

ANTIHYPERLIPIDEMIC POTENTIAL OF EREMURUS HIMALAICUS BAKER IN RATS FED WITH HIGH FAT DIET

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ABSTRACT

Eremurus himalaicus is a plant which is commonly used in case of anaemia; as a galactagogue; in the treatment of migraine with insomnia etc. Also, it has been found to possess hepatoprotective and hypoglycaemic activities. However, its antihyperlipidemic potential is yet to be evaluated. The present study was intended to explore the antihyperlipidemic potential of *Eremurus himalaicus* extracts in cholesterol induced hyperlipidemia in rats. High cholesterol diet induced significant increase in serum total cholesterol, triglycerides, low density lipoprotein- cholesterol, very low density lipoprotein- cholesterol and significant decrease in high density lipoprotein-cholesterol. Also, it induced weight gain in rats. Treatment with *Eremurus himalaicus* extracts significantly decreased the serum levels of these biochemical parameters. The weight gain was also prevented in comparison to the positive control group. Highest activity was shown by Ethyl acetate group for which the AI was 1.89, which was almost equal to the normal group (1.73) and lower than the AI of standard (2.13), which indicates EHE is highly antihyperlipidemic in nature. The AI of EHM and EHA was 2.64 and 2.50 respectively. The LDL-C/HDL-C ratio increased in the order toxic < EHM < EHA < standard < EHE < normal, with the respective values of 3.87, 1.74, 1.70, 1.48, 1.30 and 1.17. Thus, *Eremurus himalaicus* significantly prevents hyperlipidemia in rats and can be used as a good antihyperlipidemic food.

Keywords: Anthrogenic index, *Eremurus himalaicus*, Hyperlipidemia, Lipoprotein, Triglycerides

I.INTRODUCTION

The term hyperlipidemia can be defined as the rise in lipid levels in blood. It is a metabolic disorder characterized by variations occurring in serum lipid and lipoprotein profile due to elevated levels of

Triglycerides (TAG), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C-C), and very low density lipoprotein cholesterol (VLDL-C-C-C) with a concomitant fall in the concentrations of high density lipoprotein cholesterol (HDL-C-C) in the blood circulation [1]. Hyperlipidemia characterized by hypercholesterolemia is known to be the most predominant indicator for vulnerability to cardiovascular diseases [2] which is the principal cause of death in both technologically advanced and developing nations. The prime risk factors for initiation and advancement of these diseases are disorders of lipid metabolism following oxidative stress. As per World health organization (WHO) reports, the high blood cholesterol contributes to roughly 56% cases of cardiovascular diseases throughout the world and causes about 4.4 million deaths per year [2]. Hyperlipidemia can be considered responsible for development of atherosclerosis and various other conditions which arise due to atherosclerosis [3]. The main purpose of treatment in patients with hyperlipidemia is to condense the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [4]. Traditional system of medicine like Ayurveda, Unani and Chinese recommend numerous herbal drugs for cardiovascular disorders. Currently available hypolipidemic drugs have been associated with a number of side effects which include hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dries skin and abnormal liver function [5]. Now-a-days, use of alternative medicines is rapidly increasing over the globe. As herbal medicines are less damaging than synthetic drugs they have better compatibility hence improving patient tolerance even on long-term use. Recently, some medicinal plants have been testified to be useful in hyperlipidemia worldwide and have been used empirically as remedies for hyperlipidemia. In literature, a number of plant species are available having antihyperlipidemic activity [6]. However, searching for new antihyperlipidemic drugs from natural plants is still appealing as these possess substances which exert their effects through various pathways. There are many plants which contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc., which are recurrently implicated as having antidiabetic activity. The herbal treatment for hypercholesterolemia has almost no side effects and is relatively cheap, as well as locally available. They are effective in reducing the lipid levels in the system thereby play a major role in antihyperlipidemic activity.

II.MATERIALS AND METHODS

2.1 Plant material collection and extraction

The plant under study, *Eremurus himalaicus*, was collected from the local area of Kashmir and the sample was authenticated from The Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir. The voucher specimen was retained in the herbarium of The Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir under herbarium number: 1765 (KASH) of Kashmir University.

The plant material was powdered and successively extracted by Soxhlet extraction method with different solvents of increasing polarity i.e., ethyl acetate, methanol and water. The recovered extracts EHE, EHM and EHA respectively were concentrated and then kept in desiccators to remove moisture.

2.2 Experimental animals

Albino male rats weighing 200-250g were used for the study. Animals were maintained under standard environmental conditions ($22 \pm 2^\circ\text{C}$, $55 \pm 5\%$ humidity, 12hr L:D cycle) in the departmental animal house and fed with a standard feed and water *ad libitum*.

