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EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF THE PLANT CYPERUS ROTUNDUS IN POLOXAMER INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

Atherosclerosis is the major ca**u**se of morbidity and mortality in the world. The basic cause of atherosclerosis is attributed to diets rich in fats alcoholic consumption genetic factors like decreased β cell function, diabetes mellitus elevated levels of LDL, triglycerides, with reduced HDL Cholesterol. Present study focused to evaluate Antihyperlipidemic Activity of the Plant Cyperus rotundus in Poloxamer Induced Hyperlipidemic Rats. This present study shows the efficacy of Cyperus rotundus Linn. in lowering of total cholesterol, triglyceride, LDL, and VLDL cholesterol.

KEY WORDS: Antihyperlipidemic, Cyperus rotundus, Hyperlipidemic.

INTRODUCTION

CAD is a serious medical problem that affects millions of people throughout the world. People who are predisposed to a combination of risk factor (dietary habits, genetic susceptibility) are more prone to develop hyperlipidemia. Alkaloids, flavono.ids and other natural substances have also been shown to be effective in reducing lipid profile. Cyperus rotundus (cypearaceae), commonly known as nagarmotha shows antihyperlipidemic activity. The rhizomes of Cyperus rotundus on preliminary chemical analysis is found to contain flavonoids, β-sitosterol, sesquiterpenoids.

The tubers of Cyperus rotundus were collected Chhatarpur Distt, and Prof. B.K.Pathak, Head, Department of Pharmacy, Barkatullah University Bhopal identified the plant.

Preparation of drug solution

The ethanolic extract of tubers was evaporated to dryness. The dried extract was dissolved in 0.3% w/v CMC, and used for antihyperlipidemic studies. Two different dose levels i.e. 200 and 400mg/kg body weight were administered orally.

Atorvastatin, 75 mg/kg b.wt was administered in 0.3% CMC, orally.

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Induction of Hyperlipidemia

Hyperlipidemia was induced by Poloxamer (1gm/kg b. wt) administered i.p.

Experimental Setup

Acute model

The male rats weighing 250-300 gm was used for the experimental study. The animals were divided into five groups of 6 animals each.

Group I: Vehicle control (0.3% CMC orally)

Group II: Hyperlipidemic control (Poloxamer, 1gm/kg i.p)

Group III: Positive control (Atorvastatin 75 mg/kg orally)

Group IV: Hydro alcoholic extract of Cyperus rotundus (200 mg/kg orally)

Group V: Hydro alcoholic extract of Cyperus rotundus (400 mg/kg orally)

The test drug was administered orally for four days at a two different dose level 200 and 400 mg/kg. The hyperlipidemia was induced by single i.p. injection of poloxamer (1gm/kg) 48 hr prior to blood collection. On 4^{th} day the blood was collected by retro orbital sinus puncture under light ether anesthesia. The blood was centrifuged at 2500 rpm for 10 minutes. The plasma was separated and was used for various biochemical estimations. The animals were sacrificed and aorta was excised and stored in 10% buffered neutral formalin for histopathological studies.

Tissue homogenate preparation

Liver, kidney were sliced in to pieces and homogenized in appropriate buffer in cold condition to give 20% homogenate. The homogenate was centrifuged at 3000 rpm for 20 min

and the supernatant was used for LPO assay .i.e. Thiobarbituric acid reactive substances: (TBARS) and conjugated dienes were estimated.

Biochemical parameters evaluated

Lipid Profile in Plasma

The following lipids were estimated in plasma.

a) Total Cholesterol (TC)
b) HDL Cholesterol (HDL-C)
c) VLDL Cholesterol (VLDL-C)
d) LDL Cholesterol (LDL-C)
e) Triglycerides (TG)

Non-Enzymic Antioxidants (Lipid Peroxidation)

The following non-enzymic antioxidants were estimated in tissue homogenates of liver / kidney

a) Thiobarbituric acid reactive substances
b) Conjugated Dienes

ESTIMATION OF LIPIDS

Total Cholesterol (TC)

Cholesterol in plasma was estimated using an Ecoline Diagnostic Kit. reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase and its esters were released from lipoproteins by detergents. Cholesterol esterase hydrolysis the esters. enzymatic oxidation by cholesterol oxidase, H_2O_2 was formed. The reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase Cholesterol converted into a coloured quinonimine.

