or kidneys. CMFE and CMHF also decreased oil-red-O accumulation in 3T3-L1 adipocytes. Conclusion: Based on these results, it is concluded that CMA possesses anti-dyslipidemic and anti-hyperglycemic activity along with the anti-adipogenic activity. Key words: 3T3-L1 adipocytes, Cucumis melo ssp. agrestis var. agrestis, direct analysis in real time-mass spectrometry analysis, dyslipidemia, high-fat diet, Syrian golden hamster

SUMMARY

• The oral administration of Cucumis melo agrestis fruit extract (CMFE) and its fractions (CMWF and CMHF) improved serum lipid profile in HFD fed dyslipidemic hamsters.

• CMFE, CMWF and CMHF significantly attenuated body weight gain and ewAT hypertrophy.

• The CMHF decreased lipogenesis in both liver and adipose tissue.

• CMFE and CMHF also inhibited adipogenesis in 3T3-L1 adipocytes.

Abbreviation used: CMA: Cucumis melo ssp. agrestis var. agrestis, CMFE: CMA fruit extract, CMWF: CMA water fraction, CMHF: CMA hexane fraction, FAS: Fatty acid synthase, SREBP1c: Sterol regulatory element binding protein 1c, ACC: Acetyl CoA carboxylase, LXR α: Liver X receptor α.

Correspondence: Dr. Anil N. Gaikwad, BS 10/1, Sector 10, PCN-208, Division of Pharmacology, CSIR- Central Drug Research Institute, Jankipuram Extension, Lucknow - 226 031, Uttar Pradesh, India. E-mail: anil_gaikwad@cdri.res.in DOI : 10.4103/0973-1296.172945

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drugs and majority of the currently available drugs have been derived directly or indirectly from them. These days herbal drugs are being prescribed widely due to their effectiveness with minimum side effects and relatively low cost. The World Health Organization also considers medicinal plants as alternatives in the treatment of various diseases and the focus of research professionals for plants is increasing day by day.[10] *Cucumis melo* sp. *agrestis var. agrestis* (CMA) (Naudin) Pangalo var. *agrestis* Naudin, commonly known as wild melon (in English) or kachari (in Hindi) under the family cucurbitaceae.[11] CMA is a common climbing or prostrate herb, distributed almost throughout India and neighboring countries. The fruits of this plant possess the stomachic property and are also used to treat burns and abrasions. Seeds have antitussive, antioxidant, digestive, febrifuge and vermifuge properties, and seed oil extract was reported for anti-fungal activity.[11,12] Recently, we have observed that flavonoids have co-existing anti-dyslipidemic and anti-adipogenic activity, although both activities are distinct.[12] Furthermore, anti-adipogenic activity has also been reported for some of the statin classes of compound.[13,14] Syrian golden hamsters have been demonstrated as a valuable model of high fat diet (HFD) induced dyslipidemia, and it is well-suited for screening of anti-dyslipidemic agents.[12,13] In addition, hamsters also have a similarity to human plasma lipid distribution, synthesis, and excretion.[16,17] Our present study aimed to explore the anti-dyslipidemic and anti-adipogenic potential of fruit extract (excluding seed) and fractions of this plant in HFD-fed dyslipidemic hamsters. The anti-dyslipidemic activity was further assessed by gene expression and protein immunoblotting analysis in liver and adipose tissue.

**MATERIALS AND METHODS**

**Plant materials**

Ripe fruits of CMA were collected from our institute campus at Lucknow, India in July 2013. The herbarium specimen of this plant with voucher specimen number DKM24778 has been deposited in the medicinal plant herbarium of Council of Scientific and Industrial Research (CSIR)-Central Drug Research Institute (CDRI).

