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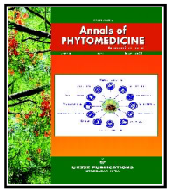

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Fruit juice of *Garcinia indica* Choisy modulates dyslipidemia and lipid metabolism in cafeteria diet based rat model

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Abstract

There is a significant rise in the incidences of dyslipidemia, leading to obesity. The therapeutics available for dyslipidemia are limited and associated with major side-effects. Thereby, researchers are shifting towards nutraceuticals compounds. In the current study, *Garcinia indica* Choisy, which is an endemic species of Western Ghats of India was evaluated for its anti-dyslipidemic properties in cafeteria diet fed obese rat model. Firstly, cafeteria diet fed rat model was developed and validated. After successful development of the model, the rats were orally fed with 1 ml *G. indica* fruit juice for 4 weeks and parameters such as OGTT, lipid profile, hormone levels of insulin and leptin, HMG CoA reductase and LCAT enzyme activities and toxicity parameters were evaluated. Identification and quantification of the hydroxycitric acid in *G. indica* fruit juice was done by HPLC method. Toxicity parameters like SGPT and creatinine were performed to evaluate the toxicity of the dose. Results showed that cafeteria diet fed animals exhibited increased body weight, increased food intake, decreased water intake, increased glucose intolerance and dyslipidemia at 10 weeks. Treatment with *G. indica* fruit juice for 4 weeks, reduced the body weight, improved the metabolic parameters like glucose sensitivity, dyslipidemia, insulin and leptin levels and lipid metabolizing levels without causing toxicity. Oral dosage of *G. indica* fruit juice for 4 weeks exhibits antiobesity potential in cafeteria diet fed dyslipidemic rats. The results obtained were better than orlistat, which is a standard mode of chemotherapy for management of dyslipidemic obesity.

1. Introduction

The prevalence of dyslipidemia leading to obesity is rapidly increasing, however, limited medications are presently available in the market (Birari and Bhutani, 2007). Obesity is a dyslipidemic disorder, wherein, derangement in lipid metabolism has been seen along with abnormal lipid levels (Bays *et al.*, 2013), often associated with higher storage of lipid in adipocytes (Arner *et al.*, 2011). It is interesting to note that dyslipidemia is associated with a cluster of diseases thereby, being a central player in development of metabolic syndrome (Jung and Choi, 2014). The manifestation of syndrome has decreased life expectancy and its quality (Katz *et al.*, 2000; Pimenta *et al.*, 2015; Taylor *et al.*, 2013). Several nutritional theories have implicated diet as an important contributor in development of abnormal lipid levels (Kamran *et al.*, 2016). It is stated that over nutrition with low metabolic output is major cause of developing dyslipidemia (Grundy and Barnett, 2004). In this context, cafeteria based diet which is enriched with high refined sugar and high fat serves as a powerhouse of excessive calories and when used in animal models mimics all features of metabolic syndrome (Gomez-Smith *et al.*, 2016; Sampey *et al.*, 2011). Data from several reports also indicate that people increase the intake of high energy snack foods when stressed, thereby leading to dyslipidemic obesity (Anderson *et al.*, 2011; Shori *et al.*, 2017). This abnormal lipid status has affected immensely other metabolic pathways leading to cardiovascular risk. This has led to urgent need for developing the new antiobesity drugs that could manage dyslipidemia with fewer side effects. Currently, potential use of nutraceuticals for the management of dyslipidemia is not fully explored and could be an outstanding substitute approach for developing safe and effective dyslipidemic drugs. India, especially north-eastern region is a rich source of various medicinal plants. In this context, several herbs have been explored, amongst which *Garcinia* is of utmost importance due to its several important biological properties. Plants from the genus *Garcinia* have been reported from Asia, Africa and Polynesia. Anti-inflammatory, antinociceptive, antioxidant, antitumoral, antifungal, anticancer, antihistaminic, antiulcerogenic, antimicrobial, antiviral, vasodilatory, hypolipidemic, hepatoprotective, nephroprotec-tive and cardioprotective properties of plants from this genus have been reported (Santo *et al.*, 2020). *G. indica* commonly known as “Kokum” is one of the *Garcinia* species used in traditional medicine in Asian countries as folk medicine to treat various ailments. In Ayurveda, it is known as Vrikshamla. Juice obtained from Kokum fruit or extracts prepared from aril or rind is used in the preparation of drugs in

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Indian, Chinese, Thai and Malaysian systems of medicine. Kokum finds its place in Ayurvedic systems of medicine and is considered to be beneficial for health (Swamy *et al.*, 2014). Also, infusion prepared from the Kokum is used to treat piles, dysentery and infections. Furthermore, Kokum is known to strengthen the cardiovascular system and stabilize liver function (Braganza *et al*., 2012). Prophylactic potential of *G. indica* fruits for ailments as varied as rheumatism, rickets, enlargement of spleen, uterine complaints and in animal disorders have been described in *Ayurveda*. Its fruit juice or syrup is used as a coolant and helps to reduce body weight (Braganza *et al*., 2012). It is also used for getting relief in stomach and liver disorders (Bhat *et al*., 2005; Krishnamurthy, 1984; Krishnamurthy *et al.*, 1982; Mishra *et al.*, 2006). The present investigation was undertaken to investigate the efficacy of fruit juice of *G. indica* as a hypolipidemic agent in cafeteria based diet dyslipidemic rodent model.

