EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF FRUIT RIND EXTRACTS OF GARCINIA GUMMI-GUTTA IN DIET-INDUCED HYPERLIPIDEMIC RATS

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INTRODUCTION

Cardiovascular disease (CVD) was previously assumed to be a problem of developed and industrialized nations, but over the past few decades, it has slowly infiltrated into the low and middle-income countries where it accounts for nearly 30% of all deaths [1]. In India, the prevalence of CVD has grown exponentially and accounts for nearly 24% of all adult deaths [2]. Reports submitted by the National Commission on Macroeconomics and Health have estimated that there would be 62 million patients with coronary artery disease by 2015 and of these 23 million would be people younger than 40 years [3].

The umbrella term CVD has multifactorial etiopathogenesis and its prevalence is attributed to lifestyle changes due to urbanization, stress, lack of physical activity, nutritional transitions, and other metabolic disorders such as diabetes and hypothyroidism.

For decades, dyslipidemia has been identified as an independent modifiable risk closely associated with the incidence of CVD. Furthermore, increased levels of low-density lipoprotein (LDL) with a concurrent decrease in high-density lipoprotein (HDL) contribute to the process of atherogenesis and evolution of atheromatous plaque [4].

Conventional preparations of lipid-lowering drugs such as statins, fibrates, bile acid sequestrants, and niacin are efficient in lowering serum lipids but not always free of side effects [5].

For many years plants and plant products have been identified as a potentially safer alternative to synthetic drugs. Despite such intensive research on various herbal medicines, many species of plants are still left unexplored.

Garcinia gummi-gutta also known as Garcinia cambogia is a diminutive purple fruit native to India and Southeast Asia. The fruit rinds find its use as a food preservative, flavoring agent, and parasitic infestations [7]. Although many of its biological effects have been explored to depth, still satisfactory studies to exploit its hypolipidemic activity have not been conducted. Therefore, the present study was planned to evaluate the hypolipidemic activity of G. gummi-gutta fruit extract using animal models.

METHODS

Collection and identification of plant materials

The fruit of G. gummi-gutta was collected during the winter season in the month of August from Palode area of Thiruvananthapuram district, Kerala, India, and was authenticated by a local botanist from Science College. The fruit rinds weighing 5 kg were separated along the grooves and deseeded. They were later cleaned, shade dried, pulverized, and stored in airtight containers until the commencement of the extraction process.

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Preparation of rind extract
Hydroalcoholic extract was prepared as per the procedure elaborated by Mahanta and Mukherjee [8]. About 40–45 g of dried powder was packed in the thimble of Soxhlet apparatus, and extraction was carried out continuously with 95% ethanol refluxing at 50–70°C which yielded a dark brown sticky mass. The extract was concentrated and dried using a ventilated oven at 45°C for 24 h. The stock powder was stored in a glass desiccator at 4°C. The total yield per cycle was around 15% (~5 g) per cycle.

Chemicals
Pure cholesterol and cholic acid were obtained from Loba Chemie Pvt., Ltd., Mumbai. Atorvastatin (Lipvas) of Cipla Company, Mumbai, was procured from a local medical store. 2-thiobarbituric acid was purchased from Sigma Aldrich, Bengaluru. All other solvents and chemicals used in the study were of analytical grade and purchased from SD fine - chem. Mumbai.

Ethical clearance
Ethical clearance was taken from Institutional Animal Ethics Committee of the institute before the commencement of the study.

Animals
Adult male Wistar albino rats 10–12 weeks of age weighing 180±20 g were procured from King’s Institute, Guindy, Chennai. They were housed and maintained in the central animal house of the institute 2 weeks before the commencement of the study so that they could adapt to the new environment. Animals were kept in polypropylene cages with dry paddy husk bedding maintaining a room temperature of 26±2°C and relative humidity of 45–55% with 12:12 h dark and light cycle. They were fed with standard pellet diet and water ad libitum. The nutritional value of the standard pellet diet was 21% of protein, 5% of lipids, 4% of crude fiber, 8% of ash, 1% of calcium, 0.6% of phosphorus, 3.4% of glucose, 2% of vitamins, 55% of carbohydrates, and produced energy of 3600 kcal/kg. Care of the animals in the central animal house was strictly in accordance with the guidelines drafted by the committee for the purpose of Control and Supervision of Experiments on Animals, New Delhi, India.