**Chemicals**

High-fat diet (Cat No. 12451) was purchased from Research Diets Inc., USA. Fenofibrate, used as positive control was purchased from Sigma-Aldrich, USA. Ethyl alcohol was procured from Merck, Germany. Oct-Dec 2015, Vol 11, Issue 44 (Supplement 4)
Division, CSIR-CDRI and were kept in controlled laboratory conditions of temperature (23 ± 2°C), relative humidity (50–60%), and light (300 lx at floor level, 12/12 h light/dark cycle). All experimental procedures adopted in this study were previously approved by the Institutional Animal Ethics Committee in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals formed by the government of India. Standard pellet food and high-fat diet (Research Diets Inc., 12451), kept at 4°C was administered daily (80 g diet/cage) at a fixed time in the morning. The leftover diet of the previous day was weighed for the net food intake before being discarded. All groups were having free access to diet and water.

Experimental design for anti-dyslipidemic and anti-hyperglycemic activity of Cucumis melo ssp. agrestis var. agrestis fruit extract, Cucumis melo ssp. agrestis var. agrestis water fraction and Cucumis melo ssp. agrestis var. agrestis hexane fraction

The animals with identification marks were acclimatized for 7 days before the experiment. The control group of hamster was fed with normal chow diet. High-fat diet (45% kcal HFD, day 1–day 10) were given in all other groups, i.e. Vehicle, fenofibrate, CMA fruit extract (CMFE), CMA water fraction (CMWF), and CMA hexane fraction (CMHF). All groups were having free access to diet and water. Likewise, the body weight of the animals was recorded daily before feeding and drug administration. Fenofibrate, CMFE or CMA water fraction (CMWF), and CMA hexane fraction (CMHF) were suspended in the 0.1% gum acacia solution and gavaged orally, once daily at a fixed time for seven consecutive days (day 4–10) to treated groups and vehicle to the parallel control. The same experimental procedure was followed for assessment of anti-dyslipidemic activity of CMWF and CMHF.

Blood collection, serum analysis, and histological analysis

The blood withdrawn from the retro-orbital plexus of anesthetized animals was collected in micro-centrifuge tubes and serum was separated by centrifugation. TG, TC, HDL-c, LDL-cholesterol, glucose, ALT, AST, and creatinine were estimated using Merck selectra junior bio-analyzer (Merck Millipore). Very low-density lipoprotein-cholesterol (VLDL-c) and creatinine were estimated using Merck selectra junior bio-analyzer (Merck Millipore). Liver and eW AT were freeze-thawed and triturated in liquid nitrogen and protein were isolated using mammalian cell lysis buffer supplemented with 100 mm EDTA, protease inhibitor, and phosphatase inhibitor (Sigma-Aldrich). Protein concentration was determined using the bicinchonic acid method (Sigma) and further western blotting procedure was done as described previously. To validate equal loading, actin was used as an internal loading control.

Statistical analysis

For in vivo studies, data were expressed as a mean ± standard error of mean. Comparisons between the treatment groups and control were performed by one-way analysis of variance followed by Bonferroni’s multiple comparison test. For in vitro studies, data were expressed as mean ± standard deviation. Comparisons between the treatment groups and control were performed using Student’s t-test. A probability value of P < 0.05 (*), 0.01 (**) and 0.001 (***) was used as a measure of statistical significance. Data were analyzed on Graph Pad Prism (Version 5.00, Graph Pad Software Inc., San Diego, CA, USA).

RESULTS

Cucumis melo ssp. agrestis var. agrestis fruit extract attenuates dyslipidemia and improves hyperglycemia

CMFE treatment in HFD-fed dyslipidemic hamster reduced increase in TC, LDL-c, however, we could not get any difference in TG [Figure 1a, b and d], HFD induced hyperglycemia was also improved with treatment of CMFE [Figure 1c]. In addition, HDL-c and HDL-c to TC ratio were also improved when treated with CMFE [Figure 1e and f]. Furthermore, we examined the intracellular toxicity of CMFE in 3T3-L1 adipocytes, using the MTT assay. The result of MTT assay proves that CMA treatment is safe [Figure 1g]. Different concentrations of CMFE (25, 50, 100 µg/ml) were supplemented with MDA during differentiation. The absorbance of extracted ORO accumulated in lipid droplets shows that CMA significantly inhibits adipogenesis [Figure 1h].

