__Original Research Article__

An Evaluation of Anti-hyperlipidemic Activity of Ethanolic extract of *Cissus quadrangularis* on High Fat Induced Hyperlipidemic Rat Model

Abstract

Herbalism is the discipline of using herbs and herbal medicines to promote and preserve optimal health, as well as to prevent, treat, or cure illnesses. In some regions, herbal remedies may be confused with herbal medicine. In rats with high-fat-induced hyperlipidemia, this study investigated the impact of a *Cissus quadrangularis* extract on lipid profiles. In groups 5 and 6, the SGPT and SGOT levels demonstrated statistically significant results ($p< 0.05$) when the high fat dose was 800 and 1200 mg/kg. The SGPT particularly demonstrated this. Nevertheless, the SGOT did not provide any statistically significant findings. During the renal function test, statistical analysis revealed that the levels of urea were significantly different ($p< 0.05$) in groups 5 and 6, which were administered dosages of 800 and 1200 mg/kg, respectively. Nevertheless, the examination of the creatinine yielded no statistically significant findings. Groups 5 and 6 showed statistically significant results ($p< 0.05$) in HDL levels when considering high-density lipoprotein (HDL) and low-density lipoprotein (LDL). More precisely, groups 5 and 6, administered dosages of 800 and 1200 mg/kg, respectively, showed statistically significant outcomes. Group 5 showed a statistically significant difference in triglyceride levels ($p < 0.05$). Group 5, which was administered a dose of 800 mg/kg, had statistically significant outcomes ($p< 0.05$) in relation to total cholesterol levels.

Keywords: Herbal medicine, *Cissus quadrangularis*, HDL, LDL, Phytochemicals.

Introduction

The liver, the largest glandular organ, regulates the majority of physiological activities in the human body. The liver is the organ that receives an individual's entire blood volume many times throughout the day. It is crucial to the metabolic activities of humans [1, 2]. Excessive alcohol consumption, drug addiction, exposure to some hazardous compounds, or infection with viruses or parasites may lead to an elevation in the levels of reactive oxygen species (ROS), such as OH, H2O2, and O2 [3]. This may lead to hepatocellular injury. The Centers for Disease Control and Prevention conducted research involving 1492 clinicians who offer ambulatory treatment in non-government facilities. The survey revealed that hyperlipidemia is the second most prevalent chronic disease seen by these doctors, with hypertension being the only condition more often encountered [4]. The study's results suggest that the primary factor leading to hyperlipidemia is the overconsumption of high-fat meals [5]. The liver plays a crucial role in metabolizing commonly used anti-hyperlipidemic drugs, such as atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, and rosuvastatin. Consequently, the bioavailability of these drugs is quite poor [6]. Statins have the ability to transiently inhibit the enzyme 3-hydroxy-3-methylglutaryl-co- A reductase (HMG-CoAR). This enzyme reduces cholesterol levels. This allows them to reduce cholesterol synthesis inside the cells. This is because statins have the ability to enter hepatocytes and inhibit HMG-CoAR, which is responsible for their pharmacological effects [7]. Statin-associated muscle symptoms (SAMS), commonly known as muscular problems, are the primary side effects that limit the use of statins. Two other potentially detrimental consequences include the onset of diabetes mellitus (DM) and complications affecting the central nervous system [8]. These synthetic medicines not only have substantial adverse effects, but they are also expensive, potentially causing financial hardships for patients who need to continue taking them during the whole therapy [9]. Therefore, it is crucial to develop highly effective antihyperlipidemic medications with minimal unwanted effects. Plants are essential in the process of discovering and synthesizing novel therapies [10]. They serve as a useful and abundant reservoir of naturally occurring chemicals for therapeutic applications. Specialists on the subject propose that certain chemical constituents extracted from medicinal plants have therapeutic properties. As a result, researchers are always searching for novel herbal remedies and other medicines derived from plants to effectively treat various ailments [4]. Traditional medicines have long been used in several nations worldwide as remedies derived from plants, dietary supplements, and alternative medical methods. In recent years, there has been a significant increase in the use of traditional medicine, with many across the country depending on it as a major form of care [11]. Plants used for medical reasons include a diverse range of chemical constituents, enabling them to exert a broad spectrum of pharmacological and therapeutic effects. These substances include many constituents, including tanning agents, glycosides, alkaloids, saponins, polysaccharides, essential oils, terpenoids, resins, and plant lipids [12–14]. Genetically engineered plants provide the ability to finely regulate chemical levels, eventually resulting in the desired therapeutic outcome. Reverse genetics has several potential uses, one of which is the augmentation of secondary metabolite synthesis, including the generation of alkaloids [15]. The global advancements in scientific research have led to an increase in the investigation of plant species' therapeutic attributes [16]. Plants are becoming more popular because of their inherent safety, potent pharmacological properties, and cost-effectiveness compared to synthetic drugs.