2.3 Experimental protocol for antihyperlipidemic activity

The initial body weights of all experimental animals were recorded and the animals were divided into six groups, with five animals in each group

Group 1 (Normal) received 1% Sodium CMC suspension.

Group 2 (Toxic) received cholesterol in coconut oil at a dose of 25mg/kg/day.

Group 3 (Standard) received cholesterol in coconut oil along with Atorvastatin at a dose of 10mg/kg/day.

Group 4 (EHE) received cholesterol in coconut oil along with EHE at a dose of 300mg/kg/day.

Group 5 (EHM) received cholesterol in coconut oil along with EHM at a dose of 300mg/kg/day.

Group 6 (EHA) received cholesterol in coconut oil along with EHA at a dose of 300mg/kg/day.

The body weight of all rats was measured on the first and last day of experiment. The animals were given required dosage of cholesterol in oil at 10 am and at 3 pm the required dosage of extracts and standard were given to the respective groups. The study was carried out for a period of 30 days. On day 31, the animals were sacrificed (after 12 hour fasting) and the blood samples were collected by cardiac puncture. The liver tissue samples were also collected and preserved in 10% formalin solution. The serum samples were analyzed for TC, TG and HDL-C levels using autoanalyser. The LDL-C and VLDL-C values were calculated using Friedewald equation [7]. The AI was calculated by the following formula, $\text{AI} = (\text{Total cholesterol} - \text{HDL-C}) / \text{HDL-C}$. LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C levels [8].

2.4 Histopathological studies

The liver sections were processed and evaluated for histopathological changes with respect to deposition of fat due to hyperglycemia and the effect of extracts of *Eremurus himalaicus* and standard atorvastatin on the same.

2.5 Statistical analysis

The values were expressed as Mean $\pm$ S.D and analyzed by ANOVA followed by post hoc Tukey's multiple comparison t-test. Values < 0.05 were considered as statistically significant.

III. RESULTS

In order to assess the antihyperlipidemic potential of *Eremurus himalaicus* the physical as well as biochemical parameters were taken into consideration. The physical parameter included measuring the body weight on first and last day of experiment. It was evident from the percentage weight gain of toxic group that administration of cholesterol in coconut oil for a period of 30 days causes an increase in body weight (16.24%), as shown in Table 1. The standard (atorvastatin) and extract treated groups on the other hand also showed an increase in body weight but the weight gain was much lower than the toxic group. The weight gain in case of ethyl acetate group (6.29%) was almost similar to that of the normal group (6.37%).

Table 1: Effect of *Eremurus himalaicus* extracts on body weight of Wistar strain albino rats

Group	Initial body weight	Final body weight	% weight gain
Normal	164.6±12.64	175.8±13.62	6.37
Toxic	185.6±12.11	221.6±13.44	16.24
Standard	173.2±16.63	186.6±16.81	7.18
EHE	178.6±13.18	190.6±14.17	6.29
EHM	170±15.82	189.2±14.87	10.14
EHA	184±13.43	201±15.06	8.45

As far as biochemical parameters are concerned, the lipid profiles in normal, standard and extract treated groups are shown in Table 2. As expected, the administration of cholesterol in coconut oil caused an increase in the levels of total cholesterol, triglycerides, low density lipoproteins (LDL-C), very low density lipoproteins (VLDL-C) and decrease in high density lipoproteins (HDL-C), as is apparent from the levels of these hyperlipidemic markers in toxic group. This increase is statistically significant when compared with normal group ($p < 0.0001$). However, the administration of standard drug atorvastatin and the extracts of *Eremurus himalaicus* prevented this increase significantly. This is clear from Table 2, where the standard and extracts of *Eremurus himalaicus* are shown to significantly decrease the cholesterol, triglycerides, LDL-C and VLDL-C levels and increase the HDL-C levels ($p < 0.001$), when compared with toxic group. The atherogenic index (AI) and LDL-C/HDL-C ratio were also calculated. The AI of EHE was 1.89 which was very near to the normal group (1.73). This value was lower than the AI of standard (2.13) which means EHE is highly antihyperlipidemic in nature. The AI of EHM and EHA was 2.64 and 2.50 respectively and that of the toxic group was 5.91.