2. Material and Methods

2.1 Chemicals

Hydroxycitric acid (HCA), calcium salt was purchased from Natural Remedies, Bengaluru, India. HPLC grade solvents (acetonitrile, trifluroacetic acid, TFA, and water) were purchased from Merck, Mumbai, India. Cholesterol (HiMedia Laboratories) and Orlistat (German Remedies) were purchased locally. Metabolic profile involving oral glucose tolerance test, lipid profile were performed using GOD-POD kit and lipid profile test kit was purchased from Enzopak (Rankem). Trizol, cDNA kit was procured from Takara Inc (PrimeScript 1st strand cDNA Synthesis Kit). Primers for metabolic enzymes were designed by primer express and synthesized by integrated DNA technologies. Serum hormones-insulin and leptin were assayed using ELISA kits (DBC Canada).

2.2 *G. indica* fruit juice

Mature fruits of *G. indica* were collected. Fruit pulp was compressed to get the juice. Collected juice was stored in glass bottles under refrigerated conditions till further use.

2.3 Apparatus and chromatographic conditions for profiling of HCA in *G. indica* fruit juice

Quantification of hydroxycitric acid was carried out using HPLC (Waters, USA) system consisting of quaternary pumps, an in-line vacuum degasser, and a photodiode array detector (PDA). The instrumentation was controlled by using Empower 3.0 software (Waters). The chromatographic separation was achieved using Sunfire™ C18 Column (4.6 x 250 mm, 5 µm, Waters, Milford, MA, USA) at ambient temperature. The mobile phases consisted of a mixture of solvents: 0.1 % trifluoroacetic acid in water (A) and 0.1 % trifluoroacetic acid in acetonitrile (B). The optimised HPLC condition for gradient elution mode is as follows: elution was initially started with 90 % of solvent (A) and 10 % solvent (B) with a flow rate of 0.8 ml/min. Further, after 10 min solvent (A) was decreased to 80 % and solvent (B) was increased to 20 %. After 25 min of solvent (A) was decreased to 70 % and solvent (B) was increased to 30 %. Solvent (A) was gradually decreased to 60 % and solvent (B) was increased to 40 % after 30 min. At 31 min composition of solvent (A) and solvent (B) was brought back to 90 % and 10 %, respectively. The flow rate through out the run was 0.8 ml/min and run time was 35 min (Kureshi *et al.*, 2018). Detection wavelength was 211 nm. Injection volume was 20 µl (Kureshi *et al.*, 2018).

2.4 Animals

Forty adult virgin female Charles Foster rats (3-4 months) showing regular estrous cyclicity were chosen for the study. Animals were housed in a standard controlled animal care facility (22-25^{o}C and 45% humidity) in cages (one rats/cage) under equal dark and light cycle (1:1). Standard nutritional and environmental conditions for animals were maintained throughout the experiment (Chidrawar *et al*., 2011). All the experiments were carried out between 9:00 and 16:00 hours, at ambient temperature. The Nations Control and Supervision of Experiments on Animals (CPCSEA) guidelines were strictly followed and all the studies were approved by the Institutional Animal Ethical Committee (IAEC) (Protocol Number: BC/11/2017).

2.5 Induction of dyslipidemia through diet

The cafeteria diet was as described by Chidrawar *et al*. (2012). It consisted of 3 different diets: diet 1-condensed milk (8 g) + bread (8 g); diet 2-chocolate (3 g) + biscuits (6 g) + dried coconut (6 g); diet 3-cheese (8 g) + boiled potatoes (10 g) for each animal. The three cafeteria diets were fed to each rat of the group that had 6 animals on days one, two, and three, respectively with repetition in the same succession for 10 weeks along with standard pellet diet. Cholesterol rich high fat diet (Kumar *et al*., 2008) was given to each rat four weeks prior to the *G. indica* fruit juice treatment.