Acute toxicity testing
A pilot study was performed using two doses 1000 mg/kg body weight and 2000 mg/kg body weight of the fruit rind extract of G. gummi-gutta. The doses selected were 10 and 20 times more than the standard pellet diet+1% CMC

Dose selection
As the limit dose did not exhibit any signs of toxicity, a dose of 200 and 100 mg/kg, p.o., which was 1/10th and 1/20th of 2000 mg/kg, was taken up for the main study. Atorvastatin at a dose of 10 mg/kg was taken as standard control.

Hyperlipidemic diet (HLD)
Method of Blank with modification was used to produce diet-induced hyperlipidemia. HLD was prepared daily by mixing standard pellet diet (92%), cholesterol (2%), cholic acid (1%), and coconut oil (5%) and was molded into pellets [9].

Experimental design
A total of 30 healthy adult Wistar albino rats were used in the study. The total duration of the study was 8 weeks. Following acclimatization for 1 week, a baseline measurement of lipid profile was performed for all the rats. In an attempt to eliminate bias, the rats were numbered and grouped based on random numbers generated using computer software.

Induction of hyperlipidemia
The study consists of five groups with six rats in each group (n=6). Except for Group 1 which received standard pellet diet and water ad libitum, all the remaining four groups received HLD for a period of 30 days.

On day 30, the lipid profile of the rats in all the four groups was estimated and induction of hyperlipidemia was confirmed.

Following successful induction, the rats belonging to those four groups were treated with the extracts, and the standard drug between 9 and 10 am daily for another 30 days suspended in 1% carboxymethylcellulose and administered using a steel gavage needle (Table 1).

Estimation of lipid profile
Blood samples for the estimation of lipid profile were taken from the lateral tail vein as per method elaborated by Diehl et al. [10]. Blood samples were collected on day 0 (baseline) and thereafter on 30th, 45th, and 60th day. Serum was separated by centrifugation at 3000 rpm for 10 min. It was then kept frozen at −20°C until analysis. Total cholesterol (TC) level was calculated by the enzymatic method and expressed in mg/dl. HDL levels were calculated using polyanion precipitation and expressed as mg/dL. LDL cholesterol level was calculated using Friedewald’s equation and expressed in mg/dl. Triglycerides (TG) in serum were converted to glycerol and then estimated using glycerol kinase enzyme based kinetic method and expressed in mg/dl. Very LDL is calculated using the formula:

\[
\text{VLDL} = \frac{\text{TG}}{5}
\]

At the end of 8 weeks, all the rats were sacrificed by an overdose of sodium pentobarbital 150 mg/kg i.p. Heart and liver were dissected and washed with the physiological salt solution and fixed with 10% formalin.

Thiobarbituric acid reactive substances (TBARS) assay
About 1 ml of serum was mixed with 500 μl of 15% trichloroacetic acid in 0.25 N hydrochloric acid (HCl) and 500 μl of 0.375% thiobarbituric acid in 0.25 N HCl. This was shaken well to mix thoroughly and was then heated in a boiling water bath at 95°C for 15 min. After bringing it down to room temperature, it was centrifuged at 1000 r.p.m for 10 min and the reddish-pink supernatant was collected. Absorbance was determined using Labman double beam UV-visible spectrophotometer (LMSP-UV1900) tuned to a wavelength (λ) at 535 nm against a blank that contained all the reagents minus the serum [11]. The concentration of malondialdehyde (MDA) in the serum was calculated using molar extinction coefficient 1.56 M/cm, and the results were expressed in μmol/ml of plasma [12].

Histopathology
The liver and heart fixed in 10% formalin were washed, dehydrated in descending grades of isopropanol and finally rinsed with xylene. The tissues were then embedded in molten paraffin wax. Sections were cut using a microtome at a thickness of 5 μm and then stained with

<table>
<thead>
<tr>
<th>Group</th>
<th>Purpose</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal control</td>
<td>Standard pellet diet+1% CMC</td>
</tr>
<tr>
<td>Group 2</td>
<td>HLD control</td>
<td>HLD+1% CMC</td>
</tr>
<tr>
<td>Group 3</td>
<td>REGG 100</td>
<td>HLD+rind extract of G. gummi-gutta at 100 mg/kg</td>
</tr>
<tr>
<td>Group 4</td>
<td>REGG 200</td>
<td>HLD+rind extract of G. gummi-gutta at 200 mg/kg</td>
</tr>
<tr>
<td>Group 5</td>
<td>Standard group</td>
<td>HLD+Atorvastatin at 10 mg/kg</td>
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</tbody>
</table>