Phytochemical screening

The bioactive fractions of CMA were investigated by LC-MS/MS. The previously reported data of Cucumis species showed the presence of carbohydrates, glycosides, phenolics, and flavonoids. Similarly, the nonpolar oily fraction contains fatty acids like 16-Octadecenoic acid, methyl ester, estra-1, 3, 5 (10)-trien-17-beta-ol, hexadecanoic acid, 2-hydroxy ethyl ester, etc. Our preliminary study and MS data also suggested the presence of carbohydrates, phenolics, and their glycosides. The compound eluted at 1.5 min having molecular weight 342 Da, was identified as a fatty acid methyl ester by comparison of the observed mass with a standard. The compound identified as a fatty acid is a component of the essential oil extracted from the fruits of Cucumis melo ssp. agrestis var. agrestis.

Table 1: Primer sequences used for real time PCR gene expression studies

| Gene name | Primer pairs |
|-----------|--------------|
| LXR α | F5′-TCAGCATCTTTCTGAGACCCGG3′<br>R5′-TCATTAGCATCCTGGAGAACA3′<br>F5′-ACCGTTCAGGATGAAACTGATAG3′<br>R5′-GTGACTAGAGTTCCTGGGATAAC3′<br>F5′-GATTCACTTTTCTGGGACTGA3′<br>R5′-AGCCACACCAGACCAGAGA3′<br>F5′-GCCACCATGTCGTCGAGG3′<br>R5′-ATGAGCTGGAGCATGTTTCTCAA3′<br>F5′-CACACAAAGGCTGTCATGCC3′<br>R5′-AGACAAACAGGTCTACACAC3′ |
| APOA1 | F5′-CATGAGATCTGGAGGAAACA3′<br>R5′-ACCGTTCAGGATGAAACTGATAG3′<br>F5′-GTGACTAGAGTTCCTGGGATAAC3′<br>R5′-GATTCACTTTTCTGGGACTGA3′<br>F5′-GCCACCATGTCGTCGAGG3′<br>R5′-ATGAGCTGGAGCATGTTTCTCAA3′<br>F5′-CACACAAAGGCTGTCATGCC3′<br>R5′-AGACAAACAGGTCTACACAC3′ |
| LPL | F5′-TCAGCATCTTTCTGAGACCCGG3′<br>R5′-TCATTAGCATCCTGGAGAACA3′<br>F5′-ACCGTTCAGGATGAAACTGATAG3′<br>R5′-GTGACTAGAGTTCCTGGGATAAC3′<br>F5′-GATTCACTTTTCTGGGACTGA3′<br>R5′-AGCCACACCAGACCAGAGA3′<br>F5′-GCCACCATGTCGTCGAGG3′<br>R5′-ATGAGCTGGAGCATGTTTCTCAA3′<br>F5′-CACACAAAGGCTGTCATGCC3′<br>R5′-AGACAAACAGGTCTACACAC3′ |
| SREBP1c | F5′-TCAGCATCTTTCTGAGACCCGG3′<br>R5′-TCATTAGCATCCTGGAGAACA3′<br>F5′-ACCGTTCAGGATGAAACTGATAG3′<br>R5′-GTGACTAGAGTTCCTGGGATAAC3′<br>F5′-GATTCACTTTTCTGGGACTGA3′<br>R5′-AGCCACACCAGACCAGAGA3′<br>F5′-GCCACCATGTCGTCGAGG3′<br>R5′-ATGAGCTGGAGCATGTTTCTCAA3′<br>F5′-CACACAAAGGCTGTCATGCC3′<br>R5′-AGACAAACAGGTCTACACAC3′ |
| LCAT | F5′-TCAGCATCTTTCTGAGACCCGG3′<br>R5′-TCATTAGCATCCTGGAGAACA3′<br>F5′-ACCGTTCAGGATGAAACTGATAG3′<br>R5′-GTGACTAGAGTTCCTGGGATAAC3′<br>F5′-GATTCACTTTTCTGGGACTGA3′<br>R5′-AGCCACACCAGACCAGAGA3′<br>F5′-GCCACCATGTCGTCGAGG3′<br>R5′-ATGAGCTGGAGCATGTTTCTCAA3′<br>F5′-CACACAAAGGCTGTCATGCC3′<br>R5′-AGACAAACAGGTCTACACAC3′ |