2.6 Treatment with *G. indica* fruit juice

Animals were divided into six major groups, Group I (C) had animals that received lab chow diet and were considered as control. Further, animals fed with cafeteria diet and high-fat diets were divided into five groups, Group II (CD) consisted of animals which were fed with cafeteria diet and were considered to be dyslipidemic animals; Group III (CD + O) consisted orlistat (standard antiobesity drug) treated animals fed with cafeteria diet; Group IV (CD + G) were *G. indica* fruit juice treated animals fed with cafeteria diet; Group V(HF) animals were high fat diet treated animals, which served as positive control for dyslipidemia and Group VI (HF + G) were high-fat diet fed animals, which received *G. indica* fruit juice treatment. All *G. indica* fruit juice treatment was given daily orally for 30 days at a dose of 1 ml.

2.7 Parameters analysed

During 30 days of *G. indica* fruit juice treatment, all animals were continuously monitored for body weight and food intake. Oral glucose tolerance test (OGTT) was performed after 12 h fasting in all rats (Buchanan *et al.*, 1991). Glucose (2g/kg body weight) was orally fed to the rats and blood samples was collected in sodium fluoride (NaF) and EDTA coated tubes considered as 0 minute. After that blood sample was collected at different time intervals (30, 60, 90, and 120 minutes) and plasma was used for the estimation of glucose. Analysis of the lipid profile was done by Enzopak kits which estimated total cholesterol, triglycerides, HDL-C, and LDL-C from serum. Serum hormones like insulin and leptin were assayed by ELISA method. Homeostatic model assessment, Insulin Resistance (HOMA-IR) was calculated based on the fasting blood glucose and fasting insulin values using the following formula HOMA IR = (Fasting insulin x Fasting glucose)/405. Normal insulin resistance: < 3; moderate insulin resistance: between 3-5; severe insulin resistance: > 5. In addition to

this, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) activity was carried out according to the protocol described by (Rao and Ramakrishnan, 1975). Also, plasma lecithin cholesterol acyl transferase (LCAT) activity was assayed by the method described by Hitz (Hitz *et al.*, 1983). In addition to this, gene expression of cholesterol biosynthetic enzymes like Acetyl-CoA carboxylase (ACC) was evaluated by RT-PCR. RNA was isolated using trizol reagent following which 2 microgram of RNA was subjected to cDNA preparation. cDNA was later used for expression studies. Primer details are indicated in Table 1. Serum glutamate pyruvate transaminase (SGPT) and creatinine were analyzed to understand the toxicity of *G. indica* fruit juice.

Table 1: Sequences of target gene specific primers

Gene name	Accession number	Sequence of primers
Acetyl-coenzyme A carboxylase	NM_022193.1	F-5'ATGGTCTACATTCCCCCACA 3'
		R-5'ATCACAACCCAAGAACCACC 3'
GAPDH (internal control)	NM_017008	F-5'CAAGGTCATCCATGACAACTTTG 3'
		R-5'GTCCACCACCCTGTTGCTGTAG 3'

2.8 Statistical analysis

Comparison of different groups was done using analysis of variance (ANOVA) and Student *t*-test. The analysis was carried out using GraphPad version 5.0. $P \geq 0.05$ was considered significant. All results are expressed as the mean ± SEM for 6-7 animals in each group.

3. Results

3.1 HPLC analysis of *G. indica* fruit juice

Identification and quantification of the HCA in *G. indica* fruit juice was carried out with the comparison of retention time and PDA spectra of the peak of standard HCA (Figure 1A). Peak of HCA eluted at 5.3 min. Furthermore, identity of peak of HCA was also confirmed by spiking studies. Quantification of HCA was carried out using a calibration curve prepared from the different concentration of standard HCA and the content of (-)-hydroxycitric acid (HCA) in *G. indica* juice was 16.57 % (Figure 1B).

