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Effect of ethanolic extract of *Cyclea peltata* Lam on a hypercholesterolemic rat model.

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Abstract :
The current investigation focuses attention on the cholesterol lowering property of the ethanolic extract of *Cyclea peltata* Lam on experimentally induced hypercholesterolemia in rats. The serum cholesterol (total and low density lipoproteins) and triglyceride levels of rats fed with a diet containing, by weight, 18% casein, 1% cholesterol and 0.5% cholic acid increased, when compared to rats fed with a normal diet. The levels of total cholesterol (TC), triglycerides (TG) and LDL cholesterol (LDL-C) were reduced significantly ($P<0.05$), while HDL cholesterol (HDL-C) levels increased ($P<0.05$) in rats given, *C. peltata* extract orally at a dose of 100 mg/kg body wt./day for 30 days. Furthermore, the serum thiobarbituric acid-reactive substance levels decreased significantly ($P<0.05$) after administration of the extract, indicating that *C. peltata* could prevent hypercholesterolemia by reducing lipid peroxidation. This study, thus demonstrates that this plant may be a useful therapy for hypercholesterolemia through reducing oxidative stress and cholesterol levels.

Key words: *Cyclea peltata* Lam, hypercholesterolemia, atherosclerosis, thiobarbituric acid-reactive substance.

Introduction

Atherosclerosis, a common cause of heart disease, accounts for 7% of the mortality worldwide (1). It is characterized by deposition of plaques of cholesterol and cholesterol esters, collectively called atheromas in the blood vessels (2). It is widely understood that a high serum cholesterol level, particularly LDL, when oxidized, is responsible for the progression of atherosclerosis (3). Approximately 50% of the cholesterol in the human body is synthesized and the remainder is by dietary intake. When sum of the cholesterol synthesized and that obtained from diet exceeds the amount required for synthesis of membranes, bile salts and steroids, pathological accumulation of cholesterol occurs in blood vessels, leading to atherosclerosis (4). This implies that, diet also plays a crucial role in the development of atherosclerosis, which led to the design of many diet-based cholesterol reduction trials. Previous studies using Indian kino tree (5), garlic (6), and flax seed oil (7) were performed and found effective in diet induced hypercholesterolemic animal models. In this study, we investigated the effect of ethanolic extract of *C. peltata* on hypercholesterolemia using a hypercholesterolemic rat model.

C. peltata, belonging to the family Menispermaceae, is a slender twining shrub distributed throughout India (8). Very few workers have investigated the shrub and hence limited reports are available. However, the available literature quotes it to contain tetradine alkaloids (9), reported as scavengers of reactive oxygen species (10) and inhibitors of lipid peroxidation (11). The role of natural antioxidants in the prevention of atherosclerosis has been already reported (12). Hence this plant, being rich in antioxidant constituents, was selected for this study.

Materials and Methods

Preparation of the extract: The tubers of *C. peltata* were collected and identified by the Department of Botany, "The American College", Madurai. The tubers were then shade dried, and coarsely powdered in such a way that it passed through sieve No. 20 and was retained on sieve No. 40. About 500g of the dry powder was extracted with ethanol (70%) in the ratio 1:5 by continuous hot percolation using Soxhlet apparatus for 72 hours. The product thus obtained was collected and distilled. The residue was further concentrated by vacuum distillation. The yield was 17.4 g. Phytochemical investigation of the extract showed the presence of alkaloids (13).

Phytochemical investigative tests for alkaloids: Small amount of the extract was stirred with a few ml of dil. HCl and filtered. The resulting filtrate was then subjected to the following tests.

a) **Mayer's test:** To small amount of the filtrate, few drops of Mayer's reagent (potassium mercuric iodide) was added, prepared by dissolving 1.36 g of mercuric chloride in 60 ml water (Soln I) and 5 g of potassium iodide in 20 ml water (soln II), followed by mixing Soln I & II and making up the volume to 100 ml with water. A white precipitate was formed indicating the presence of alkaloids.
b) **Dragendroff's test:** To small amount of the filtrate, few drops of Dragendroff's reagent (potassium bismuth iodide) was added, prepared by boiling 14 g of sodium iodide with 5.2 g of basic bismuth carbonate in 50 ml glacial acetic acid for few minutes, allow to stand overnight and then filter. To 40 ml of the filtrate add 160 ml of ethyl acetate and 1 ml of water and preserve in amber colored bottle. On addition of the reagent to the filtrate an orange red precipitate was formed, indicating the presence of alkaloids.

c) **Wagner's test:** To the filtrate, few drops of Wagner's reagent (iodine solution) was added, prepared by dissolving 2 g iodine and 3 g potassium iodide in 100 ml water. A brown precipitate was formed, indicating the presence of alkaloids.
d) **Hager's test:** To the filtrate, few drops of Hager's reagent (1% w/v of picric acid in water) was added. A yellow crystalline precipitate was formed, indicating the presence of alkaloids.

