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Potential of Blood lipid lowering effect of *barleria cristata* in chronic stress induced animal model

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Abstract

Plants have been one of the most important sources of medicines since the beginning of human civilization. Stress is a big problem among people these days. Prolonged stress can have an impact on the body's physiological processes. The purpose of this work was to evaluate the antihyperlipidemic potential of the methanolic leaf extract of *Barleria cristata* in an animal model of chronic stress. *Barleria cristata* is a shrub in the Acanthaceae family. Plant extract is used as an antispasmodic and hypoglycemic agent. Root extract is used for anemia. In case of swelling, the roots and leaves are applied. It is also useful for snake bites, rheumatism and pneumonia. Used for ear and eye diseases. The infusion of plant is given for coughs. It is said to have tonic, diuretic and blood purifying properties. The leaves of *Barleria cristata* were collected, checked, dried and then extracted with methanol. Chronic stress was induced in rats by restraint. The influence of an oral methanolic extract of *Barleria cristata* leaves (200 and 400 mg/kg) on the blood lipid profile was evaluated. The rats showed a significant change in the lipid profile of the negative control group. The results showed that administration of a methanolic extract of *Barleria cristata* leaves restored abnormal lipid profiles. The present finding indicates that methanolic extract of *Barleria cristata* leaves has antihyperlipidemic potential at low dose (200 mg/kg) and high dose (400 mg/kg).

Keywords:- *Barleria cristata*, Triglyceride, LDL, Cholesterol, HDL, Stress, activity, Hyperlipidemia, antihyperlipidemic activity.

Introduction

The ancient knowledge of medicinal herbs is valued for its well-known use in the prevention, diagnosis, treatment and cure of a variety of diseases. Herbs have a defined potency through which they can stimulate the human body to protect itself against diseases (1). These medicinal herbs serve as an excellent source of medicine in the treatment of diseases in humans and animals. There is a wealth of knowledge, information and benefits of herbal medicines in our ancient literature on Ayurveda and Unani medicine (2). One of the earliest treatises on Indian medicine, the Charaka Samhita (1000 BC), mentions the use of over 2000 herbs for medicinal purposes. According to the WHO survey, 80% of the population living in developing countries rely almost exclusively on traditional medicine for their primary health care (3). Lipid disorders are widespread worldwide. Some of their risk factors are modifiable, such as psychological and physical stress in some situations such as at work. The main cause of lipid disorders is a genetic factor and family history that cannot be changed (4). Over the past decade, researchers have been working on risk factors for lipid disorders. Hypertriglyceridemia, hypercholesterolemia and associated lipid disorders are very common, with their prevalence ranging from 20% to 50% in different population groups. There are some studies that have shown the role of environmental risk factors for dyslipidemia in addition to nutritional conditions (5). Psychological stress had effects on the human body, particularly on certain organs and parameters, as well as on physiological parameters, including lipid profile. Physical stress caused by physical work can also impact lipid profiles (6). Night shift work could be a risk factor for hyperlipidemia, but the background of well-being is important in this situation. Researchers reported lipid disorders; Association with occupational stress in professional drivers (7). Their study showed the effects of stress on triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Scientists showed the connection between work stress and dyslipidemia, including total cholesterol and LDL, as well as reduced HDL (8). *Barleria cristata* is belong to Acanthaceae family. Plant extract is used as an antispasmodic and hypoglycemic agent. Root extract is used for anemia. In case of swelling, the roots and leaves are applied. It is also useful for snake bites, rheumatism and pneumonia. Used for ear and eye diseases (9). The infusion is given for coughs. It is said to have tonic, diuretic and blood purifying properties (10). The leaves of *Barleria cristata* contain flavones, luteolin and 7-methoxyluteolin, phenolic acids, β-sitosterol, quercetin, apigenin, naringenin and malvidin (11). It is well reported in the literature that flavonoids are

responsible for lowering the blood lipid profile (12). Therefore, the present study aims to investigate the lipid-lowering effect of methanolic extract of *Barleria cristata* leaves in rat model.

Methods

The leaves of *Barleria cristata* were collected in September from the area of Amravati district, Maharashtra, India. The plant material was identified and authenticated. The leaves were dried in the shade and then pulverized to obtain a coarse powder. This powder was stored in an airtight container and used for extraction. Methanol and water were used as solvents for the extraction of *Barleria cristata* leaves. Methanol and water were used in a ratio of 7:3. A glass bottle was needed for the extraction. Dried leaves of *Barleria cristata* and methanol and water were poured into a glass bottle for extraction. In the maceration process, powdered leaves were macerated; it was occasionally stirred at regular intervals. It was filtered and concentrated. Then it was dried by evaporation. (13) Estimation of the methanolic extract for the presence of various phytoconstituents was carried out. (14).