Table 2: Effect of *Eremurus himalaicus* extracts on serum TC, TG and HDL-C levels of Wistar strain albino rats

Group	TC	TG	HDL-C	VLDL-C= TG/5	LDL-C= (TC-HDL-C)-(TG/5)
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Normal	64.16±3.49	65.09±3.52	23.46±4.39	13.01±0.70	27.68±3.33
Toxic	84.71±4.05 ^a	125.20±4.10 ^a	12.24±2.83 ^a	25.04±0.82 ^a	47.42±3.35 ^a
Standard	67.51±3.17 ^c	70.78±4.75 ^c	21.51±4.16 ^d	14.15±0.95 ^c	31.84±3.42 ^c
EHE	66.64±4.42 ^c	68.64±4.12 ^c	22.99±3.16 ^e	13.72±0.82 ^c	29.92±2.16 ^c
EHM	76.72±3.94 ^{a,e,g}	94.12±4.34 ^{a,c,f}	21.06±3.73 ^d	18.82±0.86 ^{a,c,f}	36.83±2.02 ^{a,c}
EHA	70.55±3.48 ^c	80.92±3.92 ^{a,c,g}	20.13±3.65 ^e	16.18±0.78 ^{a,c,g}	34.23±2.38 ^{b,c}

Values expressed as mean±SD, n=6, One way ANOVA followed by Tukey's multiple comparison post hoc test.

a P<0.0001, b P<0.01 when compared with normal group;

c P<0.0001, d P<0.01, e P<0.05 when compared with toxic group;

f P<0.0001, g P<0.01 when compared with standard group.

As far as LDL-C/HDL-C ratio is concerned the values increased in the order toxic < EHM < EHA < standard < EHE < Normal, with the respective values of 3.87, 1.74, 1.70, 1.48, 1.30 and 1.17 (Table 3).

Table 3: Effect of *Eremurus himalaicus* extracts on atherogenic index and LDL-C-C/HDL-C ratio

Group	Atherogenic index (AI) = (TC-HDL-C)/HDL-C	LDL-C/HDL-C
Normal	1.73	1.17
Toxic	5.91	3.87
Standard	2.13	1.48
EHE	1.89	1.30
EHM	2.64	1.74
EHA	2.50	1.70

The histopathological report of the liver tissues revealed that there was fat deposition in the toxic group. The standard and the extract treated groups however, showed much less fat deposits in the liver tissues with most evident protection shown by ethyl acetate extract.

IV.DISCUSSION

The development of cardiovascular diseases is known to be hastened up by increase in the lipid levels in the body [9]. Verlecar et al reported that coronary artery disease (CAD) is one of the most important reasons of premature death and it is likely to be the supreme cause of mortality in India by the year 2010 [10]. Many medicinal plants have been and are being used for the treatment of hyperlipidemia in Unani and Ayurvedic systems of medicine. Also, the traditional system of medicine relies on plants and plant based products [11,12].

The current study has been carried out to determine the effect of various extracts of *Eremurus himalaicus* on physical (body weight and liver histology) and biochemical parameters (serum TC, TG, LDL-C, VLDL-C and HDL-C) of hyperlipidemia induced rats. The inducer of hyperlipidemia used in the study was high cholesterol diet and coconut oil. Coconut oil is a well known saturated fatty acid containing oil which along with dietary cholesterol results in buildup of intracellular cholesterol and cholesteryl esters in the body tissues. The results revealed that the extracts of *Eremurus himalaicus* prevent this accumulation of cholesterol and its esters. Hence, these possess significant antihyperlipidemic potential in cholesterol induced hyperlipidemia. The results were quite comparable to that of the standard drug atorvastatin. The antihyperlipidemic potential of *Eremurus himalaicus* extracts may be due to various reasons which include decrease in intestinal cholesterol absorption, effect on production of lipoproteins, amplification in hepatic LDL-C catabolism through hepatic receptors etc. [13, 14, 15, 16, 17, 18, 19]. All these events may be considered responsible for the decrease in blood cholesterol and LDL-C levels.

The extracts of *Eremurus himalaicus* also lead to an increase in the serum levels of HDL-C. HDL-C, also called “good cholesterol” helps in the transfer of triglycerides and cholesterol from the peripheral cells to the liver cells where it is catabolised and eliminated from the body [20]. HDL-C levels and the incidence of cardio vascular diseases share an inverse relationship [21].

It has been reported that LDL-C and VLDL-C are highly atherogenic. The increased TG levels are known to increase their level [22]. Thus, the decrease in the levels of TG by the standard and various extracts of *Eremurus himalaicus* may be attributed to increased lipolytic activity of Plasma Lipoprotein Lipase [1; 23].

It is well known that the atherogenic index and LDL-C: HDL-C ratio are important indicators of risk of coronary heart disease, irrespective of the serum cholesterol levels [2, 24, 25]. The effect of *Eremurus himalaicus* extracts on AI and LDL-C:HDL-C ratio was also in favour of their antihyperlipidemic activity. The histopathological studies also favoured the same, proving it to be a potential antihyperlipidemic plant.

V. CONCLUSION

It can be concluded from the present study that the ethyl acetate extract followed by aqueous and methanolic extracts significantly prevent the hyperlipidemia in Wistar strain albino rats. The effect is quite comparable to the standard antihyperlipidemic drug atorvastatin. Thus further research can be performed in order to find out the anti-hyperlipidemic leads from this plant.

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