



# An Evaluation of Anti-hyperlipidemic Activity of Ethanolic Extract of *Coccinia grandis* Leaves in High Fat Induced Rodent Model

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

In the realm of health research, hyperlipidemia is a condition that has lately become prevalent and is a serious issue. Elevated lipid levels in an individual's body are the primary indicator of hyperlipidemia, a condition defined by increased levels of low-density lipoprotein (LDL), total cholesterol, triglycerides, and high lipoprotein levels. Several pharmacological agents are accessible for the treatment of hyperlipidemia. Nevertheless, human beings throughout the globe also thrive on nature-derived therapeutic items. *Coccinia grandis* is a noteworthy plant known for its

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antihyperlipidemic properties among them. In this study, we provided a high-fat diet for the experimental rats to generate hyperlipidemia conditions in this rat model. As per our research methodology, *Cocconia grandis* was administered to all the rats in the treatment group at three (low, medium and high) distinct dosages. It is eventually revealed that *Cocconia grandis* may successfully lower ( $p < 0.05$ ) the hyperlipidemic state in both medium and high dosages. Elevated and unusual Triglyceride and Total Cholesterol were observed to be restored following *Cocconia grandis* medication. However, in the case of HDL and LDL, no substantial drop was detected. A considerable restoration was noted similarly in both instances of SGPT and SGOT. These findings show that our plant has components with antihyperlipidemic action, however the concentrations did not meet the necessary threshold. So, additional investigations are needed to examine the chemical structure and the quantities of the molecule as well as find out about genetic alterations.

**Keywords:** *Hyperlipidemia; Cocconia grandis; HDL; LDL natural product.*

## 1. INTRODUCTION

Increased plasma levels of triacylglycerols and cholesterol are referred to as hyperlipidaemia. The term "hyperlipidaemia" refers to a group of inherited and acquired illnesses characterized by increased amounts of lipids in a person's body. A more objective definition of hyperlipidaemia would be defined as having low-density lipoprotein (LDL), total cholesterol, triglyceride, or lipoprotein levels that are higher than the 90th percentile relative to the general population or having HDL levels that are lower than the 10th percentile. Cholesterol levels, very low-density lipoprotein (VLDL), chylomicrons, low-density lipoprotein (LDL), lipoproteins, apolipoproteins, and high-density lipoprotein (HDL) are all lipid instances. Hyperlipidaemia and hyperlipoproteinemia are synonymous, denoting that triacylglycerols and cholesterol must be included in lipoproteins in plasma. Postprandial hyperlipidaemia, the most prevalent kind of hyperlipidaemia, is mostly caused by elevated chylomicron levels and is seen after an animal has eaten a meal high in fat. Blood samples from fasting animals must be obtained to evaluate potential anomalies in lipid metabolism and to prevent misunderstanding brought on by postprandial hyperlipidaemia [1,2]. It is a significant risk factor for stroke and heart disease in nations with both high and low incomes. The WHO estimates that elevated cholesterol is responsible for one-third of ischemic heart disease worldwide. 2.6 million fatalities (4.5% of all deaths) and 29.7 million DALYs, (Disability-adjusted life years), or 2% of total DALYs, are predicted to be caused by elevated cholesterol overall. Globally, 39% of individuals ( $\geq 5.0$  mmol/L) had elevated total cholesterol in 2008 (37% of men and 40% of women) [3].

Various lipid lowering agents, in short Antihyperlipidemic medications are available in markets to reduce blood levels of several lipids, including cholesterol. They are classified based on their mechanism of actions, like, HMG-CoA Reductase Inhibitors, known as statins: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, simvastatin; Bile Acid Sequestrants: cholestyramine, colestevlam, colestipol; Cholesterol Absorption Inhibitor: ezetimibe; Fibrates: fenofibrate, fenofibric acid, gemfibrozil; Nicotinic acid: niacin [4].

