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ANTI HYPERLIPIDEMIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *HYBANTHUS ENNEASPERMUS*

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ABSTRACT

Hybanthus enneaspermus family Violaceae, was investigated to evaluate the hyperlipidemic activity of hydroalcoholic extracts against high fat diet induced wistar albino rat. Significant anti-hyperlipidemic effects were obtained as evident from the restoration of biochemical parameters altered by cholesterol towards the normal. Almost normal histological appearance of liver was observed in treated groups. Among the three doses, 400 mg/kg showed better activity. However, the activity was found to be less than the standard atorvastatin given at 1.2 mg/kg dose. The results showed that the hydroalcoholic extract of *Hybanthus enneaspermus* has potential hypolipidemic effect along with recovery of liver functions.

KEYWORDS

Hybanthus enneaspermus, Atorvastatin and Antihyperlipidaemic.

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INTRODUCTION

Hybanthus enneaspermus, belonging to family Violaceae, is a herb or under shrub distributed in the tropical and subtropical regions of the world. It is an herb, often with woody troches, found in the warmer parts of India. The plant is popularly known as *Ratanpurus* (Hindi). An infusion of the plant extract is given in case of cholera[1]. The plant has been reported to have antiinflammatory[2], antitussive[3], antiplasmodial[4], anticonvulsant[5] and free radical scavenging activity. The plant is reported to contain aurantiamide acetate, isoarborinol, b-sitosterol and triterpene[6]. In folklore the plant is used in case of

pregnant and parturient women, and in case of gonorrhoea and urinary infections. The present study is intended to determine the antihyperlipidemic activity against high fat diet rat.

MATERIALS AND METHODS

Extraction of *Hybanthus enneaspermus*

The dried plant materials were milled into coarse powder by a mechanical grinder. The extracts were concentrated to dryness under vacuum. The plant powder (50 g) was extracted with 250 ml of hydroalcohol in a soxhlet apparatus for 72 hrs. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50 ºC) in a rotavapor.

Phytochemical analysis of *Hybanthus enneaspermus*

Test for saponins[7]

Foam test

Take 2 ml of extract with hydro alcohol in a test tube. To it add small amount of water, shake well, stable froth (foam) is formed.

Haemolysis test

Add 0.2 ml of extract (prepared in 1 % normal saline) to 0.2 ml of blood in normal saline and mix well. Centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10 % blood in normal saline diluted with 0.2 ml of normal saline.

Test for tannins

Ferric chloride test

A small amount of extract treat with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

Phenazone test

To the 5 ml of extract add 0.5 gm of sodium acid phosphate. Then warm it and filter. To the filtrate add 2 % Phenazone solution, precipitate is formed which is often coloured.

Gelatin test

To the extract add 1 % gelatin solution containing 10 % sodium chloride. Precipitate is formed.

Test for proteins

Biuret test

To the extract (2 ml) add Biuret reagent (2 ml), violet colour indicates presence of proteins.

Hydrolysis test

Hydrolyze the extract with hydrochloric acid or sulphuric acid. Then carry out the ninhydrin test for amino acid.

Xanthoproteic test

To the 5 ml of extract, add 1ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40 % sodium hydroxide solution, orange colour is formed.

Glycosides

Keller kiliani test

The test consists of extract with 10 ml 70 % alcohol for 2 to 3 minutes. The extract is filtered. To the filtrate is added, 5 ml water and 0.5 ml strong solution of lead acetate. Shake well and separate the filtrate. The clear filtrate is treated with equal volume of chloroform and evaporated to yield the extractive. The extractive is dissolved in glacial acetic acid and after cooling, 2 drops ferric chloride solution is added to it. These contents are transferred to a test tube containing 2 ml concentrated sulphuric acid. A reddish brown layer acquiring bluish-green colour after standing is observed.

Legal test

The extract is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline-pink or red colour is produced.

Baljet test

To the extract add sodium picrate solution is added. It shows yellow to orange colour.

Test for alkaloids[8]

The qualitative chemical tests used for detection of alkaloids are dependent on the characters of alkaloids to give precipitates as salts of organic acids or with compounds of heavy metals, like mercury, gold, platinum, etc. The different reagents used are Mayer's reagent (potassium mercuric iodide solution) giving cream colored precipitate. Dragendorff's reagent (potassium bismuth iodide solution) giving reddish brown precipitate. Wagner's reagent (iodine - potassium iodide solution) yielding

reddish brown precipitate. Some alkaloids also give yellow coloured precipitates with picric acid called as Hagner's reagent and picrolonic acid. Individual alkaloid gives colour or precipitate with certain specific reagent.

Dragendorff's test

To the extract, add few drops of Dragendorff's reagent. Orange brown precipitate is formed.

Mayer's test

To the extract, add few drops of Mayer's reagent give precipitate.

Hager's test

To the extract, add few drops of Hager's reagent give yellow precipitate.

Wagner's test

To the extract add few drops of Wagner's reagent give reddish brown precipitate.

Test for carbohydrates[9]

Molisch's test

The test is positive with soluble, as well as, insoluble carbohydrates. It consists of treating the compounds with alpha napthol and concentrated sulphuric acid which gives purple colour ring at the junctions of two layer.

