Asian Journal of Pharmaceutical Education and Research
Vol -11, Issue-3, July- September 2022
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND STUDY OF ANTIHYPERLIPIDEMIC POTENTIAL OF LEAVES EXTRACT OF *ADIANTUM LUNULATUM*

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Received 22 May 2022; Revised 28 May 2022; Accepted 15 June 2022, Available online 15 July 2022.

Cite this article as: Prakash S, Ghatuary SK, Prasad S, Parmar M. Extraction, phytochemical investigation and study of antihyperlipidemic potential of leaves extract of *adiantum lunulatum.* Asian Journal of Pharmaceutical Education and Research. 2022; 11(3): 135-146.

https://dx.doi.org/10.38164/AJPER/11.3.2022.135-146

ABSTRACT

Secondary metabolites produced by plant responsible for the prevention and curing of various diseases and show microbicidal activity, these secondary metabolites are produced as a part of primary metabolic pathway. The aim of this study was to investigate the possible antihyperlipidemic effect of *Adiantum lunulatum* leaf extract in triton induced hyperlipidemic rats. In the present study, we have investigated the effect of *Adiantum lunulatum* (Leaves extracts) against experimentally induced hyperlipidemia in rats. The extracts at the dose of 300mg/kg, p.o. significantly reduced serum TC and TG levels. Additionally, this decrease in TC levels corresponded significantly to a reduction in LDL-C levels. These findings were supported by a decrease in atherogenic index and an increase in the HDL-C/LDL-C ratio. Since Diet-induced hyperlipidemia is mainly due to inhibition of the extractable (heparin releasable) pool of lipoprotein lipase, the serum TG-lowering effects can be attributed to the activation of lipoprotein lipase. It is reported that cholesterol homeostasis is maintained by the control of two processes, viz., and cholesterol biosynthesis, in which HMG-CoA reeducates catalyzes the rate-limiting process, and cholesterol absorption of both dietary cholesterol and cholesterol cleared from the liver through biliary secretion.

Keywords: *Adiantum lunulatum*, Phytochemical investigation, Antihyperlipidemic potential.

INTRODUCTION

Hyperlipidemia is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these excess lipids travel in the blood attached to proteins. These fatty substances can remain dissolved while in circulation in this way only. The most recent cholesterol management guidelines (the third report of the adult treatment panel APT III), which are issued by the national cholesterol education program (NCEP) in may 2001, redefine

the levels at which blood cholesterol should be treated [1]. These new evidence-based recommendations are departure from the NCEP's previous guidelines (ATP II) in several ways[1].

The fat-protein complexes in the blood are called lipoproteins. The best-known lipoproteins are LDL (low-density lipoprotein) and HDL (high-density lipoprotein). Excess LDL cholesterol contributes to the blockage of arteries, which eventually leads to heart attack. Population studies have clearly shown that the higher the level of LDL cholesterol, the greater the risk of heart disease. This is true in men and women, in different racial and ethnic groups, and in all adult age groups. Hence, LDL cholesterol has been labeled the bad cholesterol. In contrast the lower the level of HDL cholesterol, the greater the risk of coronary heart disease. As a result, HDL cholesterol is commonly referred to as the good cholesterol. Low HDL cholesterol levels are typically accompanied by an increase in blood triglyceride levels[2]. Studies have shown that high triglyceride levels are associated with an increased risk of coronary heart disease. Although hyperlipidemia does not cause to feel bad, it can significantly increase the risk of developing coronary heart disease, also called coronary artery disease or coronary disease. People with coronary disease develop thickened or hardened arteries in the heart muscle. This can cause chest pain, a heart attack, or both. Because of these risks, treatment is often recommended for people with hyperlipidemia[3].

High lipid levels can speed up a process called atherosclerosis, or hardening of the arteries. Arteries are normally smooth and unobstructed on the inside, but as age goes, a sticky substance called plaque forms in the walls of your arteries. Plaque is made of lipids and other materials circulating in your blood. As more plaque builds up, your arteries can narrow and stiffen. Eventually, enough plaque may build up to reduce blood flow through your arteries. Hyperlipidemia has been implicated in atherosclerosis, which is the primary cause of heart disease and stroke [4].

