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Free fatty acids regulating action of *Capparis decidua* fruit on dyslipidemia in rats

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ABSTRACT

Capparis decidua belongs to family Capparidaceae in wastelands of India. The study aim was to determine the role of *C. decidua* fruits on the free fatty acids (FFA) profile in fat-rich diet (FRD) dyslipidemic rats. The methanolic extract of edible fruit of *C. decidua* (CD) was given orally to obese dyslipidemic rats at the dose of 125 mg/kg and 250 mg/kg for consecutive 28 days. CD treatment in FRD rats significantly restricts the body weight gains. Blood lipid profile was altered dose dependently and significantly after 4-week treatment with CD to FRD. rats. It significantly ($p<0.05$) enhanced serum FFA especially, γ-linolinate, α-linolinate, arachidonate, ecosapentaenoate, docosapentaenoate and docosahexaenoate. Moreover, ω3-PUFA content was also enhanced (50.3% and 78.8%) in the serum of CD treated animals, whereas MUFA was lowered (31.1% and 40%). Therefore, *Capparis decidua* fruit has a promising role on dyslipidemia and obesity and has the capabilities to regulate beneficial free fatty acids.

Keywords: obesity**,** dyslipidemia, FFA, PUFA, GC, *Capparis decidua*

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INTRODUCTION

Obesity is closely associated with insulin resistance and elevation of proinflammatory cytokines and chemokines, which leads to developing type-2 diabetes, hypertension, dyslipidemia, and disorders of blood clotting (Bhupathiraju & Hu, 2016). Blood-free fatty acid (FFA) levels are altered and elevated in obesity (Zhou et al., 2019). It has been noted that FFA creates a defect in insulin-stimulated glucose transport and insulin signaling pathway (Cusi et al., 2007; Schofield et al., 2016). Furthermore, circulating FFA activated proinflammatory NF_KB pathways resulting in oxidative damage in vital tissues (Boden et al., 2005; Yamato et al., 2007). Hence, controlling blood FFA levels can be one of the targets of obesity therapy. Several reports supported that regulation of dyslipidemia through diet and pharmacotherapy can reduce the incidence of metabolic syndrome (Onal et al., 2017).

Herbal medicines and their active constituents showed promising lipid-lowering actions. These studies showed that herbal medicines could interfere with the primary processes for manipulating lipid metabolism, including cholesterol synthesis, exogenous absorption, transport, and cholesterol excretion (Ji et al., 2019). *Capparis decidua* is a thorny xerophytic shrub belonging to the family Capparidaceae, which grows in wastelands throughout the arid and semi-arid zones of India. The edible fruit is a small ovoid-shaped multi seeded pink berry and is claimed to cure cardiac aliments (Gupta, 2010). The plant is traditionally used to treat inflammation, fever, arthritis, rheumatism, and swelling (Kirtikar and Basu, 2008). Studies have shown that *C. decidua* fruits have hypoglycemic, hypolipidaemic, antiatherosclerotic, and anti-hypertensive activities (Purohit & Vyas, 2006; Yadav et al., 1997; Zia-Ul-Haq et al., 2011). It contains glucoside, glucocapparin, stachydrine, n-triacontane, β-carotene and β-sitosterol (Rathee et al., 2010; Saxena & Goutam, 2008). It has been reported that dietary glucoside, β-carotene, and β-sitosterol inhibit intestinal cholesterol absorption via down-regulation of intestinal Niemann–Pick C1-like 1 protein or reduce bile acid reabsorption through up-regulating ileal apical sodium-dependent bile acid transporter and thereby regulate whole-body lipid excretion (Lin et al., 2009; Sun, 2020; Tsai et al., 1992; Yuan et al., 2019). Stachydrine has been shown to improve vascular microcirculation and ameliorate endothelial dysfunction (Li et al., 2020). Genetic structure and molecular information of *C. decidua* fruits have been studied using random amplified polymorphic DNA (RAPD) based matrices to identify the diversity and similarity of *C. deciduas* fruits of different varieties origin in the subcontinent (Ali et al., 2014; Kumar et al., 2013). In this context, we have planned to evaluate the role of *C. decidua* fruits on dyslipidemia, mainly on FFA levels in fat-rich diet (FRD) rats.

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MATERIALS AND METHOD

Plant extract

The fruits of *Capparis decidua* were shade dried and subjected to soxhlet extraction (6 h) with 80% methanol. The hydro-methanolic extract was then concentrated under reduced pressure to obtain a brownish crude extract (CD). The gravimetric method determined the extractive value and expressed it as a percentage yield (16.9%).

Animals

Male Wistar rats (average body weight 150 g) were used in the study. In the present experiments, the recommended guidelines for the care and use of the animals were strictly followed (CPCSEA, 2003). The institutional animal ethics committee of RG Kar Medical College & Hospital, Kolkata, India, approved the research protocol (R/N 959/c/06A/CPCSEA).

Diet-Induced Hyperlipidaemia in rats

Thirty adult male Wistar rats (150±5 g) were maintained with a fat-rich diet (FRD). Normal rats were fed with a normal diet of energy of 3.8 Kcal/gm and RD diet of energy of 5.24 Kcal/gm (Chinnasamy & Chandiran, 2016). FRD contained gram powder, wheat flour, sugar, milk powder, hydrogenated vegetable oils, cholesterol powder, and mineral and vitamin mixtures (Indu et al., 2019).

Two weeks later, my blood cholesterol level was measured. The rats were selected for further study based on their serum cholesterol level (>180 mg/dl).

Treatment with *C. decidua* fruit extract

The selected animals were then divided into the following groups for treatment: Group I: Normal diet-fed control rats (2 mL/kg distilled water); Group II: FRD-fed control rats (2 mL/kg distilled water); Group III: FRD-fed with CD (125 mg/kg BW) treated rats and Group IV: FRD-fed with CD (250 mg/kg BW) treated rats. All these test drugs were given orally, once daily, for 28 consecutive days.

Blood lipid profile assessment

After treatment, all animals were sacrificed under deep anesthesia (80 mg/kg BW, thiopentone sodium, i,p.). The body weight and liver weight were taken. Blood was collected immediately from the left ventricle and total cholesterol, HDL-cholesterol, LDL, VLDL, triglycerides, and phospholipids were determined spectrophotometrically (Chatterjee et al., 2015). Lipid peroxides were measured in 10% of liver homogenates as described elsewhere (Sur et al., 2016).

Free fatty acid profile determination

Fatty acid composition in serum was determined by gas chromatography (GC) after derivatization (Ubhayasekera et al., 2013). In brief, 0.5 mL of serum was mixed with 2-propanol: heptane: 0.5 M sulphuric acid (40:10:1v/v/v), after 10 min 1mL heptane and 1.5mL deionized water were added, and the mixture was stirred vigorously (Wen et al., 2021). The upper layer was removed, condensed under a stream of nitrogen and incubated in 0.5mL of methanolic sodium methoxide at 50°C for 10 min. Thereafter, 0.5mL of boron trifluoride methanol complex was added, and the incubation was repeated as before. Fatty acid methyl esters were extracted with 1.5mL hexane for GC analysis. The fatty acid methyl ester mixture was separated in an Agilent 6890N series gas chromatograph (USA) equipped with a capillary column (30mmx 0.25mm, Sol-Gel, Australia) and a flame ionization detector. The column temperature was programmed from160º-250°C/min. The carrier gas was nitrogen at a flow rate of 1mL/min (160°C). Methyl esters of individual fatty acids were identified in the chromatogram by comparing retention times of pure methyl esters and were quantified by comparing the area under their peaks with the aid of Agilent Chemstation ver.10.0 software.

Data Analysis

Data are shown as mean ± SEM Differences between groups have been assessed by one-way ANOVA and Post hoc analysis (LSD) using statistical software (SPSS v20, IBM, USA) 'P' value ≤ 0.05 was considered the level of statistical significance.

RESULT

Significant body weight gain (144%) was observed in FRD control rats compared to normal diet-fed control rats. In contrast, CD treatment in FRD rats restricted the body weight gains dose-dependently (35.7% and 41.7%) and significantly ($p<0.05$) as compared to FRD control rats (Table 1). FRD significantly elevated serum cholesterol (264.7%), triglycerides (332%), lipoproteins (VLDL 304.9%) and phospholipids (103.4%) levels than normal fed rats, while CD treatment in FRD rats significantly lowered the serum cholesterol (34.9% and 41.6%), triglycerides (26.2% and 30.7%), LDL-cholesterol (54.5% and 64.3%), VLDL-cholesterol (35.5% and 37.1%) and phospholipids (29.9% and 43%), while, CD treatment enhanced HDL-cholesterol level 66.9% and 72.8%. The rate of lipid peroxidation in liver tissues was enhanced by six times in FRD-treated rats compared to the normal diet-fed rats, while CD treatment in FRD rats showed dose-dependent and significant protection of 31.5% and 63.4% (Table 1).

Table 2 demonstrates the effect of CD on 15 serum FFA profiles in supplements with FRD Lipotyping of rat serum FFA in GC chromatogram is shown in Figure. 1. FRD significantly altered the composition of most of the free fatty acids in animals. MUFA content was enhanced by 186.8%, while PUFA content was lowered by 37.9%. CD treatment significantly and dose-dependently improved the free fatty acids profile. The composition of some essential fatty acids, like γ-linoleate (18:3ω6), α-linolinate (18:3ω3), arachidonate (20:4ω6), ecosapentaenoate (20:5ω3), docosapentaenoate (22:5ω3) and docosahexaenoate (22:6ω3) were significantly ($p<0.05$) enhanced after treatment. Moreover, ω3-PUFA content was also enhanced (50.3% and 78.8%) in the serum of CD-treated animals, whereas MUFA or monounsaturated fatty acids concentration was lowered (31.1% and 40%).

Table 1. Effect of methanolic extract of *Capparis decidua* on lipid profile in rats

	Normal Control (Mean± SEM)	**FRD-Control (Mean± SEM)**	**FRD-CD 125mg/kg (Mean± SEM)**	**FRD-CD 250 mg/kg (Mean± S.E.M.)**
Body weight gain (g)	22.7 ± 1.78	55.4 ± 3.08(a)* (144)	35.6 ± 6.28(b)* (-35.7)	32.3 ± 4.96(b)* (-41.7)
Total Cholesterol (mg/dl)	76.4 ± 3.19	278.7±12.36(a)* (264.7)	181.3±9.65(b)* (-34.9)	162.5±8.02(b)* (-41.6)
HDL- Cholesterol (mg/dl)	39.6 ± 2.08	32.1±4.72(a)* (-18.9)	53.6±1.02(b)* (66.9)	55.5±1.08(b)* (72.8)
LDL- Cholesterol (mg/dl)	120.7 ± 3.29	185±9.62(a)* (53.2)	84±6.12(b)* (-54.5)	66±5.30(b)* (-64.3)
VLDL- Cholesterol (mg/dl)	16.1 ± 1.22	65.2±2.92(a)* (304.9)	42±1.19(b)* (-35.5)	41±1.84(b)* (-37.1)
Serum Triglycerides (mg/dl)	72.2 ± 2.39	312 ± 9.24(a)* (332)	230 ± 6.12(b)* (-26.2)	216 ± 7.05(b)* (-30.7)
Serum Phospholipids (mg/dl)	55.4 ± 2.23	112.7 ± 8.86(a)* (103.4)	78.9 ± 5.90(b)* (-29.9)	64.1 ± 5.14(b)* (-43)
Liver Lipid peroxides (nm MDA/g tissue)	3.29 ± 0.27	19.35 ± 3.48(a)* (749.5)	9.14 ± 1.68(b)* (-31.5)	7.08 ± 0.42(b)* (-63.4)

N=10 in each group; values statistically compared in groups; a denotes Normal Control vs. FRD Control;b denotes FRD Control vs. FRD+CD; level of significant marks as an asterisk (*) p is less than 0.05 percent change given in parenthesis

DISCUSSION

Fat rich diet (FRD) is frequently used in rodents to induce obesity, increase serum fatty acids, and induce lipotoxicity in various organs because increased ectopic lipid storage is a characteristic of HFD-induced obesity and metabolic disease (Indu et al., 2019; Liu et al., 2015). In the present study, HFD caused a significant (3.6 fold) increase in serum cholesterol in rats. Administration of *C. decidua* fruit extract significantly reduced total serum cholesterol, LDL cholesterol, triglycerides, and phospholipids, suggesting its potential therapeutic role in the management of dyslipidemia. Previous studies corroborate this finding (Purohit & Vyas, 2006). The triglycerides and phospholipids are the two major compartments where fatty acids are stored. In recent years a number of studies have addressed the effects of diet and exercise on the FFA composition of animal and human tissues (Borengasser et al., 2012; Yamato et al., 2007; Zhao et al., 2016). The effect justifies the focus of these lipid classes that their FFA profile may exert on physiological function. Since the proportion of PUFA in phospholipids positively correlated with cellular metabolic activity, the unsaturation level in animals' phospholipids positively correlated with the life span (Boden et al., 2005).

Table 2.Effect of methanolic extract of *Capparis decidua* on serum free fatty acids profile in rats

Fatty acid component	Normal Control (Mean± SEM)	FRD Control (Mean± SEM)	FRD+ CD 125mg/kg (Mean± SEM)	FRD+ CD 250 mg/kg (Mean± SEM)
14:0	0.25±0.02	0.98±0.06(a)*	0.7±0.04	0.78±0.06
16:0	21.35±2.04	28.56±3.18(a)*	20.84±2.11(b)*	15.36±2.38(b)*
18:0	3.9±0.68	6.52±1.09(a)*	4.51±0.64	4.4±0.91
18:1(ω-9)	7.86±1.53	17.71±2.25(a)*	16.26±1.48	14.91±1.20
18:1(ω-7)	1.12±0.01	2.77±0.04(a)*	1.65±0.02	1.56±0.06*
18:2(ω-6)	21.98±3.90	11.27±0.82(a)*	19.97±0.75(b)*	22.23±1.72(b)*
18:3(ω-6)	1.42±0.01	0.77±0.03(a)*	1.08±0.02(b)*	1.29±0.08(b)*
18:3(ω-3)	0.46±0.01	0.18±0.08(a)*	0.36±0.02(b)*	0.48±0.06(b)*
20:0	3.13±0.16	7.53±0.68(a)*	5.7±0.28	5.53±0.22
20:2(ω-6)	3.25±0.39	6.34±0.47(a)*	6.12±0.66	5.84±0.89
20:4(ω-6)	22.7±1.24	9.35±1.5(a)*	12.34±1.08(b)*	14.73±1.67(b)*
20:5(ω-3)	0.81±0.02	0.39±0.02(a)*	0.56±0.01(b)*	0.96±0.08(b)*
22:4(ω-6)	0.57±0.01	0.3±0.01(a)*	0.48±0.02	0.62±0.04(b)*
22:5(ω-3)	9.04±0.95	6.16±0.14(a)*	7.82±0.96(b)*	9.16±1.15(b)*
22:6(ω-3)	1.92±0.02	0.61±0.02(a)*	1.06±0.06(b)*	1.91±0.01(b)*
Others	0.24±0.01	0.56±0.01	0.53±0.02	0.24±0.01
Sum	100	100	100	100
MUFA	9.58±1.02	27.48±1.05 (a)* (186.8)	18.93±0.91 (b)* (-31.1)	16.48±0.46(b)* (-40)
PUFA	53.15±3.18	35.99±2.75(a)* (-37.9)	49.59±2.24(b)* (50.3)	59.01±2.70(b)* (78.8)

MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; N=10 in each group; values statistically compared in groups; a denotes Normal Control vs. FRD Control; b denotes FRD Control vs. FRD+CD; level of significant marks as an asterisk (*) p is less than 0.05 percent change given in parenthesis

In these context, fifteen fatty acids were detected in considerable amounts by GC, namely myristate (14:0), pamitate (16:0), stearate (18:0), oleate (18:1ω9), *cis*-vaccenate (18:1ω7), linoleic (18:2ω6), γ-linolinate (18:3ω6), α-linolinate (18:3ω3), arachidate (20:0), eicosadieonate (20:2), arachidonate (20:4ω6), ecosapentaenoate (20:5ω3), adrenate (22:4ω6), docosapentaenoate (22:5ω3) and docosahexaenoate (22:6ω3). The results exhibited distinct changes in serum fatty acid composition after CD treatment. Over the last decade, a substantial number of experiments have indicated PUFA has health benefits, EPA and DHA and PUFA are known to play a major role in modulating the biosynthesis of eicosanoids and in controlling the levels of blood lipids (Onal et al., 2017; Zhao et al., 2016). In recent years, ω-3 PUFA has been acclaimed for greater potency in treating amelioration of heart disorder than ω-6 PUFA.The results indicated that CD showed a maximal beneficial effect on the serum fatty acid profiles in supplements with a fat-rich diet. It is interesting to note that CD treatment significantly enhanced several biological important fatty acids composition like γ-linolinate (18:3ω6), α-linolinate (18:3ω3), arachidonate (20:4ω6), ecosapentaenoate (20:5ω3), docosapentaenoate (22:5ω3) and

docosahexaenoate (22:6ω3). Diet and blood linoleic acid (18:2ω-6) serve as a precursor for the biosynthesis of arachidonic acid (20:4ω-6), the substrate for eicosanoid synthesis through the activity of the enzymes cyclooxygenase and 5-lipoxygenase. It has been reported that γ-linoleic acid derivatives have a hypocholesterolemic effect and are efficient in lowering low-density lipoproteins (Georgiadi & Kersten, 2012). In the present study, ω3-PUFA content was enhanced in the serum of CD-treated animals, whereas MUFA was lowered. These data clearly indicated the hypolipidaemic action of CD in animals.