HLD: Hyperlipidemic diet, REGG100: Rind extract of Garcinia gummi-gutta at 100 mg/kg, REGG200: Rind extract of Garcinia gummi-gutta at 200 mg/kg, G. gummi-gutta: Garcinia gummi-gutta, CMC: Carboxymethylcellulose
hematoxylin and eosin [13]. The sections were observed under light microscope at a magnification of ×10 (Adavision Trinocular Digital Microscope) for histopathological changes.

**Statistical analysis**

Collected data were entered in Microsoft Excel 2013 and analyzed using SPSS statistical software version 17. Results were expressed using mean±standard error of the mean as tables. Statistical analysis was performed using one way ANOVA followed by post hoc Tukey’s test. The p<0.05 was considered statistically significant at 95% confidence interval.

**RESULTS**

**Acute toxicity study of REGG in rats**

Acute toxicity studies confirmed that the fruit rind extract of *G. gummi-gutta* up to a dose of 2000 mg/kg body weight was non-toxic. No death was observed for up to 72 h. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study.

**Effect of the rind extract of *G. gummi-gutta* on serum lipid levels**

The serum levels of TC, TG, VLDL, and LDL were significantly increased, while the levels of HDL were significantly decreased when compared to normal control. The groups which received *G. gummi-gutta* rind extract at 100 mg/kg and 200 mg/kg showed a significant decrease in the mean cholesterol, TG, LDL, VLDL levels, and an increase in HDL levels when compared to the HLD group. The group which received the extract at higher dose (REGG200) was found better than the lower dose (REGG 100). REGG 200 showed significant improvement in increasing the HDL levels than all the test groups.

The standard drug atorvastatin was found better than REGG100, but there was no significant difference between a high dose of *G. gummi-gutta* rind extract and atorvastatin. REGG 200 mg/kg showed a marginal increase in HDL levels than the standard drug (Tables 2-6).

**Effect of the rind extract of *G. gummi-gutta* on TBARS levels**

Serum TBARS levels were significantly higher (3.42±0.04 nmol/ml) in rats treated with HLD. Hyperlipidemia significantly increased lipid peroxidation with the generation of reactive oxygen species due to decreased innate antioxidant activity. The groups treated with the standard drug atorvastatin and the groups treated with REGG at both doses showed a significant decrease in TBARS levels (p<0.001) when compared to HLD control. The concentration of MDA in REGG 200 (1.03±0.01 nmol/ml) was significantly lower when compared with other groups (Fig. 1).

### Table 2: Effect of fruit rind extract of *Garcinia gummi-gutta* on total cholesterol levels

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Total cholesterol levels (mg/dl)</th>
<th>Drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induction of hyperlipidemia</td>
<td>Day 0 baseline</td>
</tr>
<tr>
<td>Group 1: Normal control</td>
<td>62.67±1.50</td>
<td>61.67±1.73</td>
</tr>
<tr>
<td>Group 2: HLD control</td>
<td>64.00±0.97</td>
<td>128.83±1.08</td>
</tr>
<tr>
<td>Group 3: REGG 100</td>
<td>65.33±0.71</td>
<td>131.83±1.17</td>
</tr>
<tr>
<td>Group 4: REGG 200</td>
<td>64.17±1.17</td>
<td>129.33±0.49</td>
</tr>
<tr>
<td>Group 5: Standard control</td>
<td>65.83±1.66</td>
<td>129.00±1.18</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM. n=6 in each group. Significance at *p<0.05, **p<0.001 as compared to HLD control. SEM: Standard error of mean