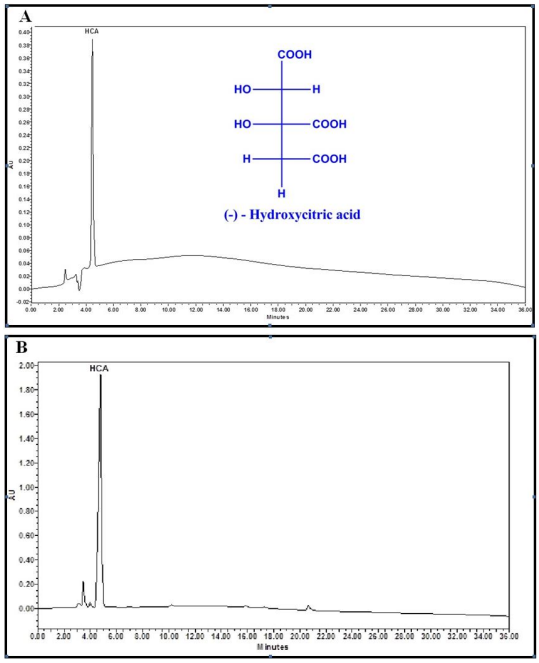
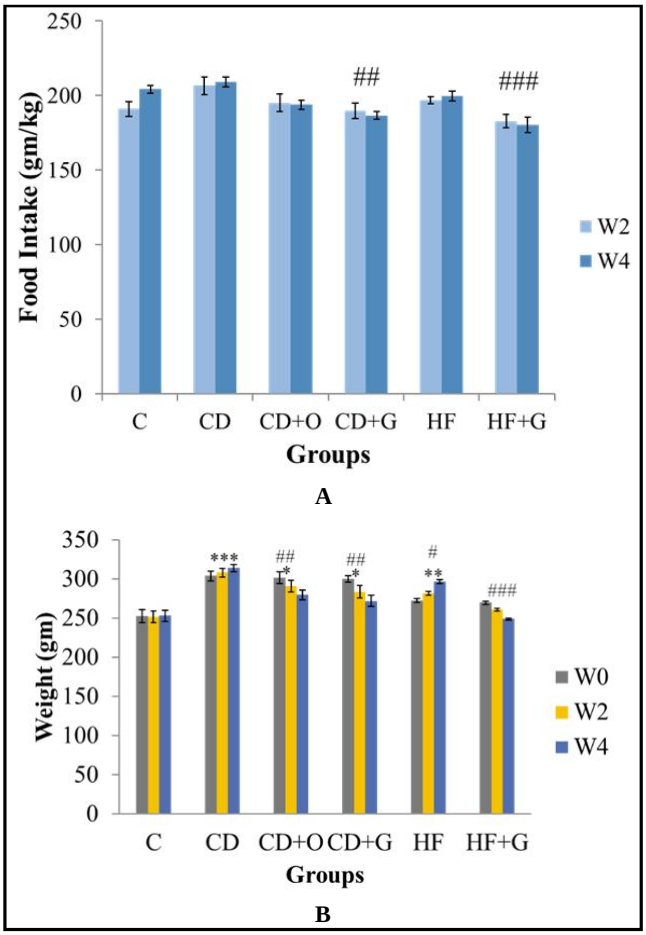


Figure 1: HPLC analysis of *G. indica* fruit juice. A peak of standard HCA B. Quantification of HCA in *G. indica* fruit juice.

3.2 Effect of *G. indica* fruit juice on body weight, food and water intake

Figure 2A. represents the effect of *G. indica* fruit juice on the food consumption of the animals. There was significant increase in body weight observed after 10 weeks of treatment in cafeteria group as compared to control group as shown in Figure 2B. After 4 weeks of treatment with *G. indica* fruit juice, it was observed that there was significant decrease in body weight as compared to non-treated cafeteria group as well as orlistat treated cafeteria group. Thereby, treatment of *G. indica* fruit juice for 4 weeks was effective in reduction of body weight in the animals.



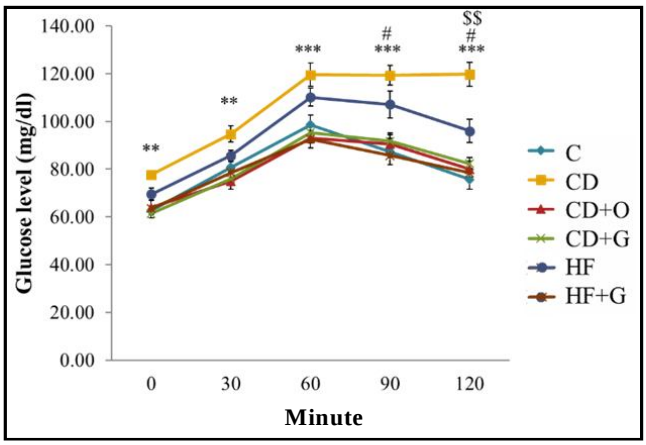


Figure 2: Effect of *G. indica* fruit juice on A. food intake, B. body weight. * comparison with control, # comparison with cafeteria diet, N=5, values are Mean ± SEM, # $p<0.05$, ## $p<0.01$, ### $p<0.001$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, W0, W2, W4 represents week 0 (before treatment), week 2 and week 4 (after treatment) C. glucose tolerance. * comparison with cafeteria diet, # comparison with high-fat diet, $ comparison between cafeteria diet and high-fat diet N=5, values are Mean ± SEM, # $p<0.05$, ** $p<0.01$, *** $p<0.001$, $$ $p<0.01$.