Reduction of fehling's solution

To the extract, equal quantity of Fehling's solutions A and B is added. After heating, brick red precipitate is obtained.

Test for flavonoids

Shinoda test

To the extract, add 5 ml 95 % ethanol, few drops concentrated HCl and 0.5 gm magnesium turnings. Pink colour observed.

Experimental procedures

Animals

Male Wistar Albino rats (200-220 g) were used for anti-hyperlipidemic activity. The animals were fed on a standard pellet diet and water *ad libitum*. Environmental conditions were standardized (temp 23 ± 2 ºC, humidity 55 – 60% with a 12 h light and dark cycle). Animals were fasted, but allowed water 12 h prior to the experiments. Animal studies were performed according to the prescribed guidelines of CPCSEA, Government of India.

Acute toxicity studies

Acute oral toxicity study in rats was carried out as per OECD-425 guidelines. Graded doses (200, 500 and 2000 mg/kg bw) of hydroalcoholic extract of *Hybanthus enneaspermus* was administered orally to various groups of rats containing ten in each group. The animals were observed for mortality, clinical signs and body weight changes for a period of 0, 1, 2, 4 and 24 hours[9].

Anti -Hyperlipidemic activity

Preparation of 2 % cholesterol diet

2 g of cholesterol and 0.5 g of Cholic acid was thoroughly mixed and mashed with 97.5 g of standard rat diet and was given in the form of pellets. Animals were divided into six groups of six animals each. Group I was served as normal control and received fed on regular standard rat diet till end of study. Groups II animals served as disease control and was treated for initial four weeks with high fat diet. After baseline lipid profile at end of four weeks, this group was fed on regular rat diet till end of study. Group III, IV and V received fed on 2 % cholesterol diet for initial six weeks and then was given hydroalcoholic extract of *Hybanthus enneaspermus* 100, 200 and 400 mg/kg, respectively. Group VI received fed on 2 % cholesterol diet for initial four weeks and then was given with Atorvastatin at the dose 1.2 mg/kg orally 10 days. All these treatments were given orally once daily for 10 days.

Induction of hyperlipidemia

The high fat diet was prepared by mixing 2 % (w/w) cholesterol and 1% (w/w) cholic acid in standard animal chow and administered for 4 weeks. All the rats except the normal control were fed with high fat diet. The normal control group was fed with standard chow only. At the end of 4th week, total cholesterol level in serum was estimated and the animal with greater than 250 mg/dl level was selected and considered as hyperlipidemia rats. At the end of the study, blood was collected by retro-orbital plexus puncture under mild ether anaesthesia from overnight fasted rats and serum was separated and analyzed for total cholesterol, HDL, LDL and TG.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett Multiple Comparison Test. P values < 0.05 were considered as significant.

RESULTS

Anti-hyperlipidemic activity of hydroalcoholic extracts of *Hybanthus enneaspermus*

The preliminary phytochemical screening like Saponins, Amino acids, Proteins, Glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the hydroalcoholic extract of *Hybanthus enneaspermus* according to the procedure. In the above chemical test the hydroalcoholic extract of *Hybanthus enneaspermus* gives positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids except glycosides. The results of preliminary test of hydroalcoholic extract of *Hybanthus enneaspermus* were shown in Table No.1. Acute toxicity results showed no clinical signs of toxicity and mortality of the animals. Therefore, an LD_{50} > 2000 mg/kg bw may be assumed. Hyperlipidemic induced rats showed significant increase in the levels of total cholesterol, LDL, TGL and VLDL and a significant reduction in the level of HDL when compared to normal animals was shown in Table No.2. Treatment with hydroalcoholic extracts of *Hybanthus enneaspermus* at all the three doses and Atorvastatin caused a significant reversal of all these changes towards the normal. Among the three doses, hydroalcohoic extract of *Hybanthus enneaspermus* at 400 mg/kg dose was found to be more active[10, 11]. However, the test drug having better activity in all the above parameters the lipid parameters was show in Figure No.1.

DISCUSSION

The *Hybanthus enneaspermus* is a rich source of flavonoids, which contributes to reduced thrombotic tendencies and also cholesterol lowering effects by alteration in cholesterol absorption, triglycerides assembly and processing of lipoproteins in plasma. Multiple functions of dietary polyphenols help in reduction of coronary heart disease risk by improving plasma lipid profile. Treatment of hyperlipidaemia is a lifelong battle. Similar side effects are a possibility with *Hybanthus enneaspermus* has found the toxic effects of *Hybanthus enneaspermus* species in liver of albino rats as shown by their significantly raised liver enzyme levels and disturbed liver histology and this finding has been confirmed with our histopathological observations**.** Future studies are needed to evaluate these issues. Whether alteration in lipid profile parameters caused by *Hybanthus enneaspermus* in experimental rats is reflected in the atherosclerotic process also needs to be addressed.

