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Original Research Article

STUDY OF PHYTOCHEMICALS AND ANTI-HYPERLIPEDEMIC ACTIVITY OF GARCINIA INDICA

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Abstract:
This study explores the phytochemical composition and anti-hyperlipidemic activity of *Garcinia indica* hydroalcoholic extract derived from its fruit rinds in a diet-induced hyperlipidemic rat model. The extraction process yielded 6.50% from petroleum ether and 14.65% from the hydroalcoholic extract, with the latter exhibiting higher concentrations of bioactive compounds. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, steroids, and phenols, with the total phenolic content measured at 0.445 mg/100 mg and total flavonoids at 0.658 mg/100 mg. The antioxidant potential was assessed using free radical inhibition assays, where the extract demonstrated promising antioxidant properties, though less potent than ascorbic acid (IC50 of 45.61 µg/ml vs 12.60 µg/ml). Treatment with Garcinia indica hydroalcoholic extract (100 mg/kg and 200 mg/kg) significantly reduced total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), while stabilizing high-density lipoprotein cholesterol (HDL-C) levels, indicating a lipid-lowering effect. Fecal analysis further revealed enhanced cholesterol and bile acid excretion, suggesting that the extract promotes the elimination of excess cholesterol. The results suggest that *Garcinia indica* extract exhibits significant lipid-lowering and antioxidant effects, supporting its potential use in managing hyperlipidemia and improving cardiovascular health. Further research is needed to explore its underlying mechanisms and clinical applicability in humans.
Keywords: Garcinia indica, Hydroalcoholic extract, Hyperlipidemia, Antioxidant activity, Lipid profiles, Cholesterol management, Fecal cholesterol excretion

Introduction

Garcinia indica, commonly known as Kokum, is a tropical fruit-bearing plant belonging to the family Clusiaceae. It is widely found in the Western Ghats of India and has been used in traditional medicine for its therapeutic properties. The fruit rind of Garcinia indica has garnered attention for its rich phytochemical composition, including bioactive compounds such as hydroxycitric acid, flavonoids, phenolic acids, and alkaloids, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties (Kumar et al., 2012; Ghosh et al., 2013).

Hyperlipidemia, characterized by elevated levels of lipids (cholesterol and triglycerides) in the blood, is a major risk factor for

cardiovascular diseases (CVDs), including atherosclerosis, stroke, and heart attacks. Despite the availability of pharmacological agents such as statins, many individuals seek natural alternatives due to side effects or insufficient efficacy of synthetic drugs (Chowdhury et al., 2017). In this context, plant-based compounds have gained interest for their potential to modulate lipid metabolism and offer a safer, more holistic approach to managing hyperlipidemia.

Several studies have demonstrated that Garcinia indica exhibits significant pharmacological activities, including antioxidant, anti-inflammatory, and lipid-lowering effects (Kusum et al., 2018; Ghosh et al., 2015). The fruit rind of Garcinia indica has been particularly noted for its high content of hydroxycitric acid (HCA), a compound that has been shown to inhibit lipogenesis and promote fat oxidation in various animal models (Ali et al., 2015). Furthermore, the antioxidant properties of the plant, attributed to its phenolic and flavonoid content, may protect against oxidative stress-induced damage, which is often associated with dyslipidemia (Soni et al., 2019).

Despite the promising phytochemical profile of Garcinia indica and its traditional use for weight management and lipid regulation, limited studies have comprehensively investigated its anti-hyperlipidemic effects in experimental models. The present study aims to evaluate the phytochemical constituents and anti-hyperlipidemic activity of Garcinia indica hydroalcoholic extract in a diet-induced hyperlipidemic rat model. Additionally, this study will explore the antioxidant potential of the extract, as oxidative stress plays a significant role in lipid metabolism disorders.

By investigating the lipid-lowering properties of Garcinia indica alongside its phytochemical content, this study seeks to provide scientific evidence supporting the plant’s therapeutic efficacy and potential as a natural supplement for managing hyperlipidemia and related cardiovascular conditions.

Material and Methods

Collection of plant material

Organoleptic, morphological and microscopic properties could all be used to distinguish between crude medicines. Garcinia indica was the chosen plant, and its Fruits rinds were collected from local market of Bhopal based on their geographic accessibility. Selected plant material was collected, cleaned, dried in the shade, ground to a fairly coarse powder, and then kept in an airtight container for later use.

Extraction of plant material

Extraction is the process of separating the parts of a plant that are medicinally effective utilizing certain solvents and accepted practices. All extraction procedures have the goal of separating the plant's soluble metabolites from its insoluble cellular marc (residue) 50 gram weight of powdered plant medicine was measured and placed in a soxhlet container. A drop from the thimble was placed on a piece of filter paper that did not have any oily spots, indicating that the plant material had been completely defatted. The plant material was defatted with petroleum ether for around 12 hours separately.

The defatted material was taken out of the Soxhlet device and allowed to air dry to get rid of any remaining petroleum ether. The medicine from the defatted plant was extracted using methanol as the solvent. For various solvents, the process was run for approximately varying lengths of time. The liquid extracts were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated (Mukherjee, 2007; Khandelwal, 2005).

Preliminary phytochemical screening

To analyze the plant material in terms of its active ingredients, one must first perform a preliminary phytochemical screening. Fruits rinds of Garcinia indica extract were put through

routine phytochemical testing in order to identify the various components they contained. The following phytoconstituents were evaluated qualitatively using established techniques: alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins, and amino acids (Kokate, 1994; Harborne, 1998).

Quantitative estimation of phenols and flavonoids

Estimation of total phenolic content

The total phenolic content of dry extracts was performed with Folin-Ciocaltaeu assay. 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of demonized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23 ^{0}C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100 mcg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20, and 25mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5min 0.3ml of 10% Aluminium chloride solution and at the 6th minute 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

In vitro DPPH radical scavenging

DPPH scavenging activity was measured by the spectrophotometer with slightly modification (Parkhe and Jain, 2018). Stock solution (6 mg in 100ml methanol) was prepared Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\% \text{ of Inhibition} = \frac{(A0 - A1)}{A0} x100$$

Where, A0 = absorbance of the control (without test samples)

A1 = absorbance of test samples.

In vivo antihyperlipidemic activity

Animals

Animal's Albino rats (SD strain) weighing 150–200g of either sex was used in the present study. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and extract

All the extracts were suspended in distilled water using 1% w/v gum acacia. The reference drugs Atorvastatin suspended in distilled water using 1% carboxymethyl cellulose (CMC). The control group received 1% w/v gum acacia in

distilled water and 1% CMC solution as vehicles.

Acute oral toxicity study

Adult Albino rats (SD strain) weighing 150–200g of either sex, fasted overnight and were used for acute toxicity study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline. Four groups of mice of both sexes were fasted overnight. The first control group mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water while the other groups received Hydroalcoholic extract of Garcinia indica in 0.5% CMC at doses of 200, 600, and 2000 mg/kg. Animals were observed closely for first 4 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 14 days and no mortality was reported within the study period.

Diet-induced hyperlipidemia in rats

The method of Blank et al. (1963), with modification, was used to produce diet-induced hyperlipidemia. Animals were divided into different groups (Table 6.1).

Briefly, the normal group received a standard chow diet and all other groups received a high-cholesterol diet consisting of normal chow diet 92%, cholesterol 2%, cholic acid 1%, and coconut oil 5% for 7 days. The reference drug (Atorvastatin 50 mg/kg) and extracts were administered once daily between 8:00 and 9:00 a.m. for 7 days. The daily food intakes were determined before treatments. On the last day, animals were deprived of food but not water. Blood samples were collected by retro orbital puncture technique under light anesthesia. The animals were sacrificed and liver tissues were collected and preserved at 40°C for further analysis.

Table 1: Diet-induced hyperlipidemia model: summary of animal groups and treatments

S. No	Groups	Treatments
1.	Normal	Vehicles (1 mL of 1% gum acacia and 1% CMC)
2.	Hyperlipidimic control	High cholesterol diet
3.	Treated with Standard (Atorvastatin)	High cholesterol diet + Atorvastatin (50mg/kg, p.o.)
4.	Treated with HAGI 100mg/kg	High cholesterol diet + HAGI (200mg/kg, p.o.)
5.	Treated with HAGI 200mg/kg	High cholesterol diet + HAGI (400mg/kg, p.o.)

HAGI: Hydroalcoholic extract of Garcinia indica

Estimation of biochemical parameters

Lipid profile

The serum lipid profile was determined on day 8 in the case of diet-induced hyperlipidemia. The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were estimated using commercially available kits (Erba; Transasia Bio-Medicals Ltd., Daman, India). Very low-density lipoprotein cholesterol (VLDL-C) was calculated as TG/5.

LDL-cholesterol (LDL-C) levels were calculated using Friedewald's *et al.,* (1972) formula. The atherogenic index was calculated using the formula: atherogenic index.

$$(\mathrm{AI}) = \frac{(\mathrm{VLDL-C} + \mathrm{LDL-C})}{\mathrm{HDL-C}}$$

Estimation of serum lipid profiles

Estimation of lipid profiles were placed major role in obesity condition. Usually, in obese condition the levels of lipids were higher than normal. So that, to know the activity of plant extract lipid profiles was studied.

Estimation of Cholesterol

Estimation of cholesterol was carried out by the method of Zlatkis *et al.,* (1953).

Ferric chloride-acetic acid (9.9 ml) reagent was added to 0.1 ml of serum for deproteinization. The contents were centrifuged at 3000 rpm for 15 min. 5 ml of the supernatant was taken and to this added 3 ml of concentrated Sulphuric acid and kept for 20 min at room temperature. The pink colour formed was read at 540 nm against a blank containing 5 ml of ferric chloride-acetic acid reagent. A set of standards were also performed in the similar manner.

Estimation of Triglycerides

Plasma triglycerides were measured by the method of Foster and Dunn *et al.,* (1973).

Plasma (0.1 ml) was taken in a glass stoppered centrifuge tube and to this added 4 ml of isopropanol and 400 mg of alumina. The tubes were tightly capped and shaken vigorously for 10 min. The tubes were centrifuged at 3000 rpm for 15 min and 2 ml of the supernatant was pipette into clean, dry test tubes. To these added 0.6 ml of alcoholic KOH and kept at 70°C for 15 min. The tubes were cooled to room temperature. To this added 0.5 ml of acetyl acetone reagent, 1.0 ml of meta periodate reagent and incubated at 50^{o}C for min. Standard was also run in the same fashion with triolein instead of plasma. The colour developed was read at 405 nm against the reagent blank.

Estimation of HDL-Cholesterol (HDL-C)

Determination of plasma HDL-Cholesterol was carried out by the method of Burstein *et al.,* (1970).

Plasma (0.5 ml) was taken in a centrifuge tube and to this added 0.25 ml of Phosphotungstic acid reagent and 0.25 ml of $MgCl_2$ and was centrifuged at 1500 x g for 30 min in a refrigerated centrifuge and the amount of cholesterol was determined in the supernatant by the method of Zlatkis *et al.,* (1953).

Estimation of VLDL and LDL cholesterol (VLDL-C and LDL-C)

By using Freidwald formula the concentration of VLDL and LDL cholesterol in serum were calculated.

LDL-C = (TC) - (HDL-C) - (TG/5)

Results and Discussion

This study investigates the phytochemical composition, antioxidant potential, and anti-hyperlipidemic effects of *Garcinia indica* fruit rind hydroalcoholic extract in a diet-induced hyperlipidemic rat model. The results demonstrate significant bioactivity of the extract in terms of lipid regulation and antioxidant effects.

The hydroalcoholic extract of *Garcinia indica* revealed the presence of several important phytochemicals, including alkaloids, glycosides, flavonoids, steroids, and phenols. These compounds are well-known for their potential therapeutic properties, such as antioxidant, anti-inflammatory, and lipid-lowering effects. The presence of flavonoids and phenolic compounds, quantified at 0.658 mg/100 mg and 0.445 mg/100 mg, respectively, supports the antioxidant potential of the extract. The phytochemical screening confirms that these constituents are likely to contribute to the observed health benefits, especially in terms of antioxidant and lipid-lowering activities.

The antioxidant activity of the hydroalcoholic extract was evaluated through its ability to inhibit free radicals. Although the extract showed promising antioxidant activity, it was less potent compared to ascorbic acid. The IC50 value for the hydroalcoholic extract was 45.61 µg/ml, while ascorbic acid had an IC50 of 12.60 µg/ml. However, at higher concentrations, the extract exhibited significant inhibition of free radicals, indicating its potential as a natural antioxidant. This activity is likely due to the presence of phenolic compounds and

flavonoids, which are known for their role in scavenging free radicals and reducing oxidative stress.

The study also evaluated the impact of *Garcinia indica* on food intake to ensure that the extract did not adversely affect feeding behavior. The results showed no significant changes in food intake across the different treatment groups, suggesting that the extract does not alter appetite or cause malnutrition. This is an important finding, as it indicates that the lipid-lowering effects of *Garcinia indica* can be achieved without impacting normal feeding patterns, which is often a concern with other pharmacological treatments.

The analysis of serum lipid profiles revealed elevated levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in the hyperlipidemic control group, with a decrease in high-density lipoprotein cholesterol (HDL-C). Treatment with *Garcinia indica* extract at doses of 100 mg/kg and 200 mg/kg significantly reduced TC, TG, and LDL-C, while stabilizing HDL-C levels. These findings suggest that *Garcinia indica* has a lipid-lowering effect, possibly through mechanisms such as inhibition of cholesterol synthesis or enhancement of cholesterol excretion.

An interesting observation in this study was the increased fecal cholesterol and bile acid excretion in the treatment groups. Specifically, the groups treated with 200 mg/kg and 300 mg/kg of *Garcinia indica* extract showed significantly higher fecal cholesterol excretion compared to the hyperlipidemic control group. This suggests that the extract may help in the elimination of excess cholesterol via the feces, which is a crucial mechanism for reducing cholesterol levels in the body. Increased bile acid excretion was also observed, further supporting the idea that the extract aids in cholesterol management.

Table 2: Extractive values obtained from *Garcinia indica*

S. No.	Extracts	Time of extraction	Color	% Yield
1	Petroleum ether	12 Hours	Brown	6.50%
2	Hydroalcoholic	12 Hours	Bark brown	14.65%

Table 3: Preliminary phytochemical screening *Garcinia indica* extract

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	Present
		Dragendorff's Test	Present
2	Glycosides	Raymond's Test	Present
		Killer Killani Test	Present
3	Carbohydrates	Molisch's Test	**Absent**
		Fehling's Test	**Absent**
4	Tannins	Vanillin- HCl Test	Present
		Gelatin Test	**Absent**
5	Flavonoids	Lead acetate	Present
		Shinoda Test	Present
6	Resins	Color detection with ferric chloride	**Absent**
		Turbidity Test	**Absent**
7	Steroids	Libermann- Bur chard Test	Present
		Salkowski Reaction	Present

8	Proteins & Amino acids	Biuret Test	Present
		Precipitation test	**Absent**
		Ninhydrin Test	Present
9.	Phenols	Ellagic Acid Test	Present

Table 4: Estimation of total phenol and flavonoids content of *Garcinia indica*

S. No.	Total phenol content	Total flavonoids content
	mg/ 100 mg of dried extract	
1.	0.445	0.658

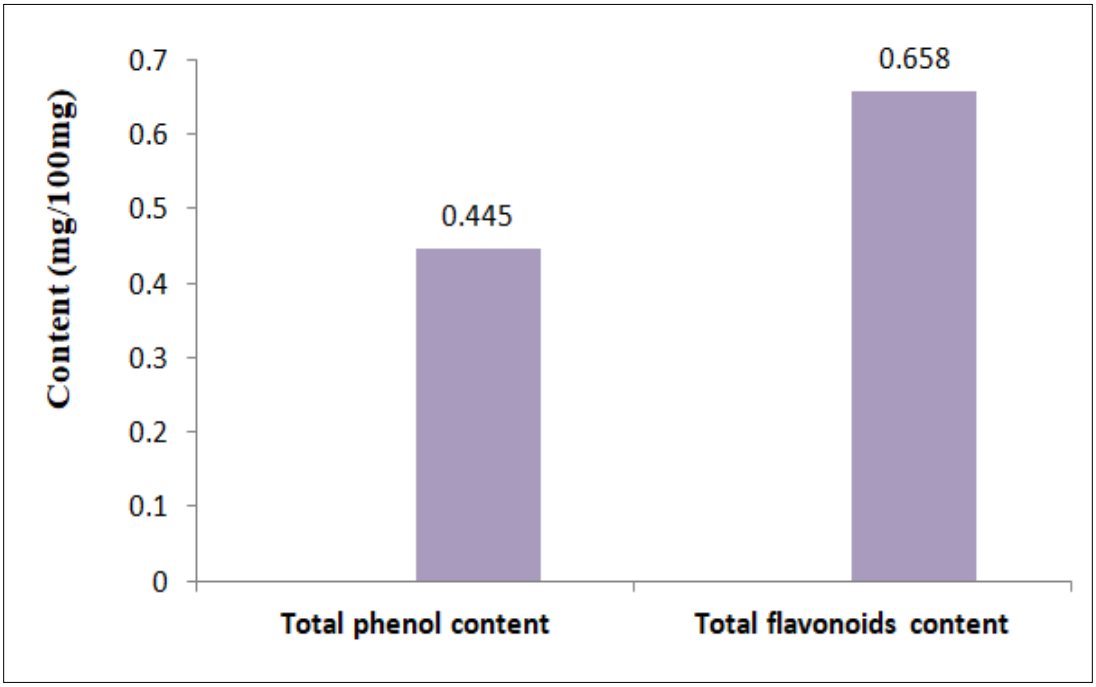

Figure 1: Estimation of total phenol and flavonoids content

Table 5: % Inhibition of ascorbic acid and Hydroalcoholic extract of *Garcinia indica*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract of *Garcinia indica*
1	10	46.58	30.25
2	20	49.98	39.98
3	40	69.98	48.87
4	60	71.12	56.65
5	80	79.98	68.85
6	100	86.65	72.23
IC 50		**12.60**	**45.61**

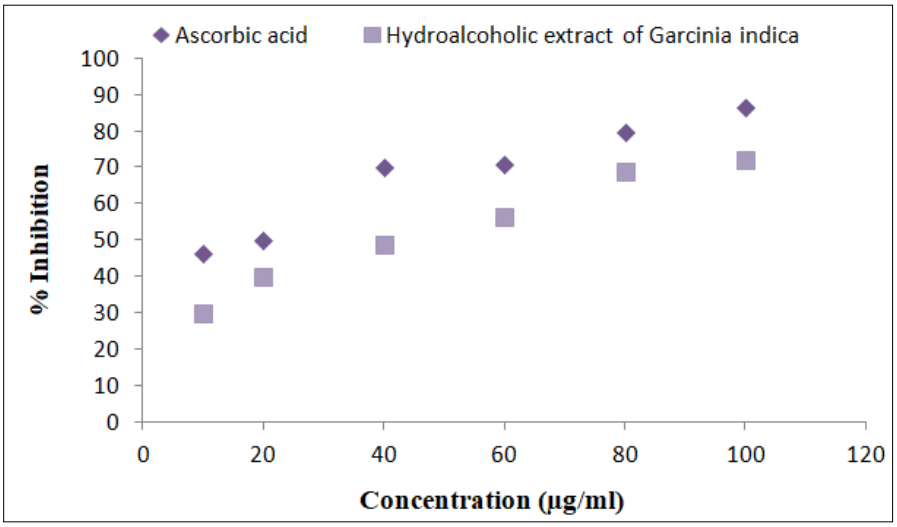

Figure 2: % Inhibition of ascorbic acid and Hydroalcoholic extract of *G. indica*

Table 6: Effects of different treatments on food intake of diet-induced hyperlipidimic rats

Group (n = 6)	Daily food intake (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Normal	20.75±0.32	21.59±0.21	20.85±0.63	22.10±0.15	21.80±0.33	21.74±0.14	21.74±0.22
Hyperlipidimic control	20.95±0.25	20.75±0.14	20.65±0.50	20.55±0.25	21.65±0.25	21.5±0.25	20.45±0.14
Atorvastatin	20.02±0.22	20.48±0.25	21.12±0.36	21.03±0.41	22.77±0.14	20.41±0.33	22.57±0.32
Treated with HAGI 100mg/kg	20.33±0.32	21.03±0.36	21.66±0.14	20.17±0.32	22.65±0.25	22.15±0.25	22.21±0.25
Treated with HAGI 200mg/kg	21.45±0.45	22.07±0.41	20.44±0.22	22.53±0.25	20.2±0.32	20.19±0.14	20.30±0.32

Note. All values represent mean ± SEM from six animals. Statistical analysis was carried out using one-way ANOVA followed by Tukey's test. $p<0.05$ was considered statistically significant.

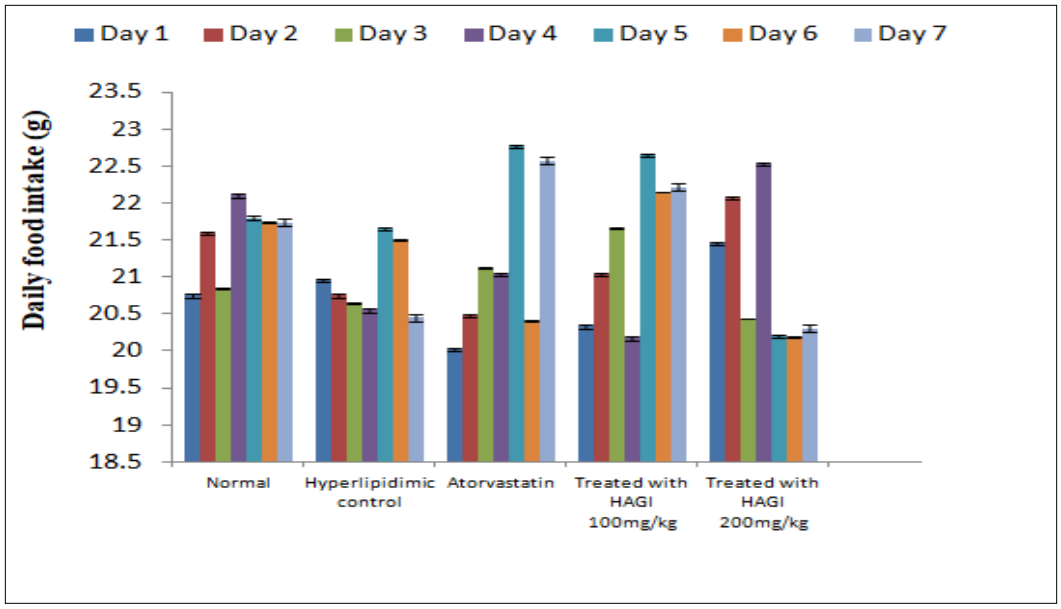

Figure 3: Effects of different treatments on food intake of diet-induced hyperlipidimic rats

Table 7: Effect of Garcinia indica fruits rinds extract on serum lipid profile of diet-induced hyperlipidemia in rats

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal	138.74±1.47	110.45±4.54	70.45±1.32	62.85±2.45	29.85±0.83
Hyperlipidemic control	337.23±7.20*	315.70±12.57*	113.45±3.54*	172.45±9.58	68.45±2.52*
Atorvastatin	172.45±6.37**	171.85±1.82**	75.00±2.47	71.60±6.48**	41.50±0.39**
Treated with HAGI 100mg/kg	251.23±3.21**	118.45±2.11**	75.58±2.41	162.15±3.61	30.56±0.42**
Treated with HAGI 200mg/kg	208.45±8.91**	131.65±5.43**	75.30±2.31†	117.30±7.73**	27.25±1.09**

Note. All values represent mean ± SEM from six animals. *Compared with normal group ($p<0.05$), **compared with hyperlipidemic control group ($p<0.05$), † significant reduction compared with hyperlipidemic control group ($p<0.05$). TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

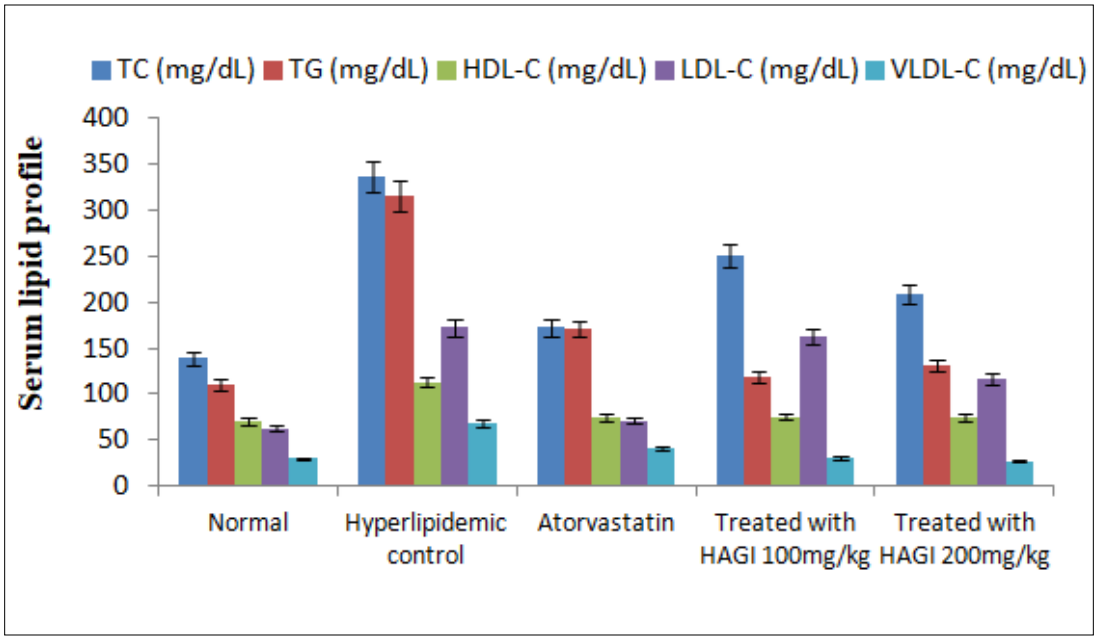

Figure 4: Effect of *Garcinia indica* Fruits rinds extract on serum lipid profile of diet-induced hyperlipidemia in rats

Table 8: Effect of *Garcinia indica* extract on fecal cholesterol and bile acid excretion in diet-induced hyperlipidimic rats

Group	Fecal cholesterol (mg/g of fecal matter)	Fecal bile acid‡ (mg/g of fecal matter)
Normal	2.10±0.09	1.35±0.08
Hyperlipidimic control	4.25±0.07*	1.10±0.05
Atorvastatin	3.75±0.07	2.60±0.06
Treated with HAGI 200mg/kg	5.85±0.13**	4.95±0.04**
Treated with HAGI 300mg/kg	9.50±0.13**	2.92±0.10**

Note. All values represent mean ± SEM from six animals. *Compared with normal group ($p<0.05$), **compared with hyperlipidimic control group ($p<0.05$), a compared with atorvastatin , ‡ as cholic acid equivalent.

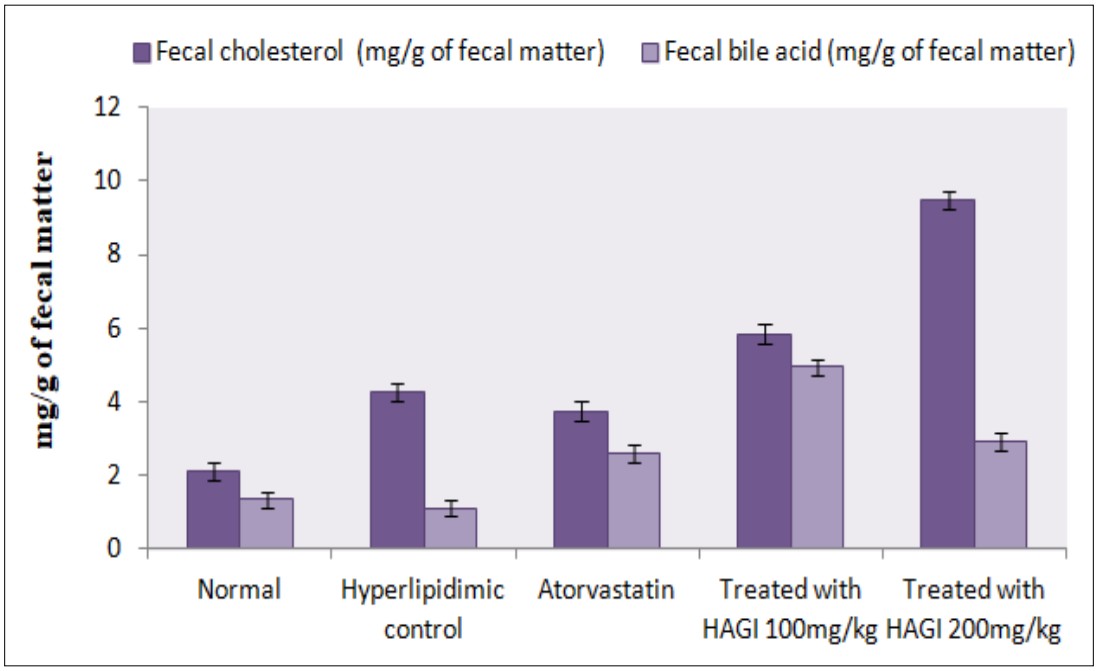

Figure 5: Graph of fecal cholesterol and bile acid

Conclusion

In conclusion, the hydroalcoholic extract of *Garcinia indica* demonstrated significant antioxidant and anti-hyperlipidemic effects in a rat model. The extract reduced lipid levels by improving the lipid profile, promoting fecal cholesterol and bile acid excretion, and exhibiting antioxidant activity. These results suggest that *Garcinia indica* could be a promising natural supplement for managing hyperlipidemia and promoting cardiovascular health, without causing adverse effects on feeding behavior. Further studies are needed to explore the underlying mechanisms of action and to confirm the clinical efficacy of the extract in human subjects.

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