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Anti Hyperlipidemic Activity of *Pedalium murex* (Linn.) Fruits on High Fat Diet Fed Rats

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Abstract: The main objective of the study was to investigate about the anti hyperlipidemic potential of the ethanolic extract from the fruits of *Pedalium murex* at doses of 200 and 400 mg/kg/p.o. in high fat diet fed rats. Biochemical parameters like serum total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (TG) levels were measured and compared with animals concurrently treated with reference standards Gemfibrozil and Atorvastatin. The ethanolic extract showed a significant decrease in triglycerides ($p<0.01$), LDL ($p<0.001$), VLDL ($p<0.01$), cholesterol ($p<0.001$) and a significant increase in HDL ($p<0.05$) levels at the tested doses.

Key words: *Pedalium murex*, anti hyperlipidemic activity, hyperlipidemia, high fat diet

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. It has been postulated that in many individuals excess weight gives rise to cardiovascular disease, type 2 diabetes mellitus, hypertension, stroke, dyslipidemia, osteoarthritis and some cancers (Brai *et al*., 2004). Hyperlipidemia is characterized by elevated serum total cholesterol and low -density and very low-density lipo protein levels. Hyper-lipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (Saravanan *et al*., 2003). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease. Lowering of circulating cholesterol especially the low density lipoprotein-cholesterol (LDL-Ch) fraction can prevent arrest or even reverse coronary atherosclerosis (Oyetayo, 2006) The main aim of treatment in patients with Hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease (Davey Smith and Pekkanen, 1992). The currently available hypolipidemic drugs have been associated with a number of side effects, including hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Speight, 1988). It has been reported that traditional systems have immune potential against various diseases. More than thirteen thousand plants have been studied for various pharmacological properties. Herbal therapy for hypercholesterolemia has no side effects and economic effective in reducing the lipid levels (Berliner and Suzuki, 1996). Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated by anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosisorhypothyroidism needs the treatment of the original disease rather than Hyperlipidemia (Suzuki and Suzuki, 2006). India has a rich heritage of medicinal herbs which are used by the local population and traditional practitioners for the treatment of several conditions. *Pedalium murex*, commonly called Gukhru in India belonging to the family Pedaliaceae is distributed in the coastal areas of south India (Nadkarani, 1982). An infusion or extract prepared from the leaves, stem and fruits in cold water is demulcent and a diuretic found useful in the disorders of urinary systems such as gonorrhea, dysuria and incontinence of urine etc (Chopra *et al*., 1999; Shukla and Khanuja, 2004). An aqueous extract from the plant has been evaluated for its analgesic and antipyretic activities (Muralidharan and Balamurugan, 2008). The diuretic potential of the fruits was taken as a base in order to assess the anti hyperlipidemic properties of *P. murex*. The ethanolic extract of the fruit was used for the study.

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MATERIALS AND METHODS

Plant material: The fruits of *P. murex* were collected from the coastal areas of Chennai dist, Tamil Nadu state, south India during the month of December, 2007 and identified by Dr. P. Jayaraman (Director), Plant Anatomy Research Centre, Chennai. The voucher specimen was deposited in the Dept.of Pharmacology, C.L. Baid Metha College of Pharmacy (Voucher Specimen No. 02/08).

Preparation of ethanolic extract: The fruits were collected and dried in shade for 15 days and made to coarse powder. The powder was passed through sieve No. 40 to achieve uniform particle size and then used for extraction process. A weighed quantity of the powder was subjected to continuous hot extraction in soxhlet apparatus with 99% ethanol. The extract was evaporated under reduced pressure using rotovac evaporator until all solvent was removed to give a molten extract with a yield of 36% w/w. The ethanolic extract of *Pedalium murex* (EEPM) was used for the study.

Semi synthetic high fat diet: Semi synthetic basal diet for the rats was prepared (Barney *et al*., 1968). It contains (g/100 g of diet) casein-30 g; agar-2 g; cellulose-8 g; sucrose-51 g; vitamin mixture-0.5 g and mineral mixture-0.5 g. Hyperlipidemia was induced by addition of cholesterol 1.5 g and coconut oil 8 mL for every 100 g of basal diet.

