Original Research Article

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

Abstract

Hyperlipidemia has been a wide spread disease recently and becoming a major concern in the field of health science. Increased amounts of lipids in a person's bodyis the most basic indication of hyperlipidemia which is characterized by low-density lipoprotein (LDL), total cholesterol, triglyceride, high lipoprotein level etc. Many antihyperlipidemic drugs are available to treat hyperlipidemia. However, People throughout the world also thrives for nature based medicinal product. In this study, We maintained high fat diet to the experimental rats to induce hyperlipidemia condition in this rat model. All the rats of the treatment group was treated with *Coccnia grandis*in 3 different doses. It is finally found that *Coccnia grandis* can effectively reduce ($p<0.05$) the hyperlipidemic condition in both medium and high dose. Elevated and abnormal Triglyceride and Total Cholesterol was found to be restored following *Coccnia grandis*administration. Although in case of HDL and LDL, no significant reduction was seen. A significant restoration was seen again in case of SGPT and SGOT. These observations depict that Our plant possesses constituents responsible for antihyperlipidemic activity, but the concentrations were not at required value. So, more studies are needed to explore the chemical structure and the concentrations of the compound as well as to know about genetic modifications.

Keywords: Hyperlipidemia, *Coccniagrandis*, HDL,LDL natural produc

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, VLDL, chylomicrons, LDL, lipoproteins, apolipoproteins, and HDL are examples of lipids. 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 (Bruss, 2008; Kris-Etherton et al., 2023). 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 (≥5.0 mmol/L) had elevated total cholesterol in 2008 (37% of men and 40% of women) (Noubiap et al., 2015).

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, colesevelam, colestipol; Cholesterol Absorption Inhibitor: ezetimibe; Fibrates: fenofibrate, fenofibric acid, gemfibrozil; Nicotinic acid: niacin ((TABANGCORA, 2023).

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 (Rayadurgam& Manikandan, 2022; C. Singh & Asija, 2017; Zhang et al., 2013).

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), *Ocimumtenuiflorum*(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(G. Singh et al., 2007). 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. (Kapw&Lkb, 2015; Ramachandran et al., 2014; Tamilselvan et al., 2011). Polyprenol-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 (S, 2013). Our research focuses on evaluating herbal medicine as a means of improving the hyperlipidaemia management system.

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.

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 + Coccinia grandis	High Fat Diet + Coccinia grandis leaves extract low dose	HFD +

5	High Fat Diet + Coccinia grandis	High Fat Diet + Coccinia grandis leaves extract medium dose	HFD +
6	High Fat Diet + Coccinia grandis	High Fat Diet + Coccinia grandis leaves extract high dose	HFD +
7	Coccinia grandis	Coccinia grandis leaves extract low dose	
8	Coccinia grandis	Coccinia grandis leaves extract medium dose	
9	Coccinia grandis	Coccinia grandis leaves extract high dose	

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.

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%)

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 Humaluzer 3000.

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).

Results and Discussion:

Table 3:Effect of *Coccnia 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 + $CG_{LowDose}$	157.55±10.66	44..92±5.09	64.26±6.90	100.62±7.89
5	HFD + $CG_{MediumDose}$	144.59±11.56*	45.26±5.53	62.63±6.59	96.56±6.60*
6	HFD $+CG_{HighDose}$	141.119.25*	45.90±4.99	61.23±6.29	92.29±6.12*
7	$CG_{LowDose}$	96.45±2.23	69.56±4.59	36.89±2.62	52.55±3.60
8	$CG_{MediumDose}$	94.29±3.29	66.62± 4.52	32.69±3.69	51.59±3.23
9	$CG_{HighDose}$	97.59±5.56	65.92±5.23	36.90±4.28	55.63±3.84

In our study, We tried to find out whether *Coccnia 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 *Coccnia grandis* possesses more or less antihyperlipidemic activity. The incident of 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 decreased was observed in case of both HDL and LDL level($P>0.05$). However, When Triglyceride level was measured, a significant reduction and deduction were observed both in group 5 and 6($P<0.05$).

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

Table 4: Effect of *Coccnia grandis* on the Liver Enzyme of Control and Experimental rats

Group number	Group	SGPT	SGOT

	Abbreviation		
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 + $CG_{LowDose}$	75.50±6.23	83.45±2.22
5	HFD + $CG_{MediumDose}$	72.29±5.90*	80.97±4.87*
6	HFD +$CG_{HighDose}$	66.69±4.92*	76.59±5.50*
7	$CG_{LowDose}$	29.96±4.56	36.12±3.97
8	$CG_{MediumDose}$	35.12±3.30	34.78±3.62
9	$CG_{HighDose}$	34.82±5.59	40.23±4.23

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 planthave greater ability to reduce the blood SGPT and SGOT level.

Table 5:Effect of *Coccnia 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 + $CG_{LowDose}$	2.3±0.08*	79.56±5.53

5	HFD + $CG_{MediumDose}$	1.8±0.09*	77.23±2.45*
6	HFD +$CG_{HighDose}$	1.20±0.0.07*	72.29±3.97*
7	$CG_{LowDose}$	0.5±0.01	28.80±3.39*
8	$CG_{MediumDose}$	0.9±0.06	26.62±3.78
9	$CG_{HighDose}$	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 *Coccnia 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($P<0.05$).

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 *Coccnia grandis*in the management of hyperlipidemic state.

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