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RESEARCH ARTICLE

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ANTIHYPERLIPIDEMIC EFFECT OF PROTOCATECHUIC ACID IN FRUCTOSE INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Hyperlipidemia is an abnormally high level of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has fewer side effects. Protocatechuic acid (PCA), which is predominantly present in the flowers of *Hibiscus sabdariffa,* possesses antihyperlipidemic and free radical scavenging activity. Taking this into consideration PCA at the dose of 25 and 50 mg/kg were evaluated against fructose induced hyperlipidemia in rats and it has showed a significant decrease ($p<0.05$, $p<0.01$ respectively) in the levels of serum TC, TG and LDL and HDL was significantly increased ($p<0.05$, $p<0.01$ respectively) in serum when compared to fructose control group. Thus Protocatechuic acid has anti-hyperlipidemic activity which may be due to increased uptake of LDL cholesterol by hepatic LDL receptor or may be due to its effect on enzymes involved in metabolism and excretion of cholesterol.

KEYWORDS

Hyperlipidemia, protocatechuic acid, antihyperlipidemic, fructose.

INTRODUCTION

Heart disease is the leading cause of death. Many of the risk factors like smoking, lack of exercise and consumption of a high fat diet are responsible for causing heart disease. The majority of risk factors involved in the causation of atherosclerotic diseases are directly or indirectly due to disturbances in the lipid and lipoprotein metabolism. Evidence from studies both in animals and humans indicates that progression can be slowed if elevated serum concentration of the atherogenic lipoprotein and triglycerides are reduced, which in turn prevents coronary heart disease [1]. Diet high in fructose induces insulin resistance (IR) in rats [2], hamsters [3] and dogs [4]. Investigators have produced IR by administering fructose in drinking water (5-10%) or by feeding a diet in which fructose contributes to more than 50% of total calories. Rats develop IR, hyperlipidemia, and hypertension as early as 2 weeks of fructose diet initiation [5]. The lipid changes reported include increased levels of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA), low density lipoprotein cholesterol (LDL-C) and decreased levels of high density lipoprotein cholesterol (HDL-C) (Kelley et al. 2004). 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 [6]. Currently available hypolipidemic drugs have been associated with number of side effects [7]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [8]. Medicinal plants are used for various research purposes. 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. An herbal treatment for hypercholesterolemia has fewer side effects and is relatively cheap, locally available. They are effective in reducing the lipid levels in the system. Consumption of much fat may lead to the production of extra VLDL, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. Therefore, improvement in human diet is highly recommended for disease prevention [7].

Benzoic acid occurs naturally free and bound as benzoic acid esters in many plant and animal species. Appreciable amounts have been found in most berries (around 0.05%). Ripe fruits of several Vaccinium species (e.g., cranberry, *V. vitis idaea*; bilberry, *V. macrocarpon*) contain as much as 0.03-0.13% free benzoic acid. Benzoic acid derivative like protocatechuic acid, which is present in the flowers of *Hibiscus sabdariffa*, has antihyperlipidemic and free radical scavenging activity. Taking this in to consideration, the present study was designed to evaluate the lipid lowering activity of protocatechuic acid in fructose induced hyperlipidemia in rats.

MATERIALS AND METHODS

(i) ***Hyperlipidemia Inducer:***

25% of D-Fructose in drinking water was used as an inducing agent for hyperlipidemia in experimental rats.

(ii) ***Chemicals:***

D-fructose was obtained from research lab fine chem. All other chemicals were of analytical grade and obtained locally. Cholesterol kit (Enzymatic Method) and HDL-C kit were procured from Biolabs diagnostics MH. Triglycerides kit was obtained from Erba diagnostics MH, India.

(iii) ***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*.

(iv) ***Dose Selection and Administration:***

Protocatechuic acid (PCA) at the dose of 25 mg/kg p.o and 50 mg/kg p.o were selected as per reported activity [9], and were suspended with 1 % carboxy methyl cellulose (CMC) in distilled water and administered orally to the animals by gastric intubation, once in a day for the period of 21 days along with 25 % fructose in drinking water daily [10].

(v) ***Protocol for Antihyperlipidemic Activity:***

In the experiment a total number of 24 rats (18 hyperlipidemia rats, six normal) were used. The rats were divided into four groups (n=6).

Group I: 1% CMC (1ml/kg) p.o.

Group II: 25% D-Fructose in drinking water daily.

Group III: PCA (25 mg/kg) p.o. +25% D-Fructose in drinking water daily.

Group IV: PCA (50 mg/kg) p.o. +25% D-Fructose in drinking water daily.

The above treatment was continued for 21 days and the protocol of the present study was approved by the Institutional Animal Ethics Committee.