Acute toxicity study: Albino rats of either sex weighing 230-250 g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines (Ecobichon, 1997). The animals were fasted overnight, provided only water, after which the drug EEPM was administered to the respective groups orally at the dose level of 5 mg kg^{-1} body weight by gastric intubation and the groups observed for 14 days. If mortality was observed in 2 or 3 animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg kg^{-1} body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h.

Experimental animals: Albino rats of either sex, weighing 230-250 g were obtained from the animal house of C.L. Baid Metha College of Pharmacy. The animals were divided into six groups of six animals each. The study was conducted after clearance from the Animal Ethical Committee.

Group 1: Served as control and received normal feed and water *ad libitum.*

Group 2: Served as high fat control and received high fat diet and water *ad libitum.*

Group 3: Served as reference standard I and received Gemfibrozil 18 mg/kg/p.o. in 1% sodium CMC concurrently with the high fat diet for a period of 30 days.

Group 4: Served as reference standard II and received Atorvastatin 1.14 mg/kg/p.o. in 1% sodium CMC concurrently with the high fat diet for a period of 30 days.

Group 5: Animals received EEPM 200 mg/kg/p.o. in 1% Sodium CMC concurrently with the high fat diet for a period of 30 days.

Group 6: Animals received EEPM 400 mg/kg/p.o. in 1% Sodium CMC concurrently with the high fat diet for a period of 30 days.

All animals had free access to the diet and water. The daily diet consumed by animals was calculated by subtracting the leftover diet the next day from the previous day's added diet. The body weight of each animal was recorded every day.

Collection of blood samples and biochemical analysis from serum: At the end of the experiment on day 30, blood was collected 4 h after the last drug administration using light ether anesthesia. Blood samples were collected separately from retro orbital sinus puncture into sterilized dry centrifugation tubes containing sodium citrate 0.01%. Samples were allowed to stand for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min using micro centrifuge. The biochemical investigation was carried out to a assess total cholesterol (McGowan *et al*., 1983), high density lipoprotein (Burstein *et al*., 1970), low density lipoprotein, very low density lipoprotein (Friedewald *et al*., 1972) and triglycerides (Buccolo and David, 1973; Fossati and Lorenzo, 1982).

Statistical analysis: The data's were analyzed by one way ANOVA followed by Dunnet's t-test using Analyse. It ANOVA software package and Microsoft Excel, $p<0.05$ was considered significant.

The percentage of lipid lowering was calculated as shown:

$$\text{Lipid lowering (\%)} = \frac{\text{HFD-fed animals lipid values-Drug+HFD-fed animals lipid values}}{\text{HFD- fed animal lipid values}}$$

RESULTS AND DISCUSSION

EEPM produced non toxic symptoms or mortality up to dose level of 2000 mg kg^{-1} body weight orally in rats and hence the drug was considered safe for further

Table 1: Effect of EEPM on various biochemical parameters in hypercholesterolemic rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
TC (mg dL^{-1})	110.252± 000.4242	163.498± 000.377**(a)	126.823± 000.322**(b) (-22.43%)	125.993± 000.263**(b) (-22.94%)	130.298± 000.243*(b)(c-ns) (-20.3%)	124.242± 000.317**(b)(c-ns) (-24.0%)
TG (mg dL^{-1})	156.664± 000.603	224.277± 000.544**(a)	162.112± 000.454**(b) (-27.71%)	170.133± 000.33**(b) (-24.14%)	194.718± 000.278*(b)(c-ns) (-13.18%)	188.825± 000.381*(b)(c-ns) (-15.8%)
LDL-c (mg kg^{-1})	92.121± 00.4824	132.29± 000.367**(a)	104.654± 000.187**(b) (-20.89%)	108.39± 000.272**(b) (-18.06%)	105.843± 000.191**(b)(c-s) (-20.0%)	101.032± 000.279**(b)(c-ns) (-23.62%)
VLDL-c (mg kg^{-1})	23.232± 00.1986	44.85± 00.108**(a)	32.986± 00.453**(b) (-26.45%)	34.02± 00.066**(b) (-24.14%)	38.94± 00.055*(b)(c-ns) (-13.17%)	37.76± 00.075*(b)(c-ns) (-15.81%)
HDL-c (mg kg^{-1})	76.232± 00.0864	68.070± 00.095*(a)	77.848± 00.183**(b) (+14.36%)	81.987± 00.163**(b) (+20.44%)	70.498± 00.149#(b)(c-ns) (+3.56%)	72.805± 00.138#(b)(c-ns) (+6.96%)
Body weight (g)	249.667± 04.1123	283.83± 4.22**(a)	264.2448± 4.435**(b) (-6.9%)	277.167± 4.46**(b) (-2.3%)	193.167± 2.725**(b)(c-ns) (-31.94%)	159.0± 002.732**(b)(c-ns) (-43.98%)
Feed intake (g/rat/day)	24.8±0.3	26.2±0.5(ns)	25.6±0.4(ns)	26.3±0.5(ns)	27.1±0.4(ns)	25.9±0.2(ns)

