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Original Article

EVALUATION OF ANTI-HYPERLIPIDEMIC ACTIVITY OF *CAPSICUM FRUTESCENS* EXTRACT

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ABSTRACT

Objective: The objective of the study was to evaluate the in-vivo anti-hyperlipidemic activity of *Capsicum frutescens* extracts.

Methods: The dried fruit powder were extracted with a three liquid phase extraction system. The acetone extract was isolated and the anti-hyperlipidemic activity was evaluated.

Results: The anti-hyperlipidemic study was carried out by inducing hyperlipidemia in rats by means of triton. The serum collected was analyzed for total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein.

Conclusion: The result of the present study revealed that the acetone extract of the fruits of *Capsicum frutescens* possess anti-hyperlipidemic activity.

Keywords: *Capsicum frutescens,* Antihyperlipidemic activity, HDL-LDL, Triton

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INTRODUCTION

Hyperlipidemia, also known as hyperlipoproteinemia or dyslipidemia, is an abnormal elevation of lipid levels in the bloodstream. These lipids include cholesterol, cholesterol compounds, phospholipids and triglycerides, all carried in blood as large molecules called lipoproteins. There are three types of hyperlipidemia namely hyperlipoproteinemia (elevated levels of lipoprotein in the blood), hypercholesterolemia (high cholesterol) and hypertriglyceridemia (high triglycerides level in blood). Hyperlipidemia affects lipid production, transportation in the bloodstream and/or deposition in body cell. It is the first and foremost factor that leads to diseases like atherosclerosis, coronary heart disease, ischemic cerebrovascular disease, hypertension, obesity and diabetes mellitus (Type-II) etc. [1, 2].

Although human beings have developed many allopathic drugs to combat the hyperlipidemia and these drugs to a certain extent have shown promising lipid-lowering activity. But these drugs have been found to be associated with side effects. As a result of side effects that are mostly associated with the allopathic system of medicine, the world population today is turning back to a traditional system of medicine as these medicines are free from side effects, easily available with less cost. Thus, in the present scenario, more research activities are required for the development of the drug from the natural sources to meet the demand of world population.

Capsicum frutescens is a vegetable used daily, and the substance capsaicin is responsible for its hot and spicy flavour, sought after in gastronomy. Capsaicin and several related molecules are known by the collective name capsaicinoids, and they are produced by all plants of the genus Capsicum, with the exception of the bell pepper (Capsicum annum), which produces no capsaicin.

The naturally occurring content of capsaicinoids in spices ranges typically from 0.1 mg/g in chilli pepper to 2.5 mg/g in red pepper and 60 mg/g in oleoresin red pepper [3]. Capsaicin and dihydrocapsaicin are the major capsaicinoids produced; however, others exist and are produced in smaller quantities.

Capsaicin is also known to inhibit Substance P, a neuropeptide that is the key transmitter of pain to the brain thus providing relief to pain. It has potent antibacterial properties that fight and prevent chronic sinus infections, or sinusitis. Capsaicin is also a thermogenic agent, which means it increases metabolic activity. This, in turn, helps to burn calories and fat. Capsaicin may help to protect the heart by reducing cholesterol, triglycerides and platelet aggregation. Capsaicin is claimed to have high antioxidant values and HDL-cholesterol-raising effect. Hence this study aims at exploring the possible effects of Capsaicin on serum TC, TG, LDL and HDL levels in experimental rats.

MATERIALS AND METHODS

Chemicals

Triton WR 1339 (Sigma-Aldrich), Cholesterol (SRL Mumbai), Atorvastatin (Micro labs Pvt Ltd, B, lore), Anaesthetic ether-SD Fine-chem Ltd., Mumbai, Chloroform-SD Fine Chem Ltd. Mumbai. Formaline-SD Fine-chem Ltd., Mumbai. All chemicals and reagents were of analytical grade. Diagnostic kits used for estimation of cholesterol, triglycerides, HDL, LDL, VLDL were procured from Robonik Diagnostic Ltd India.

Animals

Male Wistar rats aged between 8-10 w (250-300 g) were used for the study. Animals were kept in the controlled condition in the institutional animal house at an ambient temperature of 25-30 °C and relative humidity of 55-60% and 12/12 h light/dark cycle and were provided pellet di*et al.* ong with water *ad libitum*. The experimental protocol was accepted by Institutional Animal Ethics Committee.