Cholesterol level in plasma was expressed as mg/dl.

HDL Cholesterol

The HDL cholesterol was separated from plasma after precipitation of LDL and VLDL cholesterol by precipitating reagent phosphotungstic acid.

The supernatant fluid after centrifugation was estimated using Ecoline diagnostic kit.

The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. HDL Cholesterol level in plasma was expressed as mg/dl.

LDL Cholesterol

LDL Cholesterol was calculated by using the formula

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \left[\text{HDL Cholesterol} - \frac{\text{Triglycerides}}{5}\right]$$

LDL Cholesterol level in plasma was expressed as mg/dl.

VLDL Cholesterol

VLDL Cholesterol was calculated by the formula

VLDL Cholesterol = (Triglycerides)/5

VLDL Cholesterol level in serum/plasma was expressed as mg/dl.

Triglycerides (TG)

Triglyceride level in plasma was estimated using an Ecoline Diagnostic Kit.

The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. Triglyceride level in plasma was expressed as mg/dl.

Atherogenic Index

The atherogenic index was calculated using the formula.

$$\text{Atherogenic Index} = \frac{\text{(LDL + VLDL)}}{\text{HDL}}$$

HDL Ratio

$$\frac{\text{HDL Cholesterol}}{\text{Total Cholesterol} - \text{HDL Cholesterol}} \times 100$$

LIPID PEROXIDATION

Thiobarbituric acid reactive substances: (TBARS).

The reaction with thiobarbituric acid (TBA) in acidic generates a pink coloured chromophore, which was read in UV spectrophotometer at 535 nm.

To 1 ml of tissue homogenate, 2 ml of TCA-TBA-HCl reagent was added and mixed thoroughly. The mixture was kept in a boiling water bath for 15 minutes. After cooling the tubes were centrifuged at 1500 rpm for 10 minutes and the colour developed in the supernatant was measured in UV spectrophotometer at 535 nm against a reagent blank. A series of standard solutions in the concentration range of 10-100 nmoles were treated in a similar manner.

The amount of TBARS was expressed as µmoles/mg of tissue.

Conjugated Dienes (CD)

Lipid peroxidation is associated with the rearrangement of double bonds in polyunsaturated fatty acids leading to the formation of conjugated dienes, which absorb light at 233 nm. The oxidation index of the lipid sample at 233 nm and 215 nm was computed which reflect the diene content and the extent of peroxidation.

RESULTS

Result on Experimental Models

The dose of Poloxamer (P407) chosen from a preliminary dose response study in rats was 1g/kg and the optimum time for measurement of hyperlipidemia was determined to be 48 hours. In acute studies, lipid values in normal rats were compared with P407 (1 g/kg) treated rats 48 hour post hyperlipidemia induction. Triglyceride levels were increased, Total Cholestrol levels increased, no significant effect on HDL.Total cholesterol levels and LDL levels increased, VLDL levels was increased.All these increased plasma lipid levels were statistically significant ($P < 0.001$).

Atorvastatin given by oral gavage (75 mg/kg for 4 days). This group was used as a positive control. In this study three groups were compared; a normal control group (group I), Hyperlipidemic control group (group II), received 1 g/kg P407, i.p. Rats in these two groups were given an oral gavage of' 0.3% w/v CMC daily for 4 days and Positive control group (group III) given 75 mg/kg Atorvastatin, p.o for 4 days and an i.p, injection of P407 (1 g/kg) was given 48 hr prior to blood collection. Triglyceride level, Cholestrol level, LDL levels decreased VLDL levels was decreased HDL cholesterol level was increased.

Rats were given Cyperus rotundus for 4 consecutive days, after which hyperlipidemia was induced by injecting P407 48 hour prior to blood collection. Cyperus rotundus was found effective in significantly reducing both Triglyceride and Total Cholestrol levels after 4 days of pretreatment at a dose of 200 and 400 mg/kg. Cyperus rotundus significantly decreased Triglyceride levels Total Cholestrol. No significant changes were seen on HDL cholesterol all these decreased levels were statistically significant ($p<0.001$).