Animals

Eight-week-old healthy female Sprague-Dawley rats (weighing 150–250 g) were used in this study. Animals were housed in polypropylene cages with a wire mesh top and husk bedding and maintained under controlled conditions of light (12 hours of light, 12 hours of darkness), temperature (25 ± 2 °C) and humidity (60 ± 5%) and fed a standard diet Pellet feed and water ad libitum were used throughout the animal study. The experiments were carried out during the day (8:00 a.m.–4:00 p.m.). The rats were housed and treated in accordance with the rules and regulations of CPCSEA and IAEC. The protocol for all animal studies was approved by the Institutional Animal Ethics Committee (IAEC).

Experimental design

For this study animals were divided into five groups

Group I (Vehicle control group)-Rats received only saline solution.

Group II (Negative control group)-Rats were subjected to restraint stress using saline bottle for 28 days.

Group III (Low dose group)-Rats were subjected to restraint stress and treated with 200mg/kg methanolic extract of *Barleria cristata* orally for 28 days.

Group IV (High dose group)-Rats were subjected to restraint stress and treated with 400mg/kg methanolic extract of *Barleria cristata* orally for 28 days.

Group V (Standard group)-Rats were subjected to restraint stress and treated with **2mg/kg Diazepam** for 28 days.

Induction of chronic stress in rats

All groups were subjected to restraint stress for 28 days, except for the normal control group, which was housed in an animal stable in normal condition. Sprague-Dawley rats using a saline bottle. when rats were tightly packed in a salt bottle for 6 hours. daily up to 28 days. The animal model of depression is exposed to continuous stress such as food deprivation, lack of water and constant packing in saline. (15)

Drugs and dosing

Diazepam (2 mg/kg) was used as standard medication. Diazepam was diluted with distilled water. Two different concentrations (200 and 400 mg/kg) of *Barleria cristata* leaf extract were prepared by dissolving the extracts in distilled water. All solutions were freshly prepared and administered orally on test days. The dosage of *Barleria cristata* leaf extract was calculated based on the body weight of rats from different groups. An extract of 200 mg/kg was calculated for the low dose group and an extract of 400 mg/kg for the high dose group.

Biochemical Estimation

Determination of Cholesterol

Serum cholesterol levels were measured using the Ambica diagnostic kit. The kit uses the colorimetric method in which cholesterol and its esters are released from lipoproteins by

detergents. The cholesterol esterase hydrolyzed the esters. The subsequent enzymatic oxidation by cholesterol oxidase produces H2O2. This is converted into a colored quinonine in a reaction catalyzed by peroxidation with 4-aminoantipyrine and phenol. The absorbance was measured at 505 nm. (16)

Determination of Triglyceride

Serum triglyceride levels were measured using the Ambica diagnostic kit. The kit uses the colorimetric method in which enzymatic cleavage occurs with lipoprotein lipase. The indicator is quinoneimine, which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase. The absorbance was measured at 546 nm. (17).

Determination of HDL

Serum HDL levels were measured using the Ambica diagnostic kit. The kit uses the colorimetric method in which chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. When centrifuged, only the HDL remains in the supernatant. Their cholesterol content is determined enzymatically using Ecoline S and cholesterol. The absorbance was measured at 505 nm. (18).

Determination of LDL

LDL was determined according to Maruyama, C. et al. 2003 and Takeda H et al. al., 2017 and LDL were calculated using the following formula (19,20)

$$\mathbf{VLDL} = \frac{\mathbf{TG}}{\mathbf{5}}$$

$$\mathbf{LDL = TC – (HDL\text{-}VLDL)}$$

Results

Table 1. Effect of *Barleria cristata* on serum Cholesterol level in Stressed Rats

SN	Groups	Cholesterol (mg/dl)	
		0 day	28 day
1	Normal Control	48.08 + 1.92	47.31 +1.76
2	Negative Control	48.13+ 1.99	62.64+ 2.37@
3	MAP (200 mg/kg)	48.27+ 1.53	47.64 + 1.52 **
4	MAP (400 mg/kg)	48.20+ 1.43	48.35+ 1.82 **
5	Diazepam (2 mg/kg)	48.43+ 2.16	47.48 +1.96 **

All values are Mean ± SD @ p<0.01 compared with control group,**p<0.01 compared with negative control group.

Table 2. Effect of *Barleria cristata* on serum Triglyceride level in Stressed Rats

SN	Groups	Triglyceride (mg/dl)	
		0 day	28 day
1	Normal Control	67.25+2.66	67.14 + 2.52
2	Negative Control	67.15+2.46	83.64 + 2.97@
3	MAP (200 mg/kg)	66.91+2.70	69.67+ 2.85**
4	MAP (400 mg/kg)	66.95+2.73	68.48 + 2.75**
5	Diazepam (2 mg/kg)	67.37+2.70	67.48 +2.65**

All values are Mean ± SD @ p<0.01 compared with control group,**p<0.01 compared with negative control group.

Table 3. Effect of *Barleria cristata* on serum HDL level in Stressed Rats

SN	Groups	HDL (mg/dl)	
		0 day	28 day
1	Normal Control	36.81 + 0.76	38.14 + 0.76
2	Negative Control	36.90+ 0.79 ns	31.24 + 0.52@
3	MAP (200 mg/kg)	36.40+ 0.63 ns	35.93+ 0.63**
4	MAP (400 mg/kg)	36.45+ 0.65 ns	36.32+0.70**
5	Diazepam (2 mg/kg)	36.70+ 0.71 ns	37.94 + 0.76**

All values are Mean ± SD @ p<0.01 compared with control group,**p<0.01 compared with negative control group.