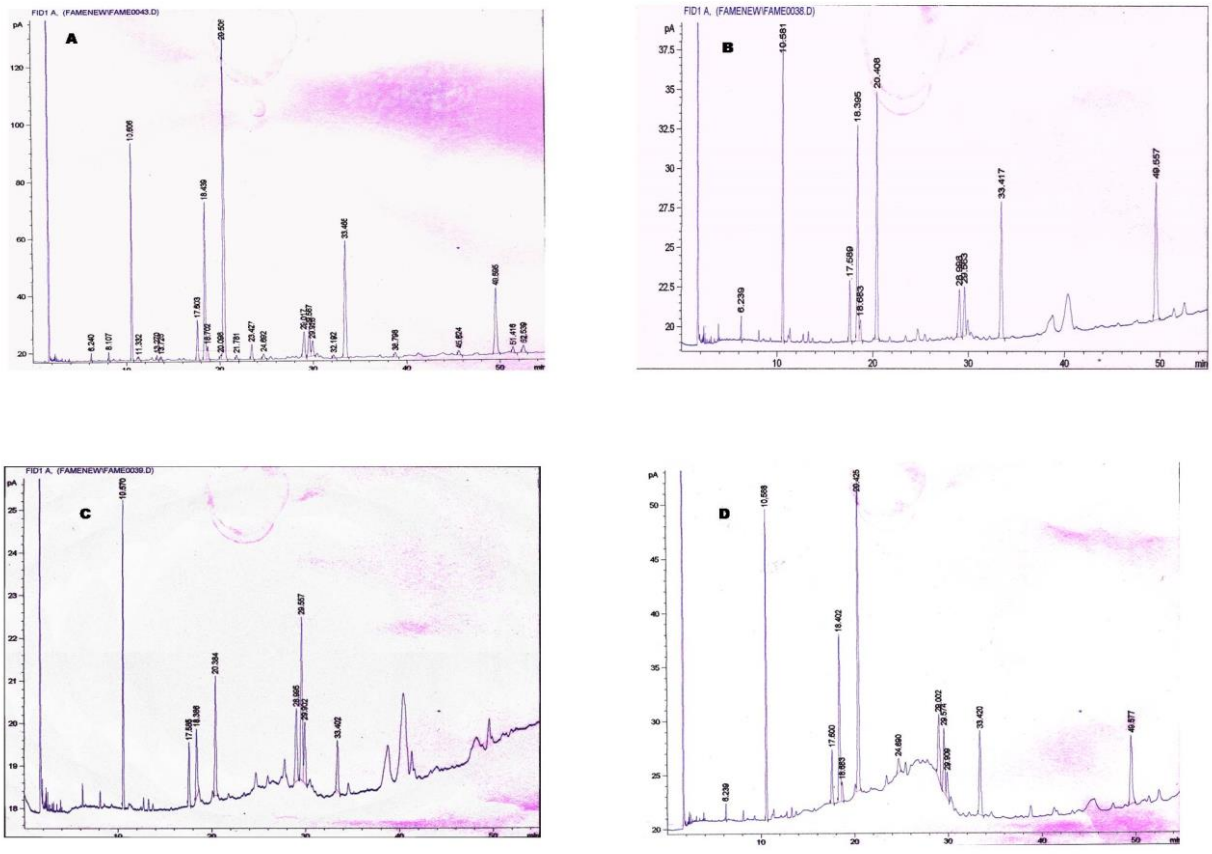

Figure 1. Gas Chromatographic chromatograms of serum FFA profile in rats

A=Normal Control, B=FRD-Control,C=FRD-CD 125 mg/kg, D=FRD-CD 250 mg/kg

CONCLUSION

Capparis decidua fruit extract significantly and dose-dependently (125 mg/kg and 250 mg/kg) lowered the lipid components and enhanced unsaturated beneficial FFAs in the blood of fat-rich diet (FRD) induced rats. FFAs profile is a good indicator to identify the risk of individuals with obesity and dyslipidemia. Therefore, *Capparis decidua* has good therapeutic properties for treating dyslipidemia and obesity and can regulate the free fatty acids pool.

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