Many synthetic hypolipidemic medications are now on the market and work well, but their usage is greatly restricted by the adverse effects they could trigger. Currently, a wide range of adverse effects have been linked to allopathic antihyperlipidemic medications which include myositis, diarrhea, nausea, and abnormal liver function. For example, synthetic drugs such as statins are used as antihyperlipidemic agents. However, long-term statin uses damages the kidneys and liver along with checking for atherosclerotic lesions already present. Because of the serious adverse effects, using statins appears improbable. In addition, certain individuals exhibit resistance or intolerance towards traditional medication. Alternative strategies are therefore desperately needed, and plant-based medicines are gaining a lot of attention because conventional medicinal practices, which mostly use plants, herbs, and shrubs, have long been important to the world health system. They are being particularly appealing since they effectively lower cholesterol levels. Moreover, herbal medications are becoming more and more in demand as natural products are often less harmful, have fewer side effects and are more readily available locally. Besides, the "backbone" of traditional medicine, medicinal plants are regarded as a beneficial

source of life for everyone because of its many therapeutic benefits of enormous compounds and natural makeup. They can be genetically modified to use it more beneficially and precisely for the needed specific therapeutic effects. So, medicinal plant can be a noteworthy replacement of available synthetic drugs [5-7].

Some indigenous plants having antihyperlipidemic potential such as seeds and leaves of *Trigonella foenum-graecum* L. (Fenugreek), leaf extract of *Cynara cardunculus* (Artichoke), *Achillea millefolium* (Yarrow), *Ocimum tenuiflorum* (Holy basil), *Zingiber officinale* (Ginger), *Coccinia grandis* (Ivy gourd), *Curcuma aromatica* (Turmeric), *Salvia rosmarinus* (Rosemary), *Hordeum vulgare* (barley), *Linum usitatissimum* (flaxseed), *Malus pumila* (apples), *Lens culinaris* (lentils), *Phaseolus vulgaris* L. (beans) etc have been utilized regularly and effectively to treat hyperlipidaemic problems according to valid scientific evidence obtained from the study of medicinal plants.

Among these *Coccinia grandis* demonstrated notable reductions in cholesterol and triglycerides [8]. *Coccinia grandis*, commonly known as Ivy gourd is a perennial vine or plant that grows widely in tropical zones of the globe from Africa to Asia specially in Pakistan, India, and Sri Lanka. The plant is a member of the Cucurbitaceae family. The roots, leaves, and fruits of this plant are traditionally used in folk medicine for a variety of ailments, including fever, asthma, coughing, burns, skin eruptive events oral sores, earaches, indigestion, eyesores, nausea, ulcers, jaundice, and wound healing. The hydroethanolic extract of *Coccinia grandis* leaves revealed the presence of flavonoids, phenols, tannins, saponins and terpenoids, according to a phytochemical evaluation. It has been stated that the leaf and its components have hypoglycemic, hypolipidemic, analgesic, antipyretic and anti-inflammatory and antioxidant qualities. [9-11]. Polyphenol-containing chloroform extract from *Coccinia grandis* leaves reduces the plasma lipid profile while boosting the ratio of total cholesterol to high-density lipid cholesterol. Leaf extracts, both aqueous and ethanolic, can be utilized to manage obesity [12]. Moreover, the methanolic extract derived from *Coccinia grandis* is found to exhibit a significant capacity to reinstate the marker enzymes, resulting in a near-normal restoration of the lipid profile [13]. Besides, treatment with *C. grandis* has been seen to

considerably decrease cholesterol levels, liver weight, and the accumulation of fat in the epididymis [14]. Our research focuses on evaluating herbal medicine as a means of improving the hyperlipidemia management system.

## 2. MATERIALS AND METHODS

### 2.1 Drugs, Chemicals, and Instruments

A gift sample of atorvastatin, an established anti-hyperlipidaemic drug, was obtained from Healthcare Pharmaceutical Limited, and ethanol was bought from Sigma Aldrich in Germany. UK-based Plasmatic Laboratory Product Ltd supplied the kits for measuring the total cholesterol level, HDL, LDL, triglycerides, SGOT, SGPT, and creatinine. The Humalyzer 3000, a semiautomated laboratory chemical analyzer manufactured in Cambodia by Medi Group Asia Limited, was utilized to assess the biochemical parameters. The required supplies for the high-fat diet were provided by supermarket purchasing.