Preparation of fruit extract

Mature *Capsicum frutescens* (bird's eye chilli) were collected and washed thoroughly with water and air dried in shade at room temperature. It was ground into powder by a miniature high-speed universal pulveriser. The three liquid phase extraction system of acetone, K2HPO4 and n-hexane was prepared by weighing acetone 22% (w/w), K2HPO4 20% (w/w), n-hexane 10% (w/w) and water (58% (w/w) and mixing. The powdered *Capsicum frutescens* was added to this three liquid phase extraction system in a mass ratio of 1:20. The mixture was thoroughly vibrated for 10 min and then settled at room temperature. After the separation of three phases, the volume of the acetone was removed from the middle layer by using a pipette and kept aside. This procedure was done again for three

times by adding fresh acetone into the same extract. The acetone extracts were pooled and dried under vacuum. The percent yield of capsaicin was 10 mg/g of *Capsicum frutescens* powder.

Electrospray mass spectrometry analysis

The electrospray mass spectrometric scans were monitored to check for the presence of Capsaicinoids using AB Sciex 4000 mass spectrometer with direct injection of sample dissolved in Methanol in MS scan mode into the mass detector.

Dose preparation of standard and leaves extracts

Atorvastatin at a dose of 10 mg/Kg was prepared by suspending in 5% sucrose in water. The extract of leaves was dissolved in 5% sucrose in water. Doses of 50 mg/Kg b.w. and 100 mg/Kg b.w. of the extract were administered by oral route to the rats.

***In vivo* acute oral toxicity studies**

In the present study, the acute oral toxicity of the extracts was performed according to OECD Guideline 423 [4]. In this method, the toxicity of the extracts was evaluated using the stepwise procedure, each step using three Wistar rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 1000 mg/Kg b.w. Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total of 14 d.

Experimental design

A total of 30 male wistar rats were utilized and the animals were randomly divided into 5 groups of 6 rats in each group:

Group I-Normal [standard diet+5% sucrose (p. o.)]

Group II–Diabetic control [standard diet+triton (i. p.)+5% sucrose (p. o.)]

Group-III-Standard drug (10 mg/Kg b.w., p. o.)+triton+standard diet.

Group-IV-Diabetic control+*Capsicum frutescens* extract (50 mg/kg body weight which is 5% of the dose used for acute toxicity studies)+triton+standard diet

Group–V-Diabetic control+*Capsicum frutescens* extract (100 mg/kg body weight which is 10% of the dose used for acute toxicity studies)+triton+standard diet

Effect in Triton-induced hyperlipidemic rats

Animals were kept for fasting for 24 h and was injected with a solution of Triton WR-1339 (dissolved in 0.9% NaCl) at a dose of 400 mg/kg body weight intraperitoneally. The plant extracts, at a dose of 50 mg/kg and 100 mg/kg body weight were administered orally through gastric intubation. The first dose was given immediately after triton injection and a second dose 20 h later.

Blood samples were collected at 6, 24 and 48h after triton injection and were used for the study of various biochemical parameters. Blood was collected through retro-orbital plexus route on anesthesia and centrifuged at 2000 rpm for 30 minutes [5-6].

Biochemical analysis

Serum samples were analyzed for

- Total serum cholesterol (TC),
- Triglyceride (TG)
- High-density lipoprotein cholesterol (HDL-C)
- Low-density lipoprotein (LDL-C)
- LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C levels.

Data analysis

Data were statistically analyzed as mean+SEM and expressed as non-significant $P>0.05$, just significant $P<0.05$, significant $P<0.01$ and highly significant $P<0.001$ by using ANOVA followed by Dunnett's t-test and unpaired t-test with Welch correction.

RESULTS

Electrospray mass spectrometry analysis

The presence of intense peaks at 306.3 and 308.3 indicated the presence of the Capsaicinoids–Capsaicin and Dihydrocapsaicin (fig. 1).