Hypercholesterolemic animal model: The method of Yokozawa et al. (2003) was followed to induce hypercholesterolemia in rats (4). Male Wistar albino rats weighing 180 ± 10g were purchased from Chellamuthu Trust (Madurai, Tamil Nadu, India) and housed in microlon cages, maintained at about 25 ± 2°C, relative humidity of 50 ± 15% and under a 12 h light-dark cycle throughout the entire period of study. The animals were acclimatized to these conditions for one week before commencing the experiment. This study was carried out according to the guidelines of the Institutional Animal Ethical Committee (K.M. College of Pharmacy, Madurai (1/IV/03))

The rats were divided into 4 groups of 6 each, avoiding any intergroup differences in body weight. G I and G II animals, served as the normal control and extract control groups respectively and were maintained on a commercial diet containing 22% protein, 4.28% oil, 3.02% fiber, 7.8% ash and 1.3% silica (Amrut laboratories, Bangalore, Karnataka, India). In addition, the extract control group was administered 100 mg/kg of the extract orally for 30 days. Hypercholesterolemia was induced in the remaining two groups (G III & G IV), by feeding the rats with the commercial diet fortified with 18% casein, 1% cholesterol and 0.5% cholic acid (4). G III rats served as the hypercholesterolemic control and did not receive any treatment. However, G IV rats were treated with 100 mg/kg of the extract dispersed in water, orally for 30 days and the total volume did not exceed 2-3 ml at a time. This extract was prepared from a stock solution containing 1 g of the extract/100 ml of water. Six hours after the last dose, the rats were sacrificed and their blood collected for measurement of serum lipid profile.

Measurement of cholesterol levels: Serum TC, TG and HDL-C levels were estimated using commercial kits (Span Diagnostic Ltd, Surat, Gujarat, India), while serum LDL-C was determined according to the method of Noma et al. (1979) (14). The procedure was as follows; Pipette 0.2 ml of serum into a test tube, add 4 ml of reagent I (prepared by dissolving 50 mg of sodium heparin and 4 g of sodium chloride in 1 liter distilled water), mixture was shaken well and allowed to stand at room temperature for 15 min. Then, 0.5 g of Amberlite IRA-400 (Organo Co. Ltd, Tokyo, Japan) was added into the tube, the mixture was shaken, allow to stand for another 10 min and then centrifuge (3000 rpm, 10 min). The supernatant was treated with buffer containing 4-amino antipyrine peroxidase and sodium azate. The optical density of the resulting pink color was measured at 505 nm.

Determination of serum thiobarbituric acid-reactive substance (TBARS) levels: The serum TBARS levels were measured using the method of Naito and Yamanaka (1978) (15). The Serum was mixed with thiobarbituric acid reagent (0.375% thiobabituric acid & 15% TCA in 0.25 N HCl). The reaction mixture of serum and TBARS reagent was placed in a boiling water bath for 15 min, cooled, centrifuged (4000 rpm for 10 min) and the optical density of the supernatant was recorded at 532 nm.

Statistical analysis: The results are expressed as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Newman-keuls multiple range test. Differences below $p<0.05$ implied significance.

Results

The rats of G III, fed with a high cholesterol diet showed a marked increase in serum TC, TG, LDL-C and a fall in HDL-C levels. The atherogenic index (LDL-C/HDL-C) and serum TBARS levels were also found high in these rats. However, following treatment with the extract (100 mg/kg), the TC, TG and LDL-C levels were reduced, while the HDL cholesterol was elevated to 31 ± 1.2 mg/dl. Likewise, the atherogenic index was reduced to 2.8 against the value of 4.25 in hypercholesterolemic rats (Table 2). The extract also caused a significant reduction in TBARS levels in of rats of G IV (Fig. 1.)

Discussion

Many observational studies suggest that, the desirable level of TC in the human body is < 200 mg/dl. These studies also indicate that the risk of coronary heart disease (CHD) is attenuated, even in the presence of additional risk factors like diet, when the TC levels are below 160 mg/dl (16). The cholesterol-diet-CHD hypothesis suggests that a diet rich in saturated fat and cholesterol, raises the serum cholesterol levels and that lowering of these elevated levels reduces the risk of developing CHD (17). Excessive dietary intake of cholesterol and saturated fatty acids lead to the deposition of LDL and oxidized LDL in the peripheral tissues, the latter being atherogenic in nature (18). All these findings suggest that, excessive dietary supplementation of cholesterol, elevates the cholesterol levels in the body.