Atherosclerosis increases your risk of heart disease, stroke, and other vascular diseases. Fortunately, may be able to reduce high lipid levels and therefore prevent or slow the progression of atherosclerosis. Lifestyle changes like exercising and eating a healthy diet can also lower your lipid levels and are often the first step in treatment. *Adiantum lunulatum* is member of division pteridophyta and the genus Adiantum contains many species. Adiantum species of ferns show terrestrial / lithophytic in growth and are commonly found in moist shady places such as rock crevices, clay, near water streams and hillocks Medicinal plant contains many of the chemical constituents present in different plant parts. This biochemical constituent's play major role in the plant as well as to other living things for their survival. Adiantacea contain increased level of phytoconstituents and this presence of biochemical constituents purely seasonal. Tribal peoples considered *Adiantum lunulatum* as curative to various diseases such as dysentery, leprosy, fever, centipede bite, blood related diseases and for other microbial diseases.

Antihyperlipidemic agents having various pharmacological actions are being tested clinically. Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis and therefore two ways are feasible to reduce hyperlipidemia; to block endogenous synthesis or to decrease absorption. Both factors can be evaluated in normal animals without artificial diets.

Groups

Normal Vehicles (1 mL of 1% gum acacia and 1% CMC)

Hyperlipidimic control-High cholesterol diet

Treated with Standard (Atorvastatin)-High cholesterol diet + Atorvastatin (50mg/kg, p.o.)

Treated with HEAL 200mg/kg-High cholesterol diet + HEAL (200mg/kg, p.o.)

Treated with HEAL 300mg/kg-High cholesterol diet + HEAL (300mg/kg, p.o.)

Estimation of biochemical parameters

Lipid profile

The serum lipid profile was determined on day 8 in the case of diet-induced hyperlipidemia. The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were estimated using commercially available kits (Erba; Transasia Bio-Medicals Ltd., Daman, India). Very low-density lipoprotein cholesterol (VLDL-C) was calculated as TG/5. LDL-cholesterol (LDL-C) levels were calculated using Friedewald's formula[7]. The atherogenic index was calculated using the formula: atherogenic index

$$(\mathrm{AI}) = \frac{(\mathrm{VLDL-C} + \mathrm{LDL-C})}{\mathrm{HDL-C}}$$

Estimation of serum lipid profiles

Estimation of lipid profiles were placed major role in obesity condition. Usually, in obese condition the levels of lipids were higher than normal. So that, to know the activity of plant extract lipid profiles was studied.

Estimation of Cholesterol

Estimation of cholesterol was carried out by the method of Zlatkis et al., 1953[8]

Reagents

1. Standard Cholesterol in aldehyde free acetic acid (2mg/ml)
2. Concentrated Sulphuric acid

3. Ferric chloride and acetic acid reagent: 0.05% in aldehyde frees acetic acid.

Procedure

Ferric chloride-acetic acid (9.9 ml) reagent was added to 0.1 ml of serum for deproteinization. The contents were centrifuged at 3000 rpm for 15 min. 5 ml of the supernatant was taken and to this added 3 ml of concentrated Sulphuric acid and kept for 20 min at room temperature. The pink colour formed was read at 540 nm against a blank containing 5 ml of ferric chloride-acetic acid reagent. A set of standards were also performed in the similar manner.

Estimation of Triglycerides

Plasma triglycerides were measured by the method of Foster and Dunn et al.,1973[9]

Results and Discussion

The screening of phytoconstituents was done subjectively and quantitatively as a preliminary step. Using the soxhletion method, preliminary screening was carried out using solvents such as Pet ether and Hydroalcohol (methanol: water). *Adiantum lunulatum* (Leaves) were studied. Phytochemical study revealed the presence of several compounds in variable amounts in the Leaves portions. Hydroalcohol solvent extract produced the best results. The Hydroalcohol contained substantial levels of alkaloids, flavonoids, and phenols. Other phytochemicals found in *Adiantum lunulatum* include tannins, saponins, quinones, and fats/oils, which are present in moderate levels.

The results of the study indicated that the activity of the enzyme was significantly depressed by the *Adiantum lunulatum* extract as was evident by the increase in the ratio. Furthermore, there was also an increase in the cholesterol content of the fecal matter, indicating that all the extract either promoted the excretion of cholesterol or prevented the absorption of cholesterol. Since the fecal bile acid levels were significantly increased, they might have promoted the cholesterol excretion

Table 1: Extractive values obtained from *Adiantum lunulatum* leaves using different solvents

S. No.	Solvent	Time of extraction (Hours)	Color of extract	% Yield
1	Petroleum ether	12	Brown	3.6%
2	Methanol: Water	28	Orange-Black	5.2%