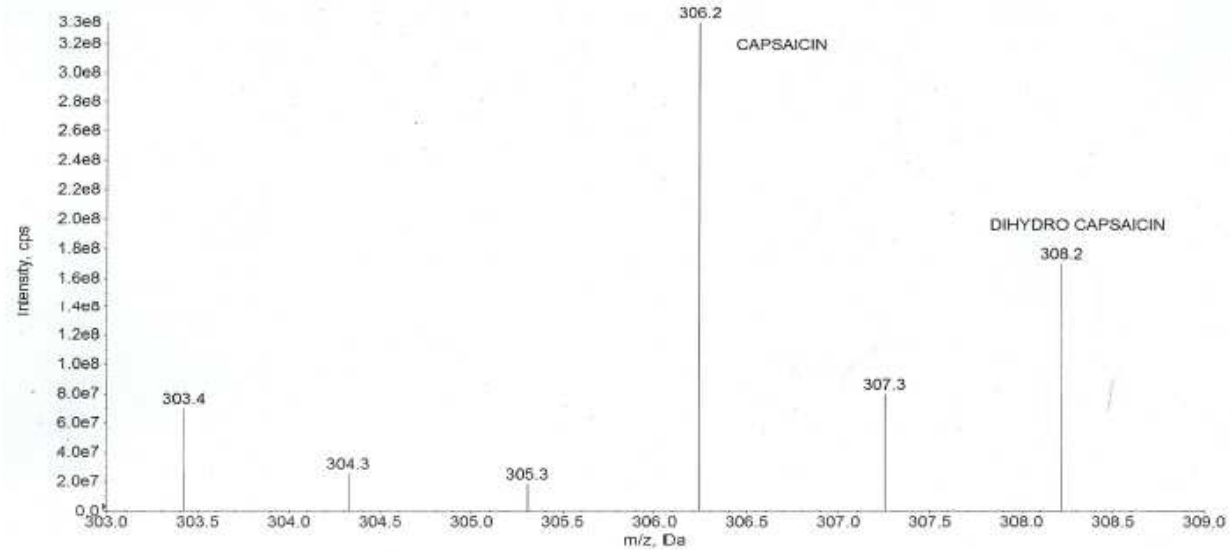

Fig. 1: MS scan showing the peaks for capsaicin and dihydrocapsaicin

***In vivo* acute oral toxicity studies**

Acute oral toxicity studies for the extracts of the leaves of *Capsicum frutescens* revealed that both the extracts were non-toxic attested dose levels and well tolerated by the experimental animals.

Effect on the lipid profile

The results of the lipid profile are tabulated in tables 1-3. The extract showed a decrease in blood lipids in hyperlipidemic rats when compared with the respective diabetic controls. The extract showed

a significant decrease in TC, TG and LDL along with an increase in HDL level dose-dependently at the tested doses (50 mg/kg and 100 mg/kg) when administered in Triton-induced hyperlipidemic rats (tables 1-3).

Table 1: Effect of capsaicinoids on total cholesterol and triglyceride levels in triton induced hyperlipidemic rats (Values are mean+SEM of 6 animals in each group)

Treatment	Cholesterol (mg/dl)			Triglycerides (mg/dl)		
	6h	24h	48h	6h	24h	48h
Normal	63.4+2.7	61.4+2.1	65.2+1.7	68.1+1.6	70.3+3.5	72.7+1.0
Diabetic control	106.4+2.1[a]	164.4+5.3[a]	119.0+2.1[a]	130.2+1.6[a]	148.7+8.6[a]	105.2+2.9[a]
Standard	91.8+2.2***	94.0+4.0***	108.6+2.4*	104.0+3.0***	81.1+2.6***	78.0+1.9***
CE(50 mg/kg)	101.0+1.9ns	142.8+6.6*	113.8+2.5ns	117.3+1.9**	124.6+5.3*	86.0+2.9***
CE(100 mg/kg)	96.2+2.7*	120.2+5.7***	111.8+2.4ns	112.3+2.8***	98.5+5.9***	79.9+2.1***

***P<0.001, **P<0.01, *P<0.05,nsP>0.05 (Dunnett's t-test) [a]P<0.001 (Unpaired t-test)

Table 2: Effect of capsaicinoids on LDL and HDL levels in triton induced hyperlipidemic rats (Values are mean+SEM of 6 animals in each group)

