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Hypolipidemic Activity of Protocatechuic Acid in Atherogenic Diet Induced Hyperlipidemic Rats

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ABSTRACT

Hyperlipidemia is an abnormally high level of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. The present study was designed to investigate the hypolipidemic effects of Protocatechuic acid in atherogenic diet induced hyperlipidemia. In atherogenic diet induced hyperlipidemic model, the rats receiving treatment of Protocatechuic acid at the dose of 25 and 50 mg/kg showed significant reduction in total cholesterol, triglyceride, total protein and elevation in high density lipoprotein cholesterol. Hence by considering the effects observed in this model, it has been suggested that Protocatechuic acid was found to possess significant hypolipidemic activity, this may be due to its effect on increasing the metabolism of the cholesterol by activating lipoprotein lipase or by increasing reverse cholesterol transport.

KEYWORDS

Atherogenic diet, Protocatechuic acid, Cholesterol, Hyperlipidemia, Hypolipidemic activity.

INTRODUCTION

Hyperlipidemia is the disorders of lipid metabolism have been ranked as one of the greatest risk factors contributing to the prevalence and severity of atherosclerosis, stroke and coronary heart diseases[1,2]. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low- density lipoprotein (LDL, VLDL) cholesterol and decreased high-density lipoprotein (HDL) levels. Atherosclerosis refers to deposition of fatty substances on the inner lining of the blood vessels. Lipids undergo peroxidative change in the arterial wall and eventually result in tissue injury. It is characterized by vascular areas containing mononuclear and proliferation of smooth muscle cells resulting in hardening and thickening of the arterial walls[3].

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The high concentration of cholesterol, particularly LDL-cholesterol is one of the principal risk factors. Coronary heart disease caused by atherosclerosis continues to be a leading cause of mortality in developed and developing nations of the world[4]. Myocardial and cerebral infarctions are also main clinical syndromes resulting from atherosclerosis and are the leading causes of death in all over the world[5]. Lipid lowering drugs like fibrates, statins and bile acid sequestrants used in the treatment of hyperlipidemia possess toxic side effect[6,7]. Therefore, there is an urgent need to have a lipid lowering drug with fewer side effects. A number of herbal medicines are used for controlling hyperlipidemia and related complications in patients[8].

Hibiscus sabdariffa L. (roselle) is extensively cultivated in Thailand where it is known as Krachiap daeng. Its red persistent calyx is the major component which has a sour taste and is used as beverage and food colorant[9]. It is

claimed as a Thai traditional medicine for kidney stones. It is also used as an antibacterial, antifungal, hypocholesterolemic, diuretic, uricosuric, mild laxative and antihypertensive substance[10].

Currently, the dried calyx extracts of these plants are commercially prepared as health food products available in the form of granules and as tea, and are claimed to be antihypertensive, hypocholesterolemic and diuretic.

Since *Hibiscus sabdariffa L.* has hypocholesterolemic activity, the present study has been undertaken to evaluate the hypolipidemic activity of its active constituent Protocatechuic acid in atherogenic diet induced hyperlipidemia in rats.

MATERIALS AND METHODS

MATERIALS

Protocatechuic acid was purchased from Sigma Aldrich, USA. All other chemicals were of analytical grade and obtained locally. Cholesterol and HDL-C kit were procured from Biolabs diagnostics MH. Triglycerides kit was obtained from Erba diagnostics MH, India.

ANIMALS

Wister albino adult male rats weighing 200-220g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark cycles were maintained.

The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. The composition of atherogenic diet used during the study was as given in Table-1. Each of these treatment groups consisted of six animals/group. The protocol of this study was approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

DOSE SELECTION AND ADMINISTRATION

According to reported activities, Protocatechuic acid at the doses of 25 and 50 mg/kg p.o. /day [11] was selected for the study.

Table: 1 Composition of normal and atherogenic diet

Composition	Normal diet (%)	Atherogenic diet (%)
Protein (Milk powder)	12	10
Carbohydrates (Wheat flour)	71	61
Sugar	05	05
Fat (Butter)	05	16
Salts	04	04
Vitamins	01	02
Fibers	02	01
Cholesterol	-	01
Total Weight	100g	100g

EXPERIMENTAL INDUCTION OF HYPERLIPIDEMIA

In order to induce hyperlipidemia, the method reported by Bopanna *et al.*[12] was followed. The animals were divided into four groups of six rats each and they received the following diets with or without treatment for 45 days orally:

Group I: Normal diet

Group II: Atherogenic diet containing 1% cholesterol.