### 2.2 Plant Collection and Extract Preparation

Leaves of *Coccinia grandis* were collected at the University of Dhaka's Faculty of Pharmacy's medicinal plant garden. Validation and taxonomic identification were accomplished. The plant specimen was kept in compliance with the rules at Bangladesh's National Herbarium. For future reference, the accession number \_\_\_56480\_\_\_, dated \_22\_-\_02\_-2023, was given by the herbarium authorities. The leaves were dried in the shade for seven to ten days before being thoroughly crushed. The leaf powder was vigorously shaken and immersed in 70% ethanol for 96 hours. Following the completion of the soaking process, the extract was filtered, and the resulting liquid was collected. A rotary evaporator was then used to concentrate the extracted solution. Lastly, the dried extract was gathered and preserved safely for further future uses.

### 2.3 Experimental Animal Handling

Male Wistar rats weighing between 125 and 150 grams were bred for fitness and kept in the University of Dhaka's Institute of Nutrition and Food Science. They were kept on a 12-hour light/dark cycle at a consistent temperature of 25°C. The rats were obtained from the

Department of Pharmacy of Jahangirnagar University in Dhaka, Bangladesh. They were regularly provided clean water and a typical pellet feed. The rats were kept there to adapt before the investigation started. Every experiment with rats was conducted in compliance with the guidelines set out by the Institutional Animal Ethics Committee (IEAC). The regulations of the Swiss Academy of Sciences (SCNAT) and the Swiss Academy of Medical Sciences (SAMS) were followed when treating and caring for the animals.

## 2.4 Experimental Guidelines

Every experiment was carried out under the 2013 Helsinki Declaration's ethical guidelines. Throughout the study, the rats received enough nutrition, and upon completion, they were humanely put to death under the influence of general anaesthesia, in compliance with the 2013 edition of the Guidelines for the Euthanasia of Animals.

## 2.5 Experimental Design

Rats were weighed individually and then divided into nine groups for the anti-hyperlipidaemic activity investigation (Table 1), with five rats in each group. The rodents were distributed evenly among the groups according to body weight. Since animals would perish if atorvastatin was the only medication utilized, Table 1 shows rats in the atorvastatin control group who were provided a high-fat diet along with the

medication. Rats in this group did not get any therapeutic treatment and was indicated by the letter N/A.

**High Fat Diet:** The composition provided by Levin and Dunn-Meynell was used to modify the high-fat diet. The percentages of fat in the high fat diet are 50% lipid, 40% carb, and 10% protein. Table 2 displays the content of the diet.

Rats were fed a high-fat diet to develop obesity for ten weeks after the components were well combined.

## 2.6 Biological Sample Collection

After the heart was sacrificed, blood samples were taken as soon as possible and placed in a microcentrifuge tube. The obtained samples were centrifuged for 5 minutes at 5,000 rpm to obtain the supernatant fluid. After that, the fluid was moved to another microcentrifuge tube so that it could be tested biochemically. After the animal was sacrificed, the liver and kidneys were quickly removed from the dead body and properly cleaned with ice-cold saline to assess the function of these organs.

## 2.7 Estimation of Biochemical Parameters

Liver, kidney function testing and lipid profile tests were conducted using the Humalyzer 3000.