Treatment	HDL (mg/dl)			LDL (mg/dl)		
	6h	24h	48h	6h	24h	48h
Normal	23.2+1.0	26.0+0.9	23.6+0.9	22.6+0.9	18.6+3.0	24.6+0.6
Diabetic Control	16.2+1.0[a]	14.1+1.6[a]	14.7+1.1[a]	43.6+2.4[a]	84.2+6.5[a]	31.7+2.3[a]
Standard	27.2+1.8***	34.7+3.8***	29.3+2.2***	31.3+2.6***	10.6+1.0***	17.4+1.0***
CE(50 mg/kg)	20.3+0.8ns	25.3+1.7*	22.0+0.9*	33.6+2.2*	26.2+2.9***	23.8+1.5**
CE(100 mg/kg)	26.8+0.6***	35.1+1.6***	28.3+2.3***	34.8+1.8*	19.9+1.1***	24.0+1.2**

***P<0.001, **P<0.01, *P<0.05,nsP>0.05 (Dunnett's t-test) [a]P<0.001 (Unpaired t-test)

Table 3: Effect of capsaicinoids on TC/HDL ratio and LDL/HDL ratio in triton induced hyperlipidemic rats

Treatment	TC/HDL ratio			LDL/HDL ratio		
	6h	24h	48h	6h	24h	48h
Normal	2.7+0.1	2.4+0.1	2.8+0.1	1.0+0.1	0.7+0.1	1.0+0.0
Diabetic Control	6.6+0.3	12.3+1.4	8.3+0.6	2.7+0.3	6.4+1.0	2.2+0.3
Standard	3.4+0.2	2.8+0.3	3.8+0.3	1.2+0.1	0.3+0.1	0.6+0.1
CE(50 mg/kg)	5.0+0.2	5.7+0.2	5.2+0.3	1.7+0.1	1.1+0.2	1.1+0.1
CE(100 mg/kg)	3.6+0.2	3.5+0.3	4.1+0.4	1.3+0.1	0.6+0.1	0.9+0.1

DISCUSSION

The three liquid phase system consisting of n-hexane, acetone, K2HPO4, and water was successfully used to extract capsanthin and capsaicin. Capsanthin and capsaicin were partitioned mainly into the n-hexane and acetone phases, respectively. They could be easily obtained after removing the solvent for further purification. TLPE was an effective method for the extraction and separation of capsaicinoids [7].

Triton WR 1339, when administered in fasted rats, causes an elevation in plasma lipid level. Initially there is a sharp increase in the lipid level reaching a peak two to three times the control value by 24h after the administration of Triton injection (Phase I–synthetic phase), this increase in lipids will fall off within the next 24h i.e. 48h after the administration of Triton (Phase II–Excretion phase) This increase in plasma lipids by Triton is thought to be due to one of the following mechanisms; as due to increase in hepatic synthesis of cholesterol or Triton physically alters very low density lipoproteins (VLDL) rendering their removal from the blood. Drugs interfering with cholesterol synthesis were shown to be active in phase I, while drugs interfering with cholesterol excretion and metabolism was active in Phase II [8-10].

Total cholesterol (TC) was determined by one step method of Wybenga and Pillegi [7] based on the reaction between cholesterol and cholesterol reagent (ferric oxide, ethyl acetate and sulfuric acid). High-density lipoprotein (HDL) from blood was determined by a two-step method i.e. initial separation of HDL from blood using a precipitating agent and then the precipitated HDL was determined by using calorimetric reaction with cholesterol reagent [11]. Triglyceride (TG) was determined calorimetrically by an enzymatic reaction using glycerol-3-phosphate oxidase. Enzymatic splitting of lipoprotein lipase along with reaction between 4-aminoantipyrin, 4-chlorophenol and H_2O_2 under the catalytic action of peroxidase generates quinimine which is used as an internal indicator in this calorimetric determination [12]. LDL was determined by using Grindelwald's formula–LDL = TC-HDL-(TG/5) [13].

The preliminary screening revealed the presence of Capsaicin and dihydrocapsaicin in the acetone extract. Capsaicinoids significantly decreased the plasma triglycerides, total cholesterol, LDL cholesterol and increased the high-density lipoprotein cholesterol. The cholesterol-lowering action of capsaicinoids was attributed to the inhibition of hepatic cholesterol synthesis, also by the stimulation of the conversion of cholesterol to bile acids by up-regulation of cholesterol 7α-hydroxylase expression and increasing the excretions of bile acids in feces [14].

In conclusion, the present study shows that Capsaicinoids extracted from *Capsicum frutescens* