

Original Research Article

An Assessment of Anti-hyperlipidemic Potentialities of Ethanollic Extract of *Hemidesmus indicus* in High Fat

Induced Rat Model

Comment [U1]: Fat-induced

Comment [U2]: model

Abstract:

The art or practice of utilizing herbs and herbal preparations to preserve health and to prevent, treat, or cure illness is referred to as herbal remedies. *Hemidesmus indicus* extract was tested for liver, kidney, and lipid profiles in rats. In groups 4,5 and 6, all dosages decreased triglycerides, although not significantly compared to the positive control group. The extract reduced total cholesterol at 1000 and 1500mg/kg doses ($p < 0.05$). Only larger dosages of 1500mg/kg significantly lowered HDL and LDL levels ($p < 0.05$) compared to positive control. In liver function tests, 1000 and 1500mg/kg doses lowered SGPT significantly. Only 1500mg/kg doses reduced SGOT concentrations ($p < 0.05$). All the doses of 500,1000 and 1500mg/kg reduced creatinine levels significantly ($p < 0.05$). Only 1500mg/kg doses lowered urea relative to the positive control group. Groups 7,8 and 9 received just the lower, medium, and higher extract dosages and had the same results as the negative control group, which is not statistically significant. These discoveries may benefit cardiovascular, liver, and renal disease patients.

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Keywords: herbal remedies, *Hemidesmus indicus*, cholesterol, cardiovascular patient, extract

Comment [U4]: phytopharmacology

Introduction

The term "hyperlipidemia" refers to a group of inherited and acquired illnesses that are characterized by high lipid levels in the human body. As an alternative, a more objective definition of hyperlipidemia states that it exists when low-density lipoprotein (LDL), total cholesterol, triglyceride, or lipoprotein levels are higher than the 90th percentile in comparison to

the general population, or when HDL levels are lower than the 10th percentile in the general population [1][2]. According to statistics from a study of 1492 doctors who offer ambulatory care in nongovernment institutions conducted by the Centers for Disease Control and Prevention, hyperlipidemia is next only to hypertension on the list of the ten most prevalent chronic illnesses spotted [3]. The vast majority of commonly used anti-hyperlipidemic medications, such as Atorvastatin, Pravastatin, Fluvastatin, Simvastatin, Lovastatin, and Rosuvastatin, are effectively absorbed but undergo significant hepatic first-pass metabolism, resulting in extremely poor absolute bioavailability [4]. Statins are reversible competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR), reducing intracellular cholesterol production. Statins' pharmacological reaction is determined by their capacity to enter the hepatocyte and inhibit HMG-CoAR [5]. The development of muscular symptoms, known as statin-associated muscle symptoms (SAMS), as well as diabetes mellitus (DM) and central nervous system disputes, is the most common adverse response limiting statin usage [6]. Apart from the serious side effects, these synthetic drugs are costly, and the patient could run into financial difficulties if the entire therapy program is continued [7]. As a result, potent antihyperlipidemic drugs with fewer adverse effects are necessary.

According to medicinal plant specialists, unique chemical compounds produced from medicinal plants might have therapeutic benefits. As a result, scientists are continually searching for alternative or plant-based herbal medicines to treat a variety of maladies. Because of the presence of numerous chemical constituents such as phenols, alkaloids, terpenoids, saponins, glycosides, tannins, flavonoids, resins, polysaccharides, plant lipids, essential oils, and so on, these medicinal plants can provide a wide range of pharmacological and therapeutic effects [8–9]. Again, the level of the plant's chemical constituents, whether increasing or decreasing, may provide the intended therapeutic benefit, which may be achieved by plant genetic manipulation. For example, using reverse genetics, we can boost the biosynthesis of secondary metabolites such as alkaloids [10].