(vi) ***Induction of hyperlipidemia and Collection of Blood Samples:***

At 21st day, two hours after the final treatment, animals were anaesthetized and blood from each animal was withdrawn from retro-orbital plexus. Serum obtained by immediate centrifugation of blood samples using Remi ultra cooling centrifuge at 3000 rpm for 5 minutes at room temperature and was directly used for estimating serum glucose and lipid profiles (serum TC, TG, LDL-C and HDL-C). All samples were stored at 4°C until analysis.

(vii) ***Biochemical Analysis:***

Serum glucose and lipid levels include TC, TG and HDL-C were carried out using commercially available kits (Biolabs diagnostics, Tarapur, India) and LDL-C in serum was calculated as per Friedewald estimation [11] LDL-C= TC-(TG/5+HDL-C)mg/dl.

RESULTS

Daily administration of 25 % of fructose in drinking water for 21 days significantly increased ($P < 0.01$) hyperlipidemia in rats by increasing the serum TC, TG, LDL- C, glucose levels and serum HDL-C levels was significantly decreased ($P < 0.01$) in fructose control group when compared to the normal control group. The effect of PCA on serum lipid profile levels was showed in Table 1. Treatment with PCA at 25 and 50 mg/kg significantly reduced ($P < 0.05$, $P < 0.01$ respectively) the serum TC, TG, LDL-C levels, glucose and serum HDL-C levels was significantly increased ($P < 0.01$) when compared to the fructose

control group. The change in lipid levels in test groups combinations were comparable with group of individual test drug treated rats.

Table 1

Effect of Protocatechuic acid on serum Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) levels in fructose induced hyperlipidemic rats.

Groups (n=6)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)
Group I: (NC)	86.19±1.17	77.41±1.59	36.97±1.12
Group II: (FC)	127.21±1.33##	130.68±1.60##	19.63±1.10##
Group III: Fructose + PCA 25	115.43±1.11*	118.40±1.36*	28.39±1.44*
Group IV: Fructose + PCA 50	98.75±1.38**	104.52±1.48**	32.10±1.02**

Values are expressed as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. ##p<0.01 Student's t test when compared with Normal Control (NC); *p<0.05, **p<0.01 when compared with Fructose Control (FC); PCA 25: Protocatechuic acid 25 mg/kg p.o. /day; PCA 50: Protocatechuic acid 50 mg/kg p.o. /day.

Table 2

Effect of Protocatechuic acid on serum Low Density Lipoprotein (LDL) and glucose levels in fructose induced hyperlipidemic rats.

Groups (n=6)	LDL (mg/dl)	Serum glucose
Group I: (NC)	33.74±1.35	74.543±1.40
Group II: (FC)	81.44±1.60##	121.76±2.19##
Group III: Fructose + PCA 25	61.36±1.35**	100.68±2.03**
Group IV: Fructose + PCA 50	53.71±1.74**	100.68±2.03**

Values are expressed as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. ##p<0.01 Student's t test when compared with Normal Control (NC); *p<0.05, **p<0.01 when compared with Fructose control (FC); PCA 25: Protocatechuic acid 25 mg/kg p.o. /day; PCA 50: Protocatechuic acid 50 mg/kg p.o. /day.

DISCUSSION AND CONCLUSION

Fructose has been reported to induce hypertriglyceridemia associated with insulin resistance, hyperinsulinemia and hypertension. After absorption in GIT, Fructose is transported *via* portal circulation to the liver, where it enters hepatocytes via the glucose transporter GLUT-5 independently of insulin and is rapidly metabolised. Fructose is metabolised into "glycerol-3-phosphate" and "acetyl CoA". These two intermediate metabolites are then used as substrates for glycerides synthesis, contributing to VLDL-TG production in liver. The exposure of liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance [12, 13].

In this model, 25 % of fructose solution was given to rats for 21 days along with PCA at 25 and 50 mg/kg to the rats and serum total cholesterol (TC) levels, serum triglyceride (TG) levels, serum HDL serum LDL and serum glucose were evaluated on 21st day.

PCA at 25 and 50 mg/kg significantly reduced ($p<0.05$, $p<0.01$respectively) serum total cholesterol (TC) levels, serum triglyceride (TG) levels, serum LDL, serum glucose levels and serum HDL was found to be increased significantly ($p<0.05$, $p<0.01$respectively) as compared to fructose control group.

Thus, Protocatechuic acid showed highly significant antihyperlipidemic and antihyperglycemic activity. The present study revealed that possible mechanism could be due

to PCA may have increased in uptake LDL cholesterol by hepatic LDL receptor or may be due to its effect on enzymes involved in metabolism and excretion of cholesterol [14].

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