3.3 Effect of *G. indica* fruit juice on metabolic parameters

High fat affects insulin sensitivity. Thereby, oral glucose tolerance test was performed after *G. indica* fruit juice treatment. Figure 2C demonstrated that the treatment with *G. indica* fruit juice for 4 weeks significant decrease in glucose intolerance was recorded. This data was comparable to group treated with orlistat, thereby, suggesting that *G. indica* fruit juice has potential to improve glucose sensitivity. However, cafeteria group and high fat group were glucose intolerant. In addition to this, serum hormone profile for insulin and leptin along with HOMO-IR demonstrated that there was significant increase in serum insulin level in cafeteria treated group (Table 2). Upon treatment with *G. indica* fruit juice, there was significant decrease in serum insulin level. Similar results were observed in orlistat treated group. In case of high-fat group, no significant change was observed as compared to control group. Serum leptin levels were significantly very high as compared to control group in cafeteria treated group. *G. indica* fruit juice treatment showed significant decrease in its level. While calculating HOMO-IR, it was observed, there is severe insulin resistance in cafeteria treated group. After treatment with *G. indica* fruit juice and orlistat, its value decreased but still it was showing moderate insulin resistance similar to control group. Thereby, it could be possible that long term treatment may improve glucose sensitivity.

Table 2: Effect of *G. indica* fruit juice on hormone profile

Groups	Insulin (mIU/ml)	Leptin (ng/ml)	HOMO-IR
C	24.09	0.74	3.73
CD	36.80**	1.17***	7.06
CD+O	32.10#	0.84###	5.07
CD+G	28.47##	0.76###	4.31
HF	29.37*##	0.98**##	5.04
HF+G	27.36	0.81	4.29

* Comparison with Control, # Comparison with cafeteria diet. n=5, Values are represented as Mean ± SEM, # $p<0.05$, ## $p<0.01$, ### $p<0.001$* $p<0.05$,** $p<0.01$,*** $p<0.001$.

Table 3 represents lipid profile after administration of *G. indica* fruit juice for 30 days. There was significant decrease in serum total cholesterol, serum tryglycerides and LDL-cholsterol and increase in HDL-cholesterol. It suggested that dyslipidemic rat model was reverting back to normal phenotype. These data when compared to standard drug shows similar result. Cafeteria treated group and high-fat treated group both shows significant alteration in lipid profile.

Table 3: Effect of *G. indica* fruit juice on lipid profile.

Groups	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-cholesterol (mg/dl)
C	55.55 ± 3.54	32.94 ± 1.09	80.83 ± 4.05	6.445 ± 4.63
CD	95.12 ± 2.90 ***	22.06 ± 0.86 **	141.9 ± 6.36 ***	44.69 ± 4.03 ***
CD + O	78.65 ± 3.75 #	32.16 ± 0.57 ##	97.98 ± 6.64 ##	26.9 ± 4.47 ##
CD + G	80.32 ± 1.58 #	31.3 ± 0.43 ##	95.3 ± 2.33 ##	29.96 ± 1.37 ##
HF	86.16 ± 2.10 ***	28.72 ± 0.78 *	118.1 ± 7.61 **	33.83 ± 1.88 **
HF+ G	82.92 ± 2.37	30.29 ± 0.96	107.5 ± 6.90	31.14 ± 1.23

* Comparison with Control, # Comparison with cafeteria diet n=5, Values are represented as Mean ± SEM, ** $p<0.01$, *** $p<0.001$, # $p<0.05$, ## $p<0.01$.

3.4 Effect of *G. indica* fruit juice on lipid metabolizing enzymes

Figure 3A represents the activity of key lipid metabolizing enzyme HMG-CoA reductase. Upon treatment of *G. indica* fruit juice, HMG-CoA reductase activity was decreased, thus indicated a decreased cholesterol biosynthesis. Ideal decrease in activity was seen on treatment with orlistat. High-fat group does not show significant change suggesting no change, in HMG-Co reductase activity. Figure 3B shows the effect of *G. indica* fruit juice on LCAT activity. On administering *G. indica* fruit juice, a significant increase in LCAT activity was observed as compared to the cafeteria group. This activity was not increased in case of orlistat treated group. While high-fat treated groups showed decrease in LCAT activity. No significant change was observed in *G. indica* fruit juice treated group as compared to high-fat treated group. Figure 3C represents transcript level of enzyme acetyl Co-a carboxylase, a lipid metabolizing enzyme. As seen in the figure, cafeteria diet treated group shows significant increase in ACC level as compared to control.