Many plants, including *Ecliptaprostrata*, *Terminalia chebula*, *Hibiscus sabdariffa L.*, and *Salvia officinalis L.*, have been shown to have antihyperlipidemic activity [11–14]. According to Ahluwalia and Amma [15], feeding the oleoresin of gum guggul (*Commiphora mukul*) reduced total cholesterol and its fractions in lipoproteins. Reshma et al. [16] discovered that corilagin and chebulinic acid from Haritaki had hypolipidemic actions. The concentrated tannins of *Solanum*

melongena decrease hyperlipidemia and hyperglycemia, according to Sudheesh et al. [17]. Several authors have reported hypolipidemic effects of proteins, saponins, gums, and beta-sitosterol.

Indian sarsaparilla, a kind of plant found in South Asia, is classified as *Hemidesmus indicus* and is a member of the Apocynaceae family. From the upper Gangetic plain in the east to Assam, as well as in a few locations in central, western, and southern India, it is present throughout the majority of the country. *H. indicus* is a thin, laticiferous, twining, perennial, wiry shrub that grows quickly and is either prostrate or semi-erect. Its roots are short, inflexible, tuberous, woody, and scented. Anantmool means "the eternal root" because its roots extend far beyond the surface of the ground [18]. Steroids, triterpenoids, alkaloids, carbohydrates, tannins, glycosides, polyphenols, and saponins are present in *Hemidesmus indicus* [19, 20]. Beta-sitosterol, which has lipid-lowering properties, is found in the roots of *Hemidesmus indicus* [21, 22]. High levels of phytosterols are seen in cell cultures obtained from *Hemidesmus indicus*. Phytosterol beta-sitosterol is said to be helpful in the treatment of hyperlipidemia [23]. The plant exhibits cytotoxic, immunostimulatory, hepatoprotective, anti-diabetic, antihypercholesterolemic, anti-ulcerogenic, cardioprotective, anti-atherogenic, and antithrombotic properties [23-29]. Our study aims to investigate the anti-hyperlipidemic activity of *Hemidesmus indicus*.

Comment [U5]: Beta-sitosterol

Materials and Methods

Drugs, Chemicals, and Instruments:

Ethanol was purchased from Sigma-Aldrich, Germany. Rosuvastatin, a common antihyperlipidemic medication, was received as a gift sample from Healthcare Pharmaceutical Limited. HDL, LDL, Triglycerides, Total Cholesterol, SGOT, SGPT, and Creatinine were provided by Plasmatic Laboratory Product Ltd. in the UK. Shahbag in Dhaka, and the Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer) were used to assess the biochemical parameters. Ingredients for the preparation of the high-fat diet were bought from a supershop.

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Plant Collection and Extract Preparation:

Hemidesmus indicus was collected from the medicinal plant garden of the University of Dhaka's Faculty of Pharmacy. The process of taxonomic identification and authentication was then finished. Plant specimens were kept in the National Herbarium of Bangladesh in compliance with their norms. The Herbarium authorities issued accession number 47380, dated 11-2-2019, for future use. The plant was then shade-dried for 7–10 days before being coarsely crushed. While steeping in 70% ethanol, the powdered plants were violently shaken for 96 hours. The extract was filtered when it had finished soaking, and the filtered liquid was collected. The

extracted solution was then concentrated using a rotary evaporator. The dried extract was then carefully collected and stored for future use [30-31]

Experimental Animal Handling: Male wistar adult rats weighing 100–150 g were obtained from the pharmacy department of Jahangirnagar University in Dhaka, Bangladesh, and housed at the University of Dhaka's Institute of Nutrition and Food Science under a 12-hour light/dark cycle at a constant temperature of 25°C. Regular supplies of a standard pellet diet and pure water were given. Before the inquiry began, the rats were left there to acclimate. Experiments involving the rats were all conducted by the institutional animal ethics committee's rules (IEAC). According to the standards set forth by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT), animals were handled and treated humanely.

Experimental Guidelines: The 2013 Declaration of Helsinki's ethical guidelines were followed in the execution of all tests [32].

Experimental Design: Each rat was weighed individually, and then the animals were divided into groups (Table 1) with an even distribution of rodents according to their body weight and five rats in each group.

Comment [U7]: Include a section of ethical aspects

Table 1: Antihyperlipidemic activity analysis

Group number	Group Status	Treatment specimen & Dose	Group Abbreviation
1	Negative Control	N/A	N
2	Positive control		
3	High fat + drug		
4	High fat + extract low		
5	High fat + extract medium		
6	High fat + extract high		

7	Low extract		
8	Medium extract		
9	High extract		

The N/A refers to the fact that rats of this group were administered with no therapeutic treatment.

High-Fat Diet: Based on the composition provided by Levin and Dunn-Meynell, the high-fat diet was adapted [33]. The high-fat diet contains 50% lipid, 40% carbohydrate, and 10% protein. The composition of the diet is presented in Table 2.

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%)
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After mixing the ingredients thoroughly, a high-fat diet was given to the rats to induce obesity for 10 weeks [33].

Experiment:

The whole duration of the experiment is ten weeks. We maintained the rats in the experimental area after collecting them to ensure reliable findings. After 2 weeks, we gave the rats a high-fat diet and a plant extract for a total of 8 months.

Biological Sample Collection: After the sacrifice, the heart was promptly punctured to obtain blood samples, which were then transferred to a microcentrifuge tube. To acquire the supernatant fluid, the collected samples were centrifuged at 5,000 rpm for 5 minutes. To perform biochemical tests, this fluid was then transferred to another microcentrifuge tube.

Estimation of Biochemical Parameters: Lipid profile, kidney, and liver function tests were carried out using Humalyzer 3000.

Statistical Analysis: The "one-way ANOVA test" was used to evaluate intergroup heterogeneity in terms of several biological parameters and to establish statistical significance for all study parameters that belonged to each group. Software called "SPSS 16" was utilized for the analysis. When the "p" value was less than 0.05 ($p < 0.05$), the outcome was deemed statistically significant.

Results:

Groups 4, 5, and 6 exhibited a statistically significant ($p < 0.05$) decrease in total cholesterol after administering plant extract at 1000 and 1500 mg/kg doses. The experimental groups receiving 500, 1000, and 1500 mg/kg doses had decreased serum cholesterol levels compared to the positive control group. While triglyceride levels were reduced across all groups while using the extract, no dosage produced a statistically significant difference when compared to the positive control group. At a dosage of 1500 mg/kg, there was a statistically significant ($p < 0.05$) reduction in LDL. HDL levels in rats were raised across all doses as compared to the positive

control group. In the cases of 1000 and 1500 mg/kg doses, there was a statistically significant reduction in SGPT levels. However, only a dosage of 1500 mg/kg reduced SGOT concentration by a statistically significant amount ($p < 0.05$). The creatinine levels of rats were reduced by a statistically significant amount ($p < 0.05$) after administration of 500, 1000, and 1500 mg/kg. However, only the 1500 mg/kg dosage reduced urea levels relative to the positive control group. The conventional medicine alone reduced the lipid profile, SGPT, SGOT, creatinine, and urea levels significantly in all the groups 3. When compared to the negative control group, the results from groups 7, 8, and 9 where all the rats were treated solely with extract, were not statistically significant.

Comment [U8]: Separate p p 0,05

Table 3: Cholesterol

C	CCL4	CCL4+S10	CCL4+VV1500	CCL4+VVI1000	CCL4+VVI1500	VVI500	VVI1000	VVI1500
100.25	167.55	130.42	161.42	140.56	137.45	102.44	105.45	101.45
107.4	155.55	121.24	147.96	142.55	130.44	105.44	103.44	102.32
104.56	155.55	126.45	162.78	137.8	140.9	96.45	96.96	104.55
97.44	157.44	117.89	153.47	151.39	135.55	90.44	102.44	105.66
97.5	162.51	119.78	152.55	144.2	137.44	99.44	99.89	101.44
101.43	159.72	123.156	155.636	143.3	136.356	98.842	101.636	103.084
4.42140	5.22181	5.15789	6.27748	5.113174	3.829768	5.76957	3.29245	1.91972
2492	0031	0073	5962	161	923	7108	0455	1334