### Table 3: Effect of fruit rind extract of *Garcinia gummi-gutta* on triglyceride levels

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Triglyceride levels (mg/dl)</th>
<th>Drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induction of hyperlipidemia</td>
<td>Drug administration</td>
</tr>
<tr>
<td></td>
<td>Day 0 baseline</td>
<td>Day 30</td>
</tr>
<tr>
<td>Group 1: Normal control</td>
<td>79.83±1.25</td>
<td>79.67±1.28</td>
</tr>
<tr>
<td>Group 2: HLD control</td>
<td>83.83±0.87</td>
<td>182.50±1.18</td>
</tr>
<tr>
<td>Group 3: REGG 100</td>
<td>81.67±0.88</td>
<td>181.50±1.12</td>
</tr>
<tr>
<td>Group 4: REGG 200</td>
<td>81.17±1.08</td>
<td>179.83±1.30</td>
</tr>
<tr>
<td>Group 5: Standard control</td>
<td>83.50±0.76</td>
<td>180.50±0.76</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM. n=6 in each group. Significance at *p<0.05, **p<0.001 as compared to HLD control. SEM: Standard error of mean

### Table 4: Effect of fruit rind extract of *Garcinia gummi-gutta* on low-density lipoprotein cholesterol levels

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>LDL levels (mg/dl)</th>
<th>Drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induction of hyperlipidemia</td>
<td>Drug administration</td>
</tr>
<tr>
<td></td>
<td>Day 0 baseline</td>
<td>Day 30</td>
</tr>
<tr>
<td>Group 1: Normal control</td>
<td>18.53±2.06</td>
<td>17.57±1.98</td>
</tr>
<tr>
<td>Group 2: HLD control</td>
<td>22.23±1.17</td>
<td>63.83±2.17</td>
</tr>
<tr>
<td>Group 3: REGG 100</td>
<td>22.17±1.31</td>
<td>66.53±1.74</td>
</tr>
<tr>
<td>Group 4: REGG 200</td>
<td>21.77±1.04</td>
<td>65.87±1.59</td>
</tr>
<tr>
<td>Group 5: Standard control</td>
<td>23.13±1.67</td>
<td>62.90±1.77</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM. n=6 in each group. Significance at *p<0.05, **p<0.001 as compared to HLD control. SEM: Standard error of mean
Effect of the rind extract of *G. gummi-gutta* on liver histopathology

Histology of liver section of normal control exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus, and nucleoli with well defined central vein and sinusoids (Fig 2a). Rats which received HLD showed micro fatty deposits in the hepatocytes, congestion with mild distortion of the architecture, and early feathery degeneration (Fig 2b). Hyperlipidemic rats which received REGG 100 showed mild cytoplasmic fatty deposits and periportal leucocyte infiltration (Fig 2c), whereas hyperlipidemic rats which received REGG 200 showed mild periporal inflammation with infiltration of lymphocytes with well-preserved liver architecture (Fig 2d).

Effect of the rind extract of *G. gummi-gutta* on myocardial histopathology

Histopathological sections of the heart did not show significant changes. Fig 3a shows normal histology of myocardial cells. Oval-elongated nucleus centrally surrounded with homogenous cytoplasm.
In our study, treatment with REGG led to a significant elevation of plasma HDL-cholesterol (HDL-C) in spite of daily HLD feed. A proportionate increase in HDL levels was observed at the end of 6 weeks with the extracts given at both doses. However, by the end of 8 weeks, the levels of HDL in Group 4 showed significant increase than the standard drug.

Of late, the crucial risk factor for CVD has been identified to be a low level of HDL-C. Decreased levels of HDL-C play a direct role in the atherogenesis, and therapeutic interventions promoting HDL-C levels is widely encouraged. HDL by counteracting LDL oxidation prevents atherogenesis and, recent studies suggest that HDL promotes the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of oxidized-LDL [18]. Furthermore, HDL not only inhibits the oxidation of LDL but also prevents the 12-lipoxygenase-mediated formation of lipid hydroperoxides [19]. Based on this, our results clearly suggest that fruit rind extract of *G. gummi-gutta* may probably exert their antiatherogenic role, partly by the inhibition of lipids oxidation (evident by reduction in the TBARS levels), and partly by the elevation of HDL-C.

For ages, LDL-C has been identified as a potential target in CVD risk reduction therapy. Elevated LDL levels augmented with oxidative stress increase the risk of atherosclerosis and CVD [20]. In our study, significant reduction in LDL levels was observed with REGG administered at both the doses. However, values obtained were comparable to that of the standard control following administration of the extracts at higher doses. The biocompound HCA may probably be responsible for the reduction in LDL levels [21].