**Table 1. Antihyperlipidemic activity analysis**

| Group number | Group Status                            | Treatment specimen & Dose  | Group Abbreviation |
|--------------|---|--|--------------------|
| 1            | Negative Control                        | Physiological Saline   | N                  |
| 2            | Positive Control                        | High Fat Diet  | P                  |
| 3            | High Fat Diet + Atrovastatin            | High Fat Diet + Atrovastatin                                       | HFD + ATV          |
| 4            | High Fat Diet + <i>Coccinia grandis</i> | High Fat Diet + <i>Coccinia grandis</i> leaves extract low dose    | HFD +              |
| 5            | High Fat Diet + <i>Coccinia grandis</i> | High Fat Diet + <i>Coccinia grandis</i> leaves extract medium dose | HFD +              |
| 6            | High Fat Diet + <i>Coccinia grandis</i> | High Fat Diet + <i>Coccinia grandis</i> leaves extract high dose   | HFD +              |
| 7            | <i>Coccinia grandis</i>                 | <i>Coccinia grandis</i> leaves extract low dose                    |                    |
| 8            | <i>Coccinia grandis</i>                 | <i>Coccinia grandis</i> leaves extract medium dose                 |                    |
| 9            | <i>Coccinia grandis</i>                 | <i>Coccinia grandis</i> leaves extract high dose                   |                    |

**Table 2. Composition of high fat diet**

| Food Ingredients   | Composition              |
|--------------------|--------------------------|
| Lipid (50%)        | Milk powder (10%)        |
|                    | Ghee (30%)               |
|                    | Mutton fat (40%)         |
|                    | Coconut oil (10%)        |
|                    | Butter (10%)             |
| Carbohydrate (40%) | Boiled rice (40%)        |
|                    | Smashed potato (40%)     |
|                    | Boiled corn (20%)        |
| Protein (10%)      | Dry powdered prone (40%) |
|                    | Dry boiled mutton (20%)  |
|                    | Cheese (20%)             |
|                    | Egg (20%)                |

### 2.8 Statistical Analysis

The "one-way ANOVA test" was utilized to examine intergroup heterogeneity based on many biological indicators to assess statistical significance. The program "SPSS 16" was used for the analysis. The result was deemed statistically significant if the "p" value < 0.05 (p 0.05), and highly significant if the "p" value < 0.01 (p 0.01).

insignificant decreased blood cholesterol level may be because, the concentrations were not up to the mark and it may possess narrow spectrum of activity.

On the other hand, no significant decrease was observed in case of both HDL and LDL levels (P>0.05). However, When Triglyceride level was measured, a significant reduction and deduction were observed both in group 5 and 6(P<0.05).

### 3. RESULTS AND DISCUSSION

In our study, We tried to find out whether *Coccinia grandis* possesses antihyperlipidemic activity or not. In Table 3, when group 2 was compared with group 4, 5 and 6 individually, It has been observed that cholesterol level was decreased significantly in group 5 & 6(P<0.05). In group 4, this level was also decreased though not in a significant amount(P>0.05). This observation depicts that the plant *Coccinia grandis* possesses more or less antihyperlipidemic activity. The incident of

With these interpretations, it can be said that *Coccinia grandis* have antihyperlipidemic activity even though the bunch of compounds responsible for antihyperlipidemic activity are present in insufficient concentrations.

In Table 4, The SGPT and SGOT levels of Positive control group were higher than groups 5 & 6, meaning that the plant was able to decrease blood SGPT and SGOT level significantly in these specific groups (P<0.05). So, our studied plant have greater ability to reduce the blood SGPT and SGOT level.

**Table 3. Effect of *Coccinia grandis* on the lipid profile of control and experimental rats**

| Group number | Group Abbreviation                     | TC            | HDL         | LDL        | TG            |
|--------------|--|---------------|-------------|------------|---------------|
| 1            | N                                      | 98.56 ±4.89   | 69.56±2.96  | 33.56±2.26 | 52.20±2.29    |
| 2            | P                                      | 159.26±8.45   | 44.56±5.59  | 64.56±3.12 | 103.56±7.79   |
| 3            | HFD + ATV                              | 123.56±9.23   | 61.59±6.23  | 44.56±6.92 | 70.41±7.98316 |
| 4            | HFD + <i>CG</i> <sub>Low Dose</sub>    | 157.55±10.66  | 44.92±5.09  | 64.26±6.90 | 100.62±7.89   |
| 5            | HFD + <i>CG</i> <sub>Medium Dose</sub> | 144.59±11.56* | 45.26±5.53  | 62.63±6.59 | 96.56±6.60*   |
| 6            | HFD + <i>CG</i> <sub>High Dose</sub>   | 141.119.25*   | 45.90±4.99  | 61.23±6.29 | 92.29±6.12*   |
| 7            | <i>CG</i> <sub>Low Dose</sub>          | 96.45±2.23    | 69.56±4.59  | 36.89±2.62 | 52.55±3.60    |
| 8            | <i>CG</i> <sub>Medium Dose</sub>       | 94.29±3.29    | 66.62± 4.52 | 32.69±3.69 | 51.59±3.23    |
| 9            | <i>CG</i> <sub>High Dose</sub>         | 97.59±5.56    | 65.92±5.23  | 36.90±4.28 | 55.63±3.84    |