Table 4. Effect of *Barleria cristata* on serum LDL level in Stressed Rats

SN	Groups	LDL (mg/dl)	
		0 day	28 day
1	Normal Control	25.21+0.64	23.40+0.78
2	Negative Control	24.92+0.73 ns	42.04+0.98@
3	MAP (200 mg/kg)	24.82+0.68 ns	29.12+0.75**
4	MAP (400 mg/kg)	25.02+0.63 ns	27.21+0.69**
5	Diazepam (2 mg/kg)	25.02+0.61 ns	25.81+ 0.65**

All values are Mean ± SD @ p<0.01 compared with control group,**p<0.01 compared with negative control group.

Interpretation of result

The impact of *Barleria cristata* methanolic extract on cholesterol levels is displayed in Table 1. When comparing the cholesterol levels of the negative control group to those of the normal control group on day 28, there was a significant increase (p<0.01). On day 28, there was a significant (p<0.01) drop in cholesterol levels following treatment with *Barleria cristata* methanolic extract when compared to the negative control group at 200 mg/kg and 400 mg/kg. Triglyceride levels are affected by the *Barleria cristata* methanolic extract, as Table 2 illustrates. Between the negative control group and the normal control group, there was a significant

increase ($p<0.01$) in the triglyceride levels on day 28. On day 28, there was a significant ($p<0.01$) decrease in triglyceride levels from the 200 mg/kg and 400 mg/kg negative control group following treatment with the methanolic extract of *Barleria cristata*.

Table 3 displays the impact of *Barleria cristata* methanolic extract on HDL levels. In comparison to the normal control group, the HDL levels in the negative control group showed a significant decrease ($p<0.01$) on day 28. A noteworthy ($p<0.01$) decrease in HDL levels was noted on day 28 following treatment with *Barleria cristata* methanolic extract in comparison to the negative control group at 200 mg/kg and 400 mg/kg.

Barleria cristata's methanolic extract's impact on LDL levels is displayed in Table 4. When comparing the LDL levels of the negative control group to those of the normal control group on day 28, there was a significant increase ($p<02$). A noteworthy ($p<0.01$) decrease in LDL levels was noted on day 28 following treatment with *Barleria cristata* methanolic extract in comparison to the negative control group at 200 mg/kg and 400 mg/kg.

Discussion

Stress is a threat that affects everyone, whether it is brought on by environmental, social, or pathological phenomena in life, or it is brought on by the progress of industrialization. A noteworthy finding from the past ten years concentrated on a collection of biochemical, neurochemical, and molecular effects of stress on the immune system, endocrine system, and central nervous system. Several studies have demonstrated a link between immune system alterations, disease progression, and stress exposure (21). The most abundant class of phytochemicals in higher plants, flavonoids have a wide range of applications in medicine. Six classes of flavonoids are distinguished by their chemical structure: isoflavonoids, anthocyanidins, flavanols, flavanones, flavones, and flavonols (22). It can be used to treat hyperlipidemia and cardiovascular disease and has been demonstrated to be effective in lowering blood lipid levels. Flavonoids are essential for promoting antihyperlidemic effects because they also have anti-inflammatory and antioxidant qualities (23).

A review of the literature reveals that *Barleria cristata* is composed of amino acids, alkaloids, steroids, phenols, flavonoids, and carbohydrates. Anthraquinones, flavonoids, tannins, phenolic compounds, carbohydrates, and saponins have all been shown to exist in recent studies (24).

Triglycerides, HDL, LDL, and cholesterol were among the biochemical markers that were investigated in this study. Comparing the negative control group to the normal control group, our findings indicated a significant decrease in HDL levels and a significant increase in triglycerides, cholesterol, and LDL levels. In contrast to the negative control group, after 28 days, the methanol-treated group, which also received diazepane (2 mg/kg) and *Barleria cristata* (at 200 and 400 mg/kg), demonstrated a significant decrease in LDL, triglycerides, and cholesterol as well as an increase in HDL levels.

Conclusion

The current study demonstrates that *Barleria cristata* leaf methanolic extract exhibits substantial antihyperlipidemic capabilities at lower doses, specifically 200 mg/kg, and at higher doses, such as 400 mg/kg.

Declarations

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Not Applicable

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's Contribution

“The Concept was discussed by Mr. Rahul Jodh while Dr P. S. Kawtikwar prepared the writing original draft of the article followed by Ms. Ankita Kawtikwar with validation and data analysis, Mr. Rahul Jodh contributes with data curation, then Dr P. S. Kawtikwar Review and edit the article and finally Mr. Rahul Jodh done the formal analysis of the article. All the author read and approved the final manuscript.”

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Ethics Statement

The Institutional Animal Ethics Committee of SNIOP, Pusad, approved all protocols utilized in this work.

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