Most of the findings, in our study, were inconsistent with previous works conducted by Mahendran and Devi who in his study observed that the rind extract of *G. cambogia* at 1000 mg/kg/day for 8 days showed notable hypolipidemic activity on dexamethasone-induced hyperlipidemic rats. He also found that the extract was capable of normalizing increased levels of cholesterol and TG as well as free fatty acid following dexamethasone administration [22]. Similar to the above study, Ramos et al. observed that supplementation of the active principle HCA significantly increased HDL-C levels with a decrease in LDL levels [21]. Preuss et al. showed that HCA salt at 4.7 g/day in moderately obese subjects was capable of reducing food intake, TC, TG, and LDL levels while significantly increasing the levels of HDL and urinary excretion of fat metabolites [23]. Koshy et al. concluded that the flavonoid-rich rind extract exhibited hypolipidemic effect probably by lowering lipid turnover and increased metabolism [24].

The observation done by Leonhardt et al. was contradicting with our study findings, which showed supplementation of 3% HCA did not result in decreased cholesterol levels [25]. Brandt et al. (2006) noted HCA (1.8%) supplementation for 4 weeks resulted in an unexpected increase in liver lipid and postprandial cholesterylamine [26]. This may be due to an insufficient dose of HCA, which has failed to lower the serum lipids levels.
Oxidative stress induced by HLD is associated with peroxidation of both cellular and circulating lipids which results in the formation of MDA. Quantifying this MDA will positively reflect the severity of an ongoing oxidative damage treatment with fruit rind extract of *G. gummi-gutta* significantly reduced the TBARS level in a dose-dependent manner. The increase in the MDA level is indicative of ongoing oxidative damage was due to oxidative stress induced by HLD [27]. In our study, a significant reduction in TBARS values was observed following treatment with the extract, wherein the maximum reduction was evident with a higher dose of the extract. Thus, it is evident that the rind extract possesses antioxidant properties at both doses, wherein the extract at higher dose exhibited strong antioxidant action compared to other groups. Our study findings were consistent with previous studies conducted by Kolodziejezyk et al. who in his study concluded that polyisoprenylated benzophenones such as garcinol and guttiferone K effectively reduced lipid peroxidation of LDL residues (reduced TBARS values), thereby indirectly counteracting oxidative stress in hyperlipidemia [28].

In our study, most of the histopathological findings were consistent with the obtained biochemical parameters. The fruit rind extract at both doses significantly reduced micro fatty deposits, periporal leukocytic infiltration, and disrupted architecture with higher doses showing evidence of hepatocellular recovery. REGG groups at both doses failed to show significant pathological changes in the myocardial cells. This may be due to the fact that hyperlipidemia primarily involves the vascular system and myocardial involvement in hyperlipidemia may be a late phenomenon. In our study, the fruit rind extracts at both the doses have effectively protected the myocardial cells from even the minor toxic insults of lipid peroxidation (signs of inflammation and fatty degeneration).

**CONCLUSION**

The present study demonstrated the hypolipidemic activity of *G. gummi-gutta*. The fruit rind extract at both doses was able to decrease the elevated serum lipids (TC, TG, LDL, and VLDL) and increase the HDL levels in a dose-dependent manner in spite of daily administration of HLD. This reduction in lipid profile and TBARS may be probably due to the presence of HCA and other antioxidants in the extracts. Hence, further studies are warranted both in animals and humans before recommending for the clinical use in humans in the prevention and treatment of hyperlipidemia.

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**AUTHORS’ CONTRIBUTIONS**

Dr. Barathane - conceptualized the study, compiled full literature search, and drafted the manuscript. Dr. Kartik J. Salwe - developed standards and edited the manuscript. Dr. Manimekalai - supervisor for search, and drafted the manuscript. Dr. Kartik J. Salwe - developed support. We would like to acknowledge Dr. Dhananjay S. Kotasthane, Professor and Head and Dr. Vaishali D. Kotasthane, Assistant professor, Department of Pathology, and Mr. Kulanthaivel, Lecturer, Department of Biochemistry, MGMRCRI, Pondicherry, India, for their valuable help and support.

**REFERENCES**


17. Offermanns F. Free fatty acid (FFA) and hydroxy carboxylic acid (HCA) receptors. Annu Rev Pharmacol Toxicol 2014;54:407-34.


25. Leonhardt M, Münch S, Westerterp-Plantenga M, Langhans W. Effects of hydroxycitrate, conjugated linoleic acid, and guar gum on food