Cissus quadrangularis L. is a shrub belonging to the Vitaceae family, often found in the hot areas of India and Sri Lanka. People commonly refer to this plant as the Devil's Backbone or Hadjora. It is cited by Ayurveda as a tonic for promoting bone health in fracture patients and accelerating tissue recovery. In addition, it has shown efficacy in treating rheumatoid arthritis, osteoarthritis, osteoporosis, scurvy, menstrual problems, otorrhoea, epistaxis, and weight management [17]. The plant's root and stem extracts exhibit antioxidant and antimicrobial properties [18]. The stem juice is abundant in minerals such as calcium and phosphorus [19], and it contains a significant amount of vitamin A, vitamin C, anabolic steroids, β-sitosterol, δ-amyrin, δ-amyrone, and flavonoids (quercetin) [20]. This plant has several pharmacological activities, including bone healing, anti-obesity, anti-ulcerative, anti-diabetic, antioxidant and free radical scavenging, gastroprotective, central nervous system, analgesic, anti-inflammatory, and stimulatory activities [21].

Our current investigation aims to evaluate the hepatoprotective properties of *Cissus quadrangularis*.

Materials and methods

Plant Collection and Extract Preparation

Cissus quadrangularis specimens were obtained from a local market in Dhaka. The National Herbarium of Bangladesh verified the authenticity of the sample. The first step was thoroughly rinsing *Cissus quadrangularis* with water, followed by allowing it to dry naturally. Finally, we pulverized the withered leaves into a fine powder. We immersed the powder in 70% ethanol for a duration of 15 days. The solution was stored for a duration of 15 days. Intermittent, vigorous shaking was also conducted. Subsequently, the solution underwent filtration. The filtrate that was collected was subjected to drying using a rotary evaporator under conditions of reduced temperature and pressure. Ultimately, the unrefined remains were submitted to the necessary pharmacological examination.

Drugs and Chemicals

Atorvastatin drug was obtained from inceptapharmacetucals as a gift sample. Ethanol were bought from Taj Scientific store.

Experimental Animal Procurement, Nursing, and Grouping

Obtained from Jahangirnagar University in Savar, Dhaka, a total of 90 male rats weighing between 120 and 150 grams were acquired. Each specimen was kept in a controlled environment with a temperature range of 25±3°C, relative humidity between 55±5%, and a 12-hour cycle of light and darkness. This environment was provided at the Institute of Nutrition and Food Science (INFS) at the University of Dhaka. They were provided with standard meals and allowed to consume purified water. All the animals were housed in this environment for a minimum of one week before the study to observe their adaptation. The experimental techniques adhered to the guidelines set out by the Institutional Animal Ethics Committee (IEAC). A total of 90 rats were randomly allocated into 9 groups, with each group consisting of 10 rats.

Experimental design

Rats were individually weighed and then divided into nine independent groups for research on anti-hyperlipidemic action. The distribution of rodents among the groups was based on their body weight, with each group consisting of five rats. The atorvastatin control group in Table 1 shows rats that were given atorvastatin with a high-fat diet since using simply atorvastatin would result in the animals dying. N/A indicates that rats in this group did not receive any therapeutic treatment.

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 +RV_{10}	High Fat Diet + Atrovastatin	HFD + ATV
4	High Fat Diet + *C. quadrangularis*	High Fat Diet+ CQ_{400}	HFD + CQ_{400}
5	High Fat Diet + *C. quadrangularis*	High Fat Diet + CQ_{800}	HFD + CQ_{800}
6	High Fat Diet + *C. quadrangularis*	High Fat Diet + CQ_{1200}	HFD + CQ_{1200}
7	*C. quadrangularis*	CQ_{400}	CQ_{400}
8	*C. quadrangularis*	CQ_{800}	CQ_{800}
9	*C. quadrangularis*	CQ_{1200}	CQ_{1200}

High Fat Diet: The high-fat diet was modified based on the composition supplied by Levin and Dunn-Meynell. The high fat diet is composed of 50% lipid, 40% carbohydrate, and 10% protein. The diet's composition is shown 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%)
	Boiled rice (40%)

Carbohydrate (40%)	Smashed potato (40%) Boiled corn (20%)
Protein (10%)	Dry powdered prone (40%) Dry boiled mutton (20%) Cheese (20%) Egg (20%)

After mixing the ingredients thoroughly, the high fat diet was given to the rats to induce obesity for 10 weeks [22].