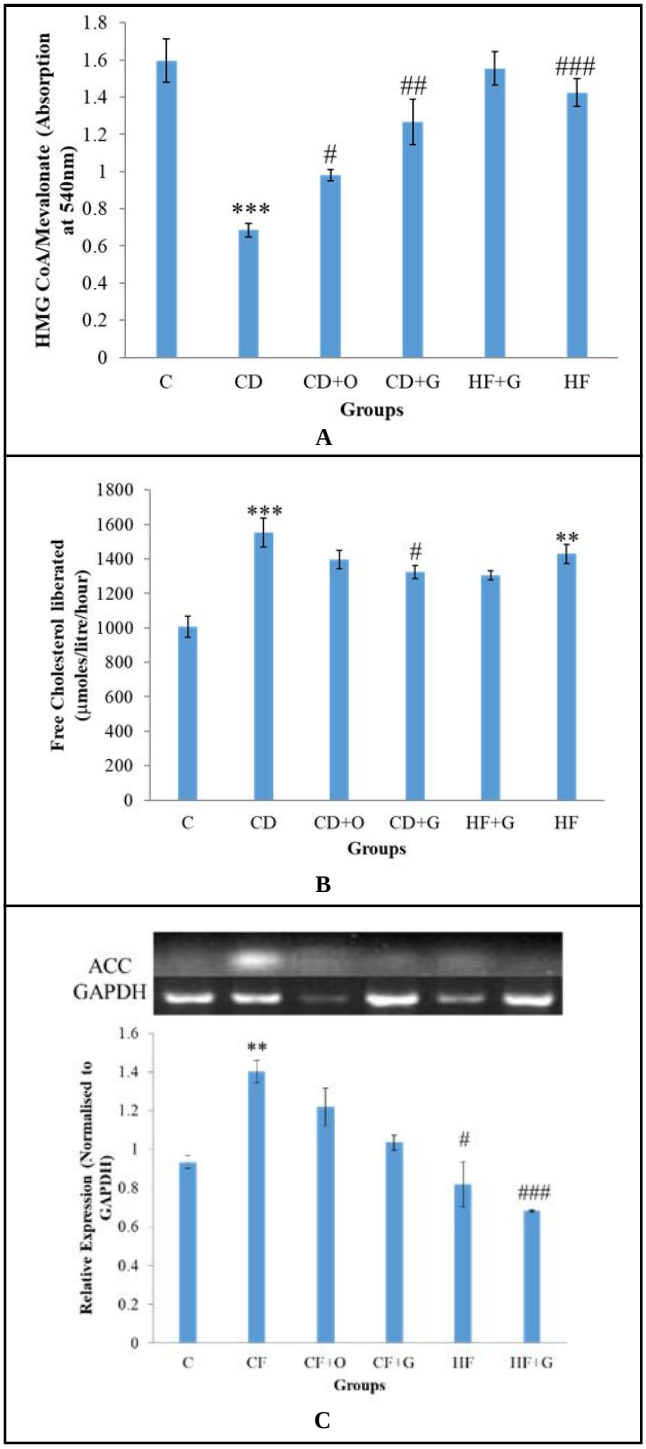


Figure 3: Effect of *G. indica* fruit juice on lipid metabolizing enzymes A. HMG CoA reductase activity B. LCAT activity and C. gene expression level of ACC enzyme. * comparison with control, # comparison with cafeteria diet, N=5, Values are Mean ± SEM, # <0.05, # *p*<0.01, ## *p*<0.001, ** *p*<0.01, * *p*<0.001, C. relative gene expression of ACC.* comparison with control, # comparison with cafeteria diet. n=5, values are Mean ± SEM, # *p*<0.05, ### *p*<0.001, ** <0.01.**

After treatment of *G. indica* fruit juice for 30 days, there was marked decrease in its level. The data is similar to the orlistat treated group. High-fat diet treated group does not show significant change in ACC level as compared to control group.

3.5 Effect of *G. indica* fruit juice on toxicity parameters

Figure 4 describes the toxicity parameters of liver and kidney upon administration with the *G. indica* fruit juice. As seen in Figure 4A, there was significant increase in SGPT activity, suggesting liver toxicity due to cafeteria diet treatment as compared to control. Upon the administration of *G. indica* fruit juice, there was significant decrease in its activity. However, there was no significant change in high-fat treated group. Figure 4B represents serum creatinine level and as seen in the Figure, there was significant increase in serum creatinine level in cafeteria treated group and the level decreased in *G. indica* fruit juice treated group. In case of high-fat treated group, significant increase in serum creatinine level was recorded.