(All values are Mean±SE of 6 rats in each group), a: Comparison of Group 1 vs. Group 2; b: Comparison of Group 2 vs. Group 3, Group 4, Group 5 and Group 6 c: Comparison of Group 3 vs. Group 3, Group 4, Group 5and Group 6; ns: non-significant; # Significant ($p<0.05$); *Significant ($p<0.01$); **Highly significant ($p<0.001$); Values in parentheses indicates percent increase (+) or decrease (-) with reference to values of Group 2 vs. Group 3, Group 4, Group 5 and Group 6

pharmacological screening. According to OECD-423 guidelines for acute oral toxicity, the LD_{50} dose of 2000 mg kg^{-1} and above is categorized as unclassified.

Administration of Ethanolic extract of *Pedalium murex* fruits (EEPM) at 200 mg/kg/p.o. to hypercholesterolemia induced animals resulted in a decrease of total cholesterol (20.3%), triglycerides (13.18%), LDL-cholesterol (20%), VLDL-cholesterol (13.17%) and an increase in HDL-cholesterol (3.56%). With 400 mg/kg/p.o. of EEPM treatment, a further reduction occurred in total cholesterol (24%), triglycerides (15.8%), LDL-cholesterol (23.62%), VLDL-cholesterol (15.81%) and an increase in HDL-cholesterol (6.96%). This reduction in total cholesterol, triglycerides, LDL-, VLDL-cholesterol and an increase in HDL-cholesterol was dose dependent and significant (Table 1). There was also a significant reduction in the body weight of the hypercholesterolemia induced animals, (31.94% at 200 mg/kg/p.o. and 43.98 at 400 mg /kg/p.o.).

It is well known that increased HDL-cholesterol levels have a protective role in Coronary artery disease (Wilson *et al*., 1988). Similarly increased level of serum LDL-cholesterol results in increased risk for the development of atherosclerosis (Warnholtz *et al*., 2001). The increased level of HDL-cholesterol and decreased cholesterol level along with its LDL fraction which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastro intestinal tract. Thus the decreasing cholesterol levels in the body under the influence of *P. murex* could have enhanced the enzymatic activity by a positive feedback mechanism. Also, the results suggests that cholesterol lowering activity of the herbal extract can be due to rapid catabolism of LDL-cholesterol through its hepatic receptors for final elimination in the form of Bile acids (Khanna *et al*., 2002). It may be concluded that EEPM at concentrations of 200 and 400 mg kg^{-1} significantly lowers the increased levels (due to high fat diet) of serum total cholesterol, triglycerides, LDL and VLDL in a dose dependent manner. EEPM treatment also raises the serum HDL levels. The above anti hyperlipidemic activity of EEPM was comparable with the effect exhibited by the standard drugs, Gemfibrozil and Atorvastatin.

Thus the anti hyperlipidemic activity of EEPM may be due to interference in the absorption of lipids from the gut, a mechanism similar to Gemfibrozil or due to interference in the enzymatic process of lipid metabolism like HMG-CoA reductase inhibitors, Atorvastatin or both. The exact mechanism involved has to be investigated through further studies.

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