Evaluation of anti-hyperlipidemic Activity

Table 3: Application of treatment efficacy

Group Number	**Group Specification**	**Treatment species**	**Dose treatment species (mg/kg)**	**Abbreviation of Groups**
1	Negative control	Physiological saline	10 ml/kg	N
2	High Fat	N/A	N/A	HF
3	$HF+RV_{10}$	Rovast 10mg/kg	10	At_{10}
4	$HF+CQ_{400}$	*Cissus quadrangularis*	400	CQ_{400}
5	$HF+CQ_{800}$	*Cissus quadrangularis*	800	CQ_{800}
6	$HF+CQ_{1200}$	*Cissus quadrangularis*	1200	CQ_{1200}
7	CQ_{400}	*Cissus quadrangularis*	400	CQ_{400}
8	CQ_{800}	*Cissus quadrangularis*	800	CQ_{800}

9	CQ_{1200}	*Cissus quadrangularis*	1200	CQ_{1200}

For this experiment, 100 rats were randomly picked and equally divided into fourteen groups

Statistical analysis:

All of our findings (raw data) in terms of numerical parameters were recorded and analyzed on a broadsheet using the MS Excel application. The gathered data were subjected to descriptive statistics, with the findings reported as mean SD. To evaluate statistical significance, we used the SPSS 16 software's "One-way Anova test" to interpret inter-group heterogenicity in terms of several biological factors. The occurrences are considered statistically significant since the 'p' value was less than 0.05 ($p<0.5$).

Results and discussion

Traditional medicine and ethnomedicine, which refers to the examination of different ethnic groups' traditional medicinal practices, have existed since the beginning of human civilization. Historically, traditional medicine used natural resources as medicinal remedies. In the past, herbs, which are plants or plant products, together with plant extracts, were the main components of the first medications employed in the traditional medical systems of many nations and civilizations. Plants and herbs have long been a prevalent source of pharmaceuticals, whether in the form of traditional extracts or as isolated active components.

This research examined the effects of a *Cissus quadrangularis* extract on lipid profiles in a rat model of high-fat-induced hyperlipidemia. Both the SGPT and SGOT levels showed statistically significant ($p< 0.05$) results in groups 5 and 6, where the high fat dosage was 800 and 1200 mg/kg, specifically in the case of the SGPT. However, the SGOT did not provide any statistically significant results. Two further studies reached the same outcomes [23, 24]. During the renal function test, it was shown that the levels of urea were statistically significant ($p< 0.05$) in groups 5 and 6, which received doses of 800 and 1200 mg/kg, respectively. However, the analysis of the creatinine did not provide any statistically significant results. Two independent inquiries [25, 26] arrived at identical findings about the topic. Regarding high-density lipoprotein (HDL) and low-density lipoprotein (LDL), groups 5 and 6 exhibited statistically significant results ($p < 0.05$) in HDL levels. Specifically, groups 5 and 6, who received doses of 800 and 1200 mg/kg, demonstrated statistically significant outcomes. The triglyceride levels in group 5 exhibited a statistically significant difference ($p < 0.05$). Group 5, which received a dosage of 800 mg/kg, had statistically significant results ($p < 0.05$) in terms of total cholesterol levels. Two further studies reached the same outcomes [27, 28].

Table 4: Lipid profil of *Cissus quadrangularis* on rat model

Groups	**SGPT**	**SGOT**	**Creati-nine**	**Urea**	**TC**	**HDL**	**LDL**	**TG**
NC	38.46±4.29	38.93±4.23	0.63±0.012	34.29±2.43	120.24±6.21	90.22±5.32	38.46±3.21	53.21±4.81
HF	92.46±5.39	93.21±8.24	3.14±0.86	103.47±8.91	209.59±12.21	48.21±6.20	147.22±14.23	114.21±12.32
HF+At$_{10}$	61.29±6.20	54.59±6.14	1.49±0.62	52.29±6.18	152.29±9.36	69.25±7.30	68.41±10.22	68.50±8.26
HF+CQ$_{400}$	88.80±5.59	90.28±5.16	2.64±0.82	100.26±6.22	204.93±8.63	50.50±6.29	142.25±11.63	110.29±11.39
HF+CQ$_{800}$	84.21±7.61*	86.29±7.28	2.29±0.73	95.24±7.32*	198.46±7.97*	55.53±7.29*	134.23±11.67*	106.21±6.21*
HF+CQ$_{1200}$	81.29±5.56*	80.17±6.29	1.87±0.63	91.50±6.32*	192.91±6.20	59.93±8.93*	127.57±10.29*	100.29±5.21*
CQ$_{400}$	36.17±5.53	37.21±3.28	0.71±0.31	37.21±3.21	122.20±7.39	93.20±6.39	36.27±4.52	55.55±2.37
CQ$_{800}$	40.20±6.17	35.50±5.21	0.84±0.26	34.20±3.19	117.70±6.29	89.18±4.20	35.20±5.29	52.21±3.39
CQ$_{1200}$	43.19±5.53	39.20±6.20	0.57±0.23	30.21±3.90	119.32±6.82	90.91±4.21	39.16±5.39	56.19±4.02

Note: The results were expressed in Mean±SEM (standard mean error) *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control.

Conclusion

This work focused on examining the hepatoprotective characteristics of an ethanolic extract derived from *Cissus quadrangularis.* According to the results of this study, it seems that an ethanol extract obtained from the *Cissus quadrangularis* plant has the potential to provide defense against high cholesterol, liver damage, and impaired kidney function. Further investigation is required to identify the specific active constituents within the complete extract that possess the capacity to mitigate hyperlipidemia and diabetes. Once the active compounds have been identified, it becomes feasible to carry out a thorough examination.

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