PCR: Polymerase chain reaction; LCAT: Lecithin-cholesterol acyltransferase; SREBP1c: Sterol regulatory element binding protein 1c; APOA1: Apolipoprotein A-1; LXR α: Liver X receptor α
unit. Thus, it could be expected as disaccharides sugar. Similarly, most of the compounds eluted subsequently showed a characteristic loss of ~162 Da from parent ion to the loss of a hexose unit. While the presence of indicative ion at m/z 91, 105, 107, 135 in MS/MS spectra designates the phenolic structure, and these compounds were identified as phenolic glycosides. Apart from this, compound eluted at 9.09 showed subsequent loss of the CH₂ unit with the losses of H₂O and CH₃OH. It suggested the presence of long carbon chain with –OH and –O-CH₃ functional group. The chromatographic and MS data of detected metabolites are shown in Table 2. However, the whole metabolites detection was not possible only from ESI source. Thus, the metabolites fingerprint of both the fraction CMWF and CMHF were recorded on DART-MS and shown in [Figure 2a]. This MS can be recognized as fingerprints to discriminate the fractions metabolite and for future reference.

**Cucumis melo ssp. agrestis var. agrestis fractions**

*suppress high-fat diet-induced weight gain, improves dyslipidemia and hyperglycemia*

The body weight of the HFD fed group significantly increased while CMWF and CMHF treatment significantly attenuated gain in body weight starting from 7th day to the end of the experimental period without any change in food intake throughout the experimental period [Figure 2b and c]. HFD significantly increased TC (4.1 fold), TG (2.2 fold), LDL-c (5.8 fold), VLDL-c (2.2 fold) in Syrian golden...
CMA: Cucumis melo ssp. agrestis var. agrestis; CMWF: CMA hexane fraction; CMWF: CMA water fraction; RT: Retention time

Cucumis melo ssp. agrestis var. agrestis fractions reduced adipose hypertrophy and hepatic lipid accumulation in vivo in Syrian golden hamsters without any toxicity

To investigate whether CMA decreases adiposity, hamsters were sacrificed and eWAT removed and weighed. The weight of eWAT was increased in the HFD-fed hamsters compared to the chow-fed hamsters, and was significantly decreased by the administration of Fenofibrate, CMWF, and CMHF (50 mg/kg) treated animals [Figure 2a]. Further, hepatic showed adipocyte hypertrophy, while the adipocyte phenotype of CMA fractions (CMWF and CMHF) treated group was similar to chow-fed fed animals. In addition to this, the liver histological sections of CMA treated group was similar to chow diet fed animals. In addition, [Figure 2b-d]. In addition, hepatic lipids and lipids which indicates that CMFE causes decrease in increased serum glucose in HFD fed hamsters [Figure 2j]. The levels of AST, ALT, and creatinine that indicates liver and kidney injury, were significantly improved in CMA fractions treated animals [Figure 3c-f]. In addition, the CMA treated hamsters did not induce any significant changes in the weight of liver (data not shown). Thus, the data indicate that administration of 50 mg/kg/day of CMA fractions for 7 days induced no biochemical detectable adverse toxic effects in the hamsters.

Cucumis melo ssp. agrestis var. agrestis fractions inhibited adipogenesis and lipogenesis, increased lipid metabolism and reverse cholesterol transport

Different concentrations of both CMA fractions (CMWF and CMHF at 25, 50, 100 µg/ml) were supplemented with MDI during differentiation of 3T3-L1 adipocytes. The absorbance of extracted ORO accumulated in lipid droplets shows that CMHF significantly inhibits adipogenesis [Figure 4a]. Further, EAS protein level was also reduced more prominently in CMHF treated eWAT [Figure 4b]. CMWF and CMHF treatment also decreased EAS, and ACC in the liver. Hepatic ATP citrate-lyase was reduced significantly when treated with CMHF. However, we could not get any significant change in ATP-citrate lyase protein expression when treated with CMWF [Figure 4c]. Hence, we further analyzed the mRNA expression of genes involved in lipogenesis, lipid metabolism, and reverse cholesterol transport only in CMHF treated animals. The CMHF treatment increased hepatic mRNA expression of liver X receptor α (LXR α), LPL and significantly decreased expression of sterol regulatory element binding protein 1c (SREBP1c) [Figure 4d-f]. Further, hepatic mRNA expression of lecinthin-cholesterol acyltransferase (LCAT) and apolipoprotein A-I (APOA1), involved in reverse cholesterol transport were significantly enhanced by treatment of CMHF [Figure 4g and h].

**DISCUSSION**

Traditionally, India has very long history of using natural extracts in the form of Ayurvedic medicines. Currently, people throughout the world are recognizing the value of natural compounds. Our recent study also showed that the anti-adipogenic compounds also show anti-dyslipidemic activities. Hence further, we performed an anti-adipogenic activity of CMFE in 3T3-L1 adipocytes and found that CMFE decreases concentration-dependent oil-red-O accumulation, a dye used for staining of neutral TGs and lipids which indicates that CMFE causes decrease in lipid accumulation during 3T3-L1 adipogenic differentiation. Based on
the data presented in this study, CMA fractions (CMWF and CMHF) treatment significantly improved serum dyslipidemia by decreasing TG, TC, and LDL-c with increase in HDL-c, and the ratio of HDL-c to TC. Diabetic dyslipidemia is a complex cluster of abnormalities.[2] In addition to the hypolipidemic effects, CMWF and CMHF treatment also showed hypoglycemic activity in dyslipidemic hamsters. Treatment of CMA fractions also attenuated body weight and eWAT weight, a marker of obesity. Histological analysis also proved that CMA fractions decreased the hypertrophy in adipocyte, a marker of dysfunctional adipose tissue. Moreover, hepatic lipid accumulation a marker of hepatic insulin resistance is also reduced with treatment of CMWF and CMHF.[2,3] Hence taken together, CMA fractions attenuated body weight gain, ameliorated dyslipidemia, and hyperglycemia in HFD fed dyslipidemic hamsters. FAS is a very well-known lipogenic target for