Table 2: Preliminary phytochemical screening of *Adiantum lunulatum* leaves

S. No.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	Present
		Dragendorff's Test	Present
2	Glycosides	Raymond's Test	Present
		Killer Killani Test	Present
3	Carbohydrates	Molisch's Test	Absent
		Fehling's Test	Absent
4	Tannins	Vanillin- HCl Test	Present
		Gelatin Test	Absent
5	Flavonoids	Lead acetate	Present
		Shinoda Test	Present
6	Resins	Color detection with ferric chloride	Absent
		Turbidity Test	Absent
7	Steroids	Libermann- Bur chard Test	Present
		Salkowski Reaction	Present
8	Proteins & Amino acids	Biuret Test	Present
		Precipitation test	Absent
		Ninhydrin Test	Present
9.	Phenols	Ellagic Acid Test	Present

Table 3. Effects of different treatments on food intake of diet-induced hyperlipidimic rats

Group (n = 6)	Daily food intake (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Normal	20.58±0.44	21.58±0.23	20.75±0.21	21.92±0.44	21.15±0.40	20.78±0.45	20.91±0.55
Hyperlipidimic control	20.79±0.21	20.47±0.41	20.52±0.39	20.68±0.78	21.75±0.31	21.13±0.50	20.48±0.54
Atorvastatin	19.95±0.52	20.48±0.42	21.12±0.34	21.03±0.32	22.77±0.75	20.41±0.41	22.58±0.48
Treated with HEAL 200mg/kg	20.22±0.31	21.03±0.52	21.66±0.32	20.17±0.42	22.65±0.58	22.15±0.29	22.21±0.54
Treated with HEAL 300mg/kg	21.08±0.35	22.08±0.35	20.43±0.42	22.52±0.47	20.20±0.45	20.19±0.62	20.30±0.52

Note. All values represent mean ± SEM from six animals. Statistical analysis was carried out using one-way ANOVA followed by Tukey's test. $p<0.05$ was considered statistically significant.

Table 4: Effect of *Adiantum lunulatum* leaves extracts on serum lipid profile of diet-induced hyperlipidemia in rats

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal	132.64±2.97	101.46±4.16	62.12±1.32	53.43±2.45	21.09±0.83
Hyperlipidemic control	329.23±6.95*	304.80±12.59*	105.56±3.54*	163.91±9.58	61.76±2.52*
Atorvastatin	163.00±6.97**	163.99±1.95**	67.87±2.47†	63.53±6.48**	33.60±0.39**
Treated with HEAL 200mg/kg	243.26±3.45**	109.42±2.12**	67.50±2.41†	155.08±3.61	22.68±0.42**
Treated with HEAL 300mg/kg	208.56±8.91**	131.51±5.43**	65.99±2.31†	117.46±7.73**	27.10±1.09**

Note. All values represent mean ± SEM from six animals. *Compared with normal group ($p<0.05$), **compared with hyperlipidemic control group ($p<0.05$), † significant reduction compared with hyperlipidemic control group ($p<0.05$). TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

Table 5: Effect of *Adiantum lunulatum* leaves extract on fecal cholesterol and bile acid excretion in diet-induced hyperlipidimic rats

Group	Fecal cholesterol (mg/g of fecal matter)	Fecal bile acid‡ (mg/g of fecal matter)
Normal	2.05±0.09	1.29±0.08
Hyperlipidimic control	3.18±0.07*	1.12±0.05
Atorvastatin	2.27±0.07	2.56±0.06
Treated with HEAL 200mg/kg	4.83±0.13**	3.69±0.04**
Treated with HEAL 300mg/kg	8.50±0.13**	3.97±0.10**

Note. All values represent mean ± SEM from six animals. *Compared with normal group ($p<0.05$), **compared with hyperlipidimic control group ($p<0.05$), a compared with atorvastatin , ‡ as cholic acid equivalent.

CONCLUSION

The results of the present study also indicated that lipid per oxidation was significantly reduced by all the extracts. Additionally, they improved the liver antioxidant status by improving the activities of the various enzymes. Ascorbic acid is the only biologically active form, playing a vital role as a natural antioxidant against a variety of stress conditions including lipid per oxidation. It is easily converted into

dehydroascorbic acid, thereby regenerating vitamin E. It also maintains high intracellular levels of glutathione. The findings of this study showed significantly higher levels of the serum total and ascorbic acid levels in all the extract-pretreated groups, suggesting a marked reduction in oxidative stress.

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