Table 4: Triglyceride

NC	CCI4	CCI4+Atv	CCI4+HI low	CCI4+HI medium	CCI4+HI High	HI Low	HI medium	HI High
49.23	110.23	74.56	110.23	108.45	105.92	53.21	48.429	50.5
4.291	4.259	3.269	3.425	2.239	3.214	2.292	1.298	2.3962

Table 5: LDL

NC	CCI4	CCI4+Atv	CCI4+HI low	CCI4+HI medium	CCI4+HI High	HI Low	HI medium	HI High
37.43	70.48	51.45	68.48	70.892	60.23	33.48	39.4528	41.23
1.97	3.4598	3.1498	2.4582	4.2328	3.149	1.298	2.23914	2.239

Table 6: HDL

NC	CCI4	CCI4+Atv	CCI4+HI low	CCI4+HI medium	CCI4+HI High	HI Low	HI medium	HI High
67.88	48.52	58.23	48.89	51.047	56.76	65.47	67.89	69.74
1.268	5.21492	4.623	3.489	3.598	2.948	4.216	3.1296	3.635

Table 7: SGOT

NC	CCI4	CCI4+Atv	CCI4+HI low	CCI4+HI medium	CCI4+HI High	HI Low	HI medium	HI High
42.23	90.41	63.45	87.45	85.45	81.452	41.25	37.88	38.45
2.452	4.1256	3.2285	4.225	4.255	2.455	2.33	3.2285	2.33

Table 8: SGPT

NC	CCI4	CCI4+Atv	CCI4+HI low	CCI4+HI medium	CCI4+HI High	HI Low	HI medium	HI High
29.7	74.63	58.9	71.485	68.48	63.125	30.46	31.29	33.22
1.983	3.4982	4.9827	3.254	2.48	3.245	2.982	1.769	1.439

Table 9: UREA

NC	CCI4	CCI4+Atv	CCI4+HI low	CCI4+HI medium	CCI4+HI High	HI Low	HI medium	HI High
28.47	84.52	59.47	81.4	80.54	75.4581	27.58	32.32	31.48
2.12465	5.264189	3.26188	6.45129	4.296521	4.52973	1.4982	2.14622	2.15938

Table 10: Creatinine

C	CCL4	CCL4+S 10	CCL4+V VI 500	CCL4+VVI10 00	CCL4+VVI15 00	VVI500	VVI1000	VVI150 0
0.52	2.4	0.9	1.8	1.4	1.1	0.7	0.8	0.5
0.1492321 73	0.5.18 94	0.43297 1	0.4941 87	0.24844	0.0297252	0.0.224 78	0.025874 41	0.0284 11

Discussion:

Hyperlipidemia is one of the important risk factors involved in the development of cardiovascular diseases. Atherosclerosis and congestive heart diseases are strongly associated with disorders of lipid metabolism and plasma lipoproteins. In this study, we evaluated the antihyperlipidemic activity of the plant (roots) of *Hemidesmus indicus*.

In the case of groups 4,5 and 6, all the doses of extract lowered the triglyceride level but it was not statistically significant when compared to the positive control group.

But the extract at medium and higher doses lowered the total cholesterol level in the body which is statistically significant ($p < 0.05$). The HDL and LDL level was decreased only on higher doses which is also statistically significant when compared to the positive control group.

In the case of the liver function test, SGPT levels were decreased statistically significantly in the case of medium and high doses. But in the case of SGOT only high doses lowered the concentration of SGOT which is statistically significant ($p < 0.05$).

In the kidney function test, after administration of all the doses, lower, medium, and high the creatinine level in the rats decreased statistically significantly level ($p < 0.05$). But urea was decreased only on high doses when compared to the positive control group.

The group 7,8 and 9 which were treated only the lower, medium and higher doses of the extract showed the result as same as the negative control group which is not statistically significant. so we can say that, it has no impact in the change of normal physiological function of the body.

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Comment [U9]: I suggest to actualize some of the bibliography to the last 5 years (2019 – 2013)

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