Group III: Atherogenic diet + Protocatechuic acid (25 mg/kg/day).

Group IV: Atherogenic diet + Protocatechuic acid (50 mg/kg/day).

At the end of the treatment the rats were fasted overnight, blood was drawn from retro orbital plexus as per CPCSEA guidelines. Serum was separated and stored in refrigerator until assay.

MEASUREMENT OF SERUM LIPID PROFILE

Biochemical estimation kits (Biolabs diagnostics MH) were used for the photometric estimation of Total cholesterol (TC), total triglyceride (TG), total protein (TP) and total high density lipoprotein (HDL). The atherogenic index was calculated by using the following formula[13].

Atherogenic index = Total serum cholesterol/ Total serum HDL – Cholesterol

STATISTICAL ANALYSIS

Values are given as means±S.D. for six rats in each group. Statistical analysis was carried out by Student's t- test.

RESULT AND DISCUSSION

The results showed that feeding of atherogenic diet increased serum total cholesterol, total triglyceride and total protein and decreased serum HDL-cholesterol level when compared to normal group at over a period of 45 days (Table: 2).

Administration of Protocatechuic acid at the dose of 25 and 50 mg/kg per day showed statistically significant decrease in total cholesterol (P<0.05), triglyceride (P<0.001) and total protein (P<0.001) level as compared to hyperlipidemic animals (Table: 2).

At this time an increase in HDL-cholesterol level was also observed. Both 25 and 50 mg/kg body wt. Protocatechuic acid treated animals showed decrease in the atherogenic index and increased percentage of protection (Table: 3).

Table: 3 Atherogenic index in various groups

Groups	Atherogenic index	Protection * (%)
Group I (Normal)	3.152	-
Group II (Control) (Atherogenic diet only)	4.410	-
Group III (Atherogenic diet + Protocatechuic acid 25 mg/kg)	1.918	57.62
Group IV (Atherogenic diet + Protocatechuic acid 50 mg/kg)	1.326	65.93

High fatty diet is a very common cause of heart disease. Particularly, with an increase in tendency towards fast foods, which are rich in saturated fats, an increase in coronary heart disorder (CHD) is being observed in the developing countries since past few decades[14].

Table: 2 Effect of Protocatechuic acid treatment for 45 days on plasma lipid profile of normal and atherogenic diet induced hyperlipidemic rats

Groups	Total Cholesterol (mg/dl)	Total triglyceride (mg/dl)	Total protein (mg/dl)	Total HDL (mg/dl)
Group I (Normal)	160.0+14.4*	51.68+1.09**	5.41+0.07**	50.41+2.96**
Group II (Control) (Atherogenic diet only)	240.0+16.0	182.48+1.82	12.42+0.09	55.81+0.46
Group III (Atherogenic diet + Protocatechuic acid 25 mg/kg	139.1+34.8*	84.71+0.98**	5.74+0.06**	72.89+0.98**
Group IV (Atherogenic diet + Protocatechuic acid 50 mg/kg	130.2+30.0*	36.23+0.74**	5.43+0.07**	97.41+0.38**

Values are given as means ±S.D. from six rats in each group; * p <0.05, ** p <0.001, Statistical significance in comparison to group – III, IV with group II.

$$*\text{Protection (\%)} = \frac{\text{Atherogenic index of control} - \text{Atherogenic index treated group}}{\text{Atherogenic index of control}} \times 100$$

A one percent decrease in HDL-cholesterol is associated with a 3-4% increase in the risk of heart disease. In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease in other lipid parameters were observed (Tables 2 and 3).

It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and total protein which are actually raised in atherogenic diet, can be lowered significantly with Protocatechuic acid. Protocatechuic acid can be utilized for providing dietary management in the prevention of atherosclerosis in hyperlipidemic patients.

CONCLUSION

Treatment with Protocatechuic acid produced a significant decrease in the serum level of lipids in atherogenic diet induced hyperlipidemia in rats. Hence by considering the effects observed in this model, the possible mechanism of Protocatechuic acid may involve increase of HDL-cholesterol, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT) enzyme[15]. LCAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL. Thus from the above results we can conclude that Protocatechuic acid has hypolipidemic activity.

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