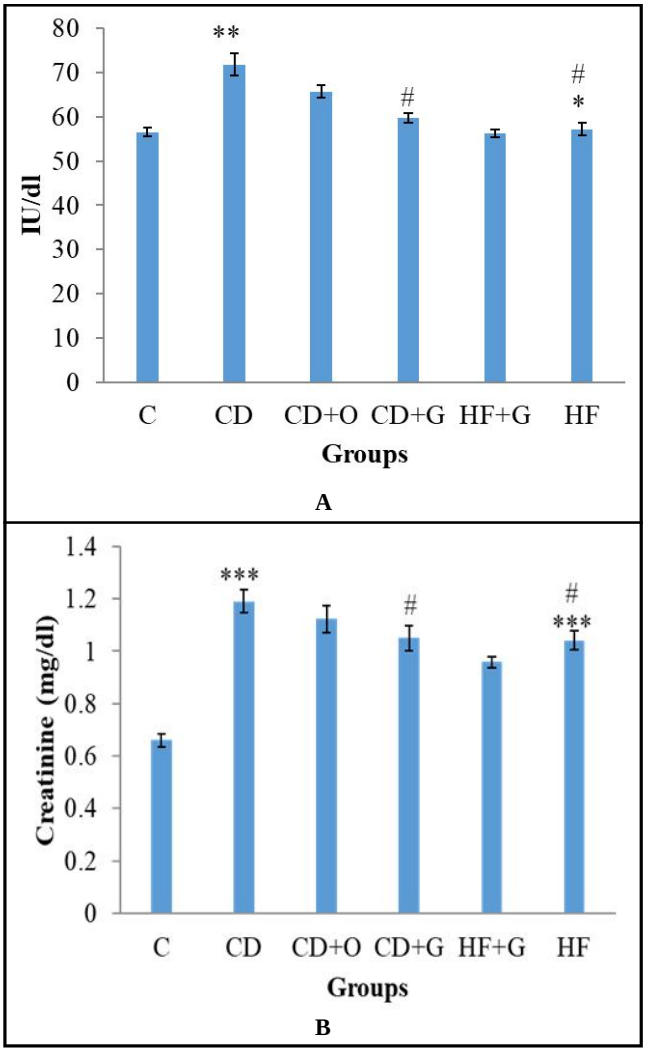


Figure 4: Effect of *G. indica* fruit juice on toxicity parameters of A. liver by SGPT activity and B. kidney by creatinine level. *comparison with control, # comparison with cafeteria diet. N=5, values are Mean ± SEM, ** *p*<0.01, * *p*<0.001, # *p*<0.05.**

4. Discussion

Obesity is a one of major dyslipidemia associated metabolic syndrome. Dyslipidemia is one of the initial steps for the development of obesity. In present study, cafeteria diet was used to generate a metabolic syndrome model for dyslipidemia to study initial stages for obesity. During dyslipidemia, there is increase in glucose intolerance, which is one of the important factor of metabolic syndrome and this may be due to increase in free fatty acid (Nambi *et al.*, 2002). OGTT profile was observed upon cafeteria diet administration, suggesting that enriched fat diet for 10 weeks is sufficient to cause dyslipidemia. In dyslipidemia, lipoprotein metabolism is altered which results in either overproduction or deficiency in certain lipoprotein molecule (Meisinger *et al.*, 2006). There may be alteration in the levels of either one or more lipoprotein molecules followed by elevated levels of total cholesterol, low density lipoprotein and triglycerides along with decreased levels of high density lipoprotein which later on can be developed in obesity (Mishra *et al.*, 2005; Misra *et al.*, 2006; Snehalatha, 2003; Vikram *et al.*, 2003). In our model, it was observed that there was significant increase in total cholesterol, LDL-cholesterol and triglycerides. Also, significant decrease in HDL-cholesterol was also observed. These all parameters are major characteristics of dyslipidemia (Nambi *et al.*, 2002).

HMG-CoA-Reductase catalyses the rate limiting step of liver cholesterol biosynthesis (Bucher *et al.*, 1960; Siperstein,. 1966). In dyslipidemia, it was observed that HMG-CoA-reductase activity was higher as compared to control group (Wu *et al.*, 2013). In our study, it was observed that there was significant decrease in substrate to product ratio suggesting an increase in HMG-CoA-reductase activity. Increase in its activity has resulted in increase in serum cholesterol levels after 10 weeks of treatment. LCAT is an enzyme that catalyses transfer of fatty acids from phosphatidylcholine to the hydroxyl residue (Assmann *et al.*, 1978). Acetylation of cholesterol is helpful for clearance of excess cholesterol. LCAT promotes maturation of HDL particles in plasma and transport cholesterol maintaining a concentration gradient for the diffusion of cellular un-esterified cholesterol to HDL-cholesterol (Shigematsu *et al.*, 2001). In diet induced obese rats, it was observed that there was significant decrease in LCAT activity which correlates with decrease in HDL-cholesterol (Subash and Augustine, 2014). Current study demonstrates that in dyslipidemia, rats there was significant decrease in LCAT activity which can be correlated with low HDL-cholesterol.

Dyslipidemia is a major symptom in development of metabolic syndrome (Mishra *et al.*, 2007). There are several medications available for dyslipidemia. Two major classes are statins and fibrtaes (Wierzbicki *et al.*, 2003). But, due to high cost and hazardous side-effects, there is need for new and better therapy in management of dyslipidemia. Plant derived products or nutraceuticals can be potential targets for the development of new drugs (Chidrawar *et al.*, 2012), as it renders few side-effects. In this context, several plants have been explored for the hypolipidemic effects like *Aloe barbadensis* Mill., *Enicostemma littorale*, *G. cambogia* (Desai *et al.*, 2012; Oluyemi *et al.*, 2002; Vasu *et al.*, 2005). *G. indica* is an indigenous plant however, medicinal properties of this plant are not well studied. Thereby, our present study, involves evaluating efficacy of *G. indica* for its hypolipidemic property.

After the treatment of *G. indica* fruit juice, there was significant decrease in body weight as compared to non-treated groups, suggesting that the *G. indica* fruit juice could be used for the development of anti-dyslipidemic drugs. Similar result was observed in the rats treated with standard drug. Similarly glucose intolerance was reduced in the *G. indica* fruit juice treated group as compared to non-treated group. In case of positive control (high-fat) group, no significant change in the body weight was observed as compared to control group, suggesting that dyslipidemic rat model was not developed. Glucose intolerance is a characteristic feature of insulin resistance, therefore, serum insulin levels were measured (Vikram *et al.*, 2003). There was significant increase in serum insulin level in cafeteria group as compared to control group. After the treatment with *G. indica* fruit juice, there was significant reduction in insulin level. It can be correlated with serum triglycerides level. HOMO-IR is used for evaluation of insulin resistance. While calculating HOMO-IR, it was found that the animals treated with cafeteria diet showed significant increase in index suggesting severe insulin resistance. While treatment with *G. indica* fruit juice resulted in decrease in index but the values were still in the range of moderate insulin resistance. During dyslipidemic obesity, there is increased level of circulating leptin level (Dobrian *et al.*, 2000). Serum leptin levels were elevated in cafeteria treated and high-fat diet treated groups as compared to control. After treatment with *G. indica* fruit juice, it was observed that there was significant reduction in serum leptin levels.

Lipid profile showed significant change in the *G. indica* fruit juice treated groups as compared to non-treated ones. Serum total cholesterol, triglycerides and LDL-cholesterol were back to normal in *G. indica* fruit juice treated group as compared non-treated group. It suggests that *G. indica* fruit juice is altering the lipid metabolism pathway resulting in normal phenotype.

HMG-CoA-reductase and LCAT activity were measured to check effect of *G. indica* fruit juice on lipid metabolising enzymes. After treatment, it was observed that in there was significant increase in the ratio of substrate to product resulting in decreased HMG-CoA-reductase activity. This might be the reason for decreased cholesterol level. Group treated with standard drug also showed the similar result, but to a lower extent. LCAT activity was significantly increased in *G. indica* fruit juice treated group as compared to non-treated group. In case of group treated with standard drug, no significant change was observed. mRNA transcripts levels of acetyl-coenzyme A carboxylase (ACC) were also checked. ACC is an enzyme responsible for the production and regulation of fatty acid. It is predominately expressed in the liver and it is inducible in response to feeding. Transcript level of ACC is increased in obesity resulting in production of free fatty acid (Jeffery *et al.*, 2013). mRNA levels of ACC were increased in cafeteria treated group. As a result of administration of *G. indica* fruit juice, significant reduction in its level was observed, suggesting decrease in free fatty acid levels. However, other transcriptional factors need to be evaluated for further confirmation. Thereby, present study has clearly shown the *G. indica* fruit juice could be a strong candidate for a hypolipidemic drug. Further, investigations needs to be done to mark the product as a dyslipidemic nutraceutical. Liver and kidney function tests showed that after 10 weeks of treatment with cafeteria diet there was significant increase in SGPT activity and serum creatinine, respectively, suggesting that prolong dose of cafeteria diet can alter liver and kidney function (Chidrawar *et al.*, 2012). After treatment with *G. indica* fruit juice, it was observed that there was significant

decrease in SGPT activity and serum creatinine levels. It suggests that *G. indica* fruit juice may have potential to restore liver and kidney functions. These results were not observed in the group treated with standard drug.

5. Conclusion

Increasing evidences are suggesting the significant impact of plant derived phytocomponents in the regulation of different aspects of human physiology and thereby, aid in the treatment and management of cardiovascular diseases and metabolic syndrome like obesity and dyslipidemia. In the current study, cafeteria diet induced obesity model was successfully developed which exhibited the associated complications of metabolic syndrome such as increased body weight, dyslipidemia, and insulin resistance after 10 weeks. Also, the potential of *G. indica* fruit juice to ameliorate dyslipidemia was evaluated in cafeteria diet fed obese rat model. It was observed that treatment with *G. indica* fruit juice at a dose of 1 ml for 4 weeks could restore the body weight, dyslipidemia, glucose sensitivity, insulin resistance and expression and activities of key lipid metabolizing enzymes in cafeteria diet induced dyslipidemic rat model. The observed therapeutic potential was better than orlistat, which is the standard drug used for treatment of dyslipidemia and obesity. Thereby, suggesting that phytocomponents such as HCA present in *G. indica* fruit juice is a potent anti-dyslipidemic agent without inducing toxicity. However, phytocompounds from *G. indica* can be further analysed for understanding its molecular targets towards amelioration of dyslipidemia.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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