**Table 4. Effect of *Cocccnia grandis* on the liver enzyme of control and experimental rats**

| Group number | Group Abbreviation                     | SGPT        | SGOT        |
|--------------|--|-------------|-------------|
| 1            | N                                      | 28.89±2.24  | 39.24±3.98  |
| 2            | P                                      | 77.89±4.49  | 85.56±5.20  |
| 3            | HFD + ATV                              | 63.69±6.47  | 62.50±4.00  |
| 4            | HFD + <i>CG</i> <sub>Low Dose</sub>    | 75.50±6.23  | 83.45±2.22  |
| 5            | HFD + <i>CG</i> <sub>Medium Dose</sub> | 72.29±5.90* | 80.97±4.87* |
| 6            | HFD + <i>CG</i> <sub>High Dose</sub>   | 66.69±4.92* | 76.59±5.50* |
| 7            | <i>CG</i> <sub>Low Dose</sub>          | 29.96±4.56  | 36.12±3.97  |
| 8            | <i>CG</i> <sub>Medium Dose</sub>       | 35.12±3.30  | 34.78±3.62  |
| 9            | <i>CG</i> <sub>High Dose</sub>         | 34.82±5.59  | 40.23±4.23  |

**Table 5. Effect of *Cocccnia grandis* on the kidney functioning of control and experimental rats**

| Group number | Group Abbreviation                     | Creatinine level | Urea level  |
|--------------|--|------------------|-------------|
| 1            | N                                      | 0.7±0.02         | 26.26±2.23  |
| 2            | P                                      | 2.9±0.30         | 82.26±5.19  |
| 3            | HFD + ATV                              | 1.01±0.05        | 52.52±5.50  |
| 4            | HFD + <i>CG</i> <sub>Low Dose</sub>    | 2.3±0.08*        | 79.56±5.53  |
| 5            | HFD + <i>CG</i> <sub>Medium Dose</sub> | 1.8±0.09*        | 77.23±2.45* |
| 6            | HFD + <i>CG</i> <sub>High Dose</sub>   | 1.20±0.0.07*     | 72.29±3.97* |
| 7            | <i>CG</i> <sub>Low Dose</sub>          | 0.5±0.01         | 28.80±3.39* |
| 8            | <i>CG</i> <sub>Medium Dose</sub>       | 0.9±0.06         | 26.62±3.78  |
| 9            | <i>CG</i> <sub>High Dose</sub>         | 0.8±0.02         | 28.45±2.34  |

In Table 5, Creatinine level was also seen to be decreased respectively in group 4, 5 & 6 (P<0.05) in comparison to positive control group due to the effect of *Cocccnia grandis*, though it was probably because of narrow spectrum.

In group 5, 6 and 7, where there's a markable decrease in urea level when compared with group 2 (P<0.05).

#### 4. CONCLUSION

As our ethanolic whole extract is found to effectively reverse disturbed pathological state in case of triglyceride and total cholesterol level in group 5 & 6, It can be concluded that this extract possesses compound or bunch of compounds that can exert antihyperlipidemic activity. So, more vigorous studies are needed in order to identify the specific compound responsible for antihyperlipidemic activity and finally to introduce *Cocccnia grandis* in the management of hyperlipidemic state.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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