Figure 2: Cucumis melo ssp. agrestis var. agrestis fractions (Cucumis melo ssp. agrestis var. agrestis water fraction and Cucumis melo ssp. agrestis var. agrestis hexane fraction) attenuated body weight gain, improved dyslipidemia and hyperglycemia without altering diet intake. The direct analysis in real time-mass spectrometry was recorded on a JEOL-Accu TOF JMS-T100LC mass spectrometer having a direct analysis in real time source. The samples were subjected as such in front of direct analysis in real time source. Dry Helium gas was used for ionization at 4 L/min flow rate and source temperature kept at 350°C. The orifice 1 was set at 28 V and spectra collected as average of 6-8 scan. (a) Direct analysis in real time-mass spectrometry fingerprint of both fractions. Syrian golden hamsters (n = 8), fed with chow or high fat diet were kept on either fenofibrate (100 mg/kg), on Cucumis melo ssp. agrestis var. agrestis fruit extract (50 mg/kg) or on Cucumis melo ssp. agrestis var. agrestis hexane fraction (50 mg/kg) for 7 days. (b) Body weight of chow or high fat diet fed dyslipidemic hamsters treated with or without Cucumis melo ssp. agrestis var. agrestis fractions. Hamsters were treated with Cucumis melo ssp. agrestis var. agrestis fractions or fenofibrate for 7 days and body weight was measured every day in morning before providing diet. (c) Average food intake amount of chow and high fat diet diet mice in 10 days feeding. Diet intake was recorded every day. (d) Total cholesterol. (e) Triglyceride (f) low-density lipoprotein-cholesterol. (g) high-density lipoprotein-cholesterol cholesterol. (h) Very low-density lipoprotein-cholesterol. (i) High-density lipoprotein-cholesterol cholesterol/total cholesterol ratio. (j) Serum Glucose. Values are means (n = 8), with their standard error of mean represented by vertical bars. Mean values were significantly different from the high fat diet diet-fed animals (one-way analysis of variance): *P < 0.05, **P < 0.01, ***P < 0.001. *Denotes that the mean values are significantly different
SREBP-1c, which was significantly decreased when treated with CMWF and CMHF in eWAT as well as in the liver, further contributing to the effect of CMA fractions on modulating circulating lipids. CMWF and CMHF treatment also decreased the protein expression of ACC. Hepatic ATP-citrate lyase was decreased when treated with CMHF, but we could not get any significant difference in ATP-citrate lyase with CMWF treatment. We could not get any significant changes in adipogenesis and other markers (e.g., ATP-citrate lyase) with CMWF. Thus, further mRNA expression analysis was performed with CMHF only. LXR α is a sensor of cholesterol excess and its activation in dyslipidemic hamsters led to an increase in reverse cholesterol transport and reduced lipid accumulation.[14] In this study, CMHF treatment in HFD-fed hamsters increased the hepatic mRNA expression of LXR α. Further, SREBP-1c is considered as a central mediator of obesity and insulin resistance and master regulator of ATP-citrate lyase, ACC, and FAS. ATP-citrate lyase is a lipogenic enzyme that converts citrate to acetyl-coenzyme A. Acetyl-coenzyme A is further converted to malonyl-CoA. Malonyl-CoA is known to play a key role for chain elongation in fatty acid biosynthesis.[15] Liver-specific ATP-citrate lyase downregulation via adenovirus-mediated RNA interference has been reported to reduce expression of peroxisome proliferator-activated receptor-gamma and the entire lipogenic program in the liver.[16] Hence, we hypothesize that

**Figure 3:** *Cucumis melo ssp. agrestis var. agrestis* fractions ameliorates high fat diet induced adipose tissue weight gain without any toxicity. Hamsters were fed either a chow or high fat diet for 7 days in the presence of fenofibrate (100 mg/kg) or *Cucumis melo ssp. agrestis* var. *agrestis* fractions (50 mg/kg) (n = 8). (a) Images of hamster showing *Cucumis melo ssp. agrestis* var. *agrestis* fruit extract and *Cucumis melo ssp. agrestis* var. *agrestis* hexane fraction mediated decrease in epididymal white adipose tissue (b) epididymal white adipose tissue weight (c) histological analysis of the epididymal white adipose tissue and liver after staining with hematoxylin and eosin. *Cucumis melo ssp. agrestis* var. *agrestis* does not cause any toxicity, in vivo in Syrian golden hamster (d) serum alanine aminotransferase (e) serum aspartate aminotransferase (f) serum creatinine.
CMWF and CMHF increase expression of LXRα and inhibits expression of the transcription factor SREBP1c, leading to a reduced expression of its all major targets including FAS, ACC, and ATP-citrate lyase to inhibit lipogenesis in the liver as well as in adipose tissue. Overexpression of APOA-I limits progression and reduces pre-existing atherosclerosis hence, increasing the levels of APOA1 A-I has been a therapeutic target in CVDs.\textsuperscript{17,18} CMHF treatment increased the mRNA expression of APOA1 and LCAT, the genes involved in reverse cholesterol transport, proving that CMHF not only decreases lipogenesis but also increases reverse cholesterol transport. Hence, it is concluded that apart from adipose tissue, the liver is also an important player for \textit{in vivo} activity of CMA. Hypertriglyceridemia (>150 mg/dL) is a common lipid abnormality associated with several other metabolic disorders.\textsuperscript{19,20} LPL plays an important role by catalyzing first reaction in TG metabolism and increase in LPL causes a reduction in hypertriglyceridemia.\textsuperscript{41} Our results show that CMHF increased lipoprotein lipase mRNA expression in the liver that might be a reason for the decrease in serum TG level.