Effect of Cyperus rotundus on serum lipid profile in poloxamer induced hyperlipidemic rats.

Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL- C (mg/dl)	LDL –C (mg/dl)	VLDL –C (mg/dl)
Control, 0.3% w/v CMC, p.o	43.0±0.7071	56.6±1.568	42.8±0.7348	55.80±2.709	12.72±0.3137
Hyperlipidemic Control, Poloxamer (1 g/kg, i.p)	182.8±2.478*	566.8±2.871*	43.4±0.9274*	761.2±4.974*	23.38±0.5741*
Positive Control, Atorvastatin (75 mg/kg, p.o)	147.6±3.945	468.0±4.183	64.6±1.3270	548.0±5.431	9.536±0.8367
Cyperus rotundus (200mg/kg, p.o)	166.0±3.450*	494.4±4.675*	39.8±0.7348*	622.8±6.674*	17.97±0.9351*
Cyperus rotundus (400mg/kg, p.o)	130.8±3.625*	487.8±3.007*	45.8±1.0680*	591.8±5.643*	16.67±0.6013*

All the values were expressed in Mean ± S.E.M. of six animals.

*denotes statistical significance in comparison to treated group with control group at p<0.001.

Effect of Cyperus rotundus on Atherogenic index and HDL ratio in poloxamer induced hyperlipidemic rats.

Treatment	Atherogenic index	HDL ratio in%
Control, 0.3% w/v CMC, p.o	1.728	264.6
Hyperlipidemic Control, Poloxamer (1 g/kg, i.p)	24.208*	54.20*
Positive Control, Atorvastatin (75 mg/kg, p.o)	9.585	105.20
Cyperus rotundus (200mg/kg, p.o)	16.970*	58.90*
Cyperus rotundus (400mg/kg, p.o)	14.674*	84.140*

All the values were expressed in Mean ± S.E.M. of six animals.

*denotes statistical significance in comparison to treated group with control group at p<0.001.

Table. 5. Effect of Cyperus rotundus on lipid peroxidation (TBARS and CD) in poloxamer induced hyperlipidemic rats.

Treatment	TBARS (in µmoles/mg of tissue)	Conjugated Dienes (CD) (in µmoles/mg of tissue)
Control, 0.3% w/v CMC, p.o	83±2.00	0.267±0.004
Hyperlipidemic Control, Poloxamer (1 g/kg, i.p)	218±3.75*	0.661±0.005*
Positive Control, Atorvastatin (75 mg/kg, p.o)	152.4±3.69	0.464±0.003
Cyperus rotundus (200mg/kg, p.o)	197.2±2.57*	0.602±0.004*
Cyperus rotundus (400mg/kg, p.o)	188.2±4.01*	0.565±0.005*

All the values were expressed in Mean ± S.E.M. of six animals.

*denotes statistical significance in comparison to treated group with control group at p<0.001.

DISCUSSION

Triglyceride level, Cholestrol level, LDL levels decreased VLDL levels was decreased HDL cholesterol level was increased.

Cyperus rotundus was found effective in significantly reducing both Triglyceride and Total Cholestrol levels after 4 days of pretreatment at a dose of 200 and 400 mg/kg. Cyperus rotundus significantly decreased Triglyceride levels Total Cholestrol. Current model (Poloxamer- P 407 induced hyperlipidemic rat model) appears to be reproducible, sensitive, and may have applicability for screening of various sub-fractions of herbal drugs, traditional medicines, for their anti-hyperlipidemic activity.

The present study was designed to examine whether Cyperus rotundus show hyperlipidemic response. The effect of Cyperus rotundus on lipid levels was tested using the P407 model at two dose levels of 200 and 400 mg/kg. Cyperus rotundus was effectinive acute (4 day) administration to reduce TG and TC levels.

CONCLUSION

This present study demonstrated the efficacy of Cyperus rotundus in lowering of total cholesterol, triglyceride, LDL, and VLDL cholesterol in Poloxamer induced hyperlipidemic in acute rat models. These results were very well correlated with the histopathological findings i.e. absence of foam cells indicated that the antihyperlipidemic activity of Cyperus rotundus at 400 mg/kg dose.

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