In experimental animals also, consumption of a diet rich in cholesterol, resulted in elevated LDL-C levels and atherosclerosis (19). Based on this finding, hypercholesterolemia was induced in male Wistar albino rats by feeding them with a cholesterol rich diet. Using this animal model, the effect of *C. peltata* on hypercholesterolemia was investigated.
Following the administration of cholesterol rich diet to rats, a remarkable rise in TC and LDL-C were observed (Table 1). Previous reports suggest that, elevated LDL-C levels in the plasma are a prime cause of coronary atherosclerosis (20). Many epidemiological studies also reveal a significant relationship between LDL-C and coronary artery disease (CAD) (21) and that drug or diet induced reduction of LDL-C could reduce the risk of CAD associated mortality (22).

In the present study, it was found that treatment with the ethanolic extract of *C. peltata* significantly reduced the serum TC, TG and LDL-C levels, in addition to

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Table 1. *Effect of ethanolic extract of C. peltata on the lipid profile of hypercholesterolemic rats.*

Treatment Groups	TG	TC	HDL-C	LDL-C
G I	46.7 ± 9.0	54.0 ± 2.0	18.0 ± 1.70	27.3 ± 2.10
G II	48.3 ± 2.10	49.8 ± 1.30	19.0 ± 2.80	26.0 ± 1.40
G III	83.8 ± 1.90*a	163 ± 2.98*a	22.0 ± 1.80*a	125 ± 4.50*a
G IV	55.8 ± 1.60*b	129 ± 3.20*b	31.0 ± 1.20*b	89 ± 3.20*b

The values are expressed in mg/dl. Values are expressed as mean ± SEM for six animals in each group.
**a values are significantly different from normal control (G I) rats.*
**b values are significantly different from hypercholesterolemic (G III) rats.*
Newman-Keuls multiple range test (P<0.05) was used.

Table 2. *Effect of ethanolic extract of C. peltata on atherogenic index and body weight in hypercholesterolemic rats.*

Treatment Groups	Atherogenic Index (LDL-C/HDL-C)	Body weight (g)
G I	1.5 ± 0.06	156 ± 5
G II	1.3 ± 0.02	153 ± 4.2
G III	4.25 ± 0.32*a	203 ± 6
G IV	2.8 ± 0.22*b	176 ± 3.3

Values are expressed as mean ± SEM for six animals in each group.
**a values are significantly different from normal control (G I) rats.*
**b values are significantly different from hypercholesterolemic (G III) rats.*
Newman-Keuls multiple range test (P<0.05) was used.

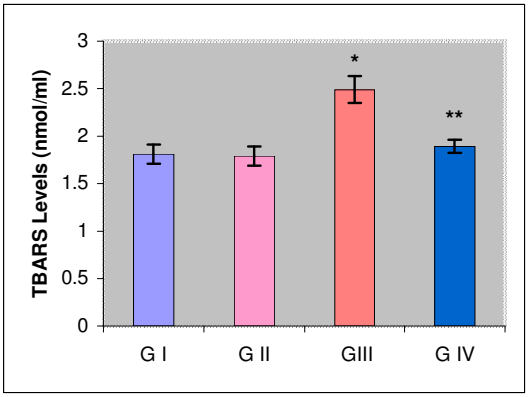

Fig. 1. *Effect of ethanolic extract of C. peltata on serum TBARS levels in hypercholesterolemic rats. Values are expressed as mean ± SEM for six animals in each group. G I (normal control), G II (extract control), G III (hypercholesterolemia induced rats), G IV (hypercholesterolemic rats treated with the extract). * values are significantly different from G I rats. ** values are significantly different from G III rats. Newman-Keuls multiple range test (P<0.05) was used.*

elevating the HDL-C levels. Hence the atherogenic index of the treated rats was reduced to 2.8 against the value of 4.25 in hypercholesterolemic rats. Reduction of LDL-C by the extract suggests it to reduce the pathological process of atherosclerosis. Another interesting observation is that, this extract reduced the serum TC, TG and LDL-C levels even in normal rats.

Hypercholesterolemic atherosclerosis is associated with an increase in the level of the lipid peroxidation product malondialdehyde, which is an index of the level of reactive oxygen species (23), (24). A decrease in lipid peroxidation leads to a reduction in arterial wall cholesterol content. Therefore, reduction of hypercholesterolemic atherosclerosis was associated with a decrease in lipid peroxidation, as the serum TBARS levels decreased after administration of the extract.

On the basis of these observations, though the lipid profile was not totally corrected, as the TBARS level was normalised, we hypothesize that *C. peltata* would reduce the development of hypercholesterolemic atherosclerosis by reducing lipid peroxidition. Further studies on the active components of *C. peltata* and mechanism(s) of its protective effect against hypercholesterolemia are needed.

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