**CONCLUSION**

The administration of CMFE, CMWF, and CMHF significantly attenuated body weight gain and eWAT hypertrophy and improved serum TC, TG, and LDL-c levels in HFD-induced dyslipidemic hamsters. CMFE and CMHF inhibited adipogenesis in 3T3-L1 adipocytes. The dyslipidemic effects of CMHF altered the expression of LXRα, SREBP1c in the liver, and FAS in both liver and adipose tissue. These results suggest that CMA treatment is useful for treating metabolic diseases such as obesity and...
hyperlipidemia. Our study shows the effects of CMWF and CMHF on TC, TGs, and LDL-α along with an analysis of liver and kidney adverse reactions indicates that CMA could be a cheap, efficient, and safe lipid-lowering drug in metabolic syndrome patients.

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Conflicts of interest
The authors declare no conflicts of interest.

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ABOUT AUTHORS

Kripa Shankar, is Senior Research Fellow at Pharmacology Division, CSIR-Central Drug Research Institute. His research interest is in the area of insulin resistance, dyslipidemia and obesity.

Sumit Kumar Singh, is Project Fellow at Botany Division, CSIR-Central Drug Research Institute. His interests are Plant tissue culture, Conservation biology, Medicinal plant diversity and Ethno-botany.

Durgesh Kumar, is Senior Research Fellow at Pharmacology Division, CSIR-Central Drug Research Institute. His research interest is in the area of insulin resistance, dyslipidemia and obesity.

Salil Varshney, Salil Varshney is Project Fellow at Pharmacology Division, CSIR-Central Drug Research Institute. His research interest is in the area of insulin resistance, dyslipidemia and obesity.

Abhishek Gupta, is Senior Research Fellow at Pharmacology Division, CSIR-Central Drug Research Institute. His research interest is in the area of insulin resistance, dyslipidemia and obesity.

Sujith Rajan, is Senior Research Fellow at Pharmacology Division, CSIR-Central Drug Research Institute. His research interest is in the area of insulin resistance, dyslipidemia and obesity.

Anurag Kumar Srivastava, is Senior Research Fellow at Pharmacology Division, CSIR-Central Drug Research Institute. His research interest is in the area of insulin resistance, dyslipidemia and obesity.

Sanjeev Kanojiya, is Senior Scientist at Mass spectrometry Laboratory, Sophisticated Analytical Instruments Facilities, CSIR-Central Drug Research Institute. His research interest includes development of analytical methods for chemical analysis (metabolic profiling of bioactive natural product extracts) using high performance liquid chromatography mass spectrometry.

Dipak K. Mishra, is Senior Scientist at Botany Division, CSIR-Central Drug Research Institute and Assistant Professor, Academy of Scientific and Innovative Research. His research interest includes Plant tissue culture, Conservation biology, Medicinal plant diversity and Ethno-botany.

Dr. Anil N. Gaikwad, is Senior Scientist at Pharmacology Division, CSIR-Central Drug Research Institute, Lucknow, India. His research interest is in the area of insulin resistance and obesity. He is also secretary of Indian Pharmacological Society: Lucknow Chapter.