Table No.1: Phytochemical screening results of *Hybanthus enneaspermus*

S.No	Phytoconstutient	Result
1	Saponins	Present
2	Tannins	Absent
3	Amino acids	Present
4	Proteins	Present
5	Glycosides	Present
6	Alkaloids phytosterols	Present
7	Carbohydrates	Present
8	Flavonoids	Present

Table No.2: Effect of the hydroalcoholic extract of *Hybanthus enneaspermus* on cholesterol levels in hyperlipidemic rats

S.No	Mean	Total cholesterol	HDL	LDL	TGL	VLDL	AI
1	Normal	109.00±14.14	12.00±02.83	100.00±14.14	117.50±10.61	11.00±1.41	1.00±0.06
2	HFD	310.00±28.28	08.00±01.41	130.00±09.60	167.50±03.54	16.70±3.54	1.32±0.08
3	ATOR (1.2 mg/kg)	185.00±07.07*	12.50±02.12*	095.00±07.07*	104.50±06.36*	12.50±3.54*	0.85±0.04*
4	*HE* (100 mg/kg)	265.00±14.14**	09.50±00.71**	115.00±09.08**	125.00±07.07**	15.00±1.41**	1.00±0.01**
5	*HE* (200 mg/kg)	195.00±21.21**	11.00±00.71**	105.00±05.10**	110.00±14.14**	13.76±1.48**	0.94±0.03**
6	*HE* (400 mg/kg)	120.00±14.14***	12.00±02.83***	085.00±07.07***	097.50±10.61***	13.50±2.12***	0.87±0.16***

Between groups comparison was done using one way ANOVA, Values are in mean ± SE; Number of animals in each group = 6; *p < 0.05 Vs Group I; ** p <0.05 Vs Group III,
*** p <0.05 Vs Group I.

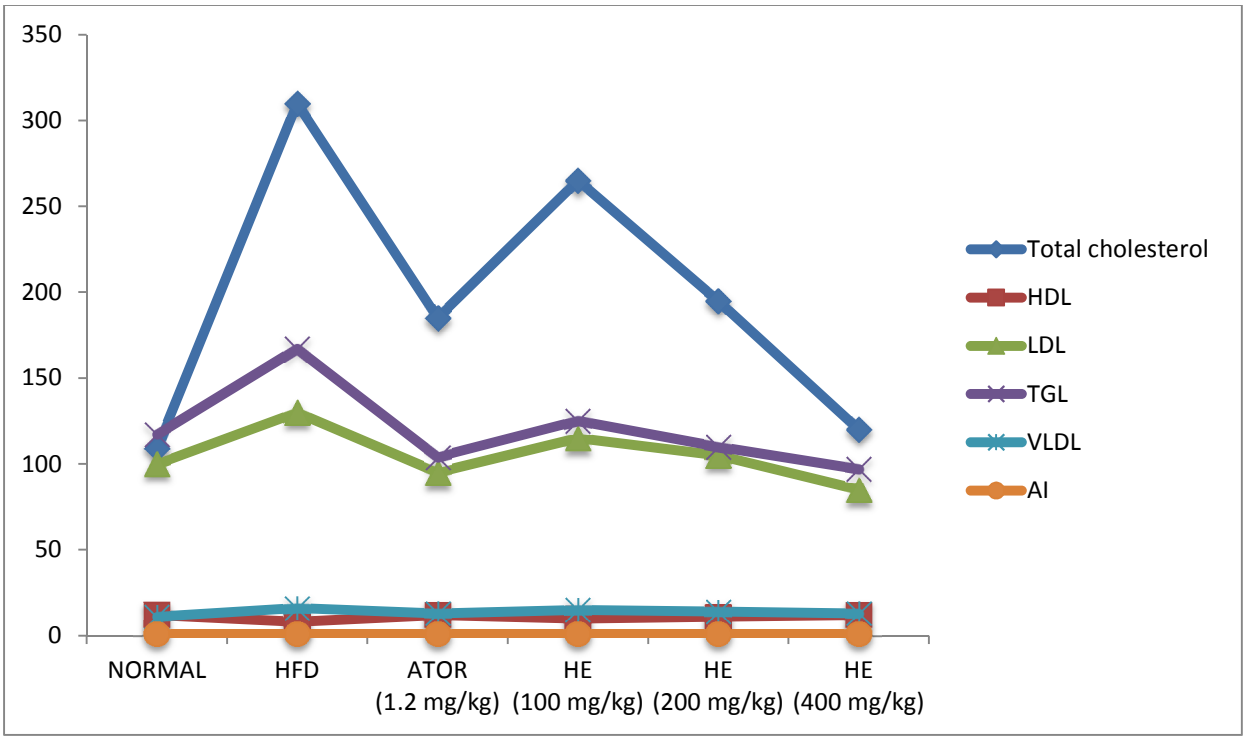

Figure No.1: Effect of the hydroalcoholic extract of *Hybanthus enneaspermus*

CONCLUSION

The *Hybanthus enneaspermus* has a potential to be used in the treatment of hyperlipidemia as this is the risk factor for cardiovascular disease. Data on the short and long term adverse effects of *Hybanthus enneaspermus* ingestion needs to be collected. Further pharmacological investigations are in progress to support that *Hybanthus enneaspermus* is a kind of promising extract and can be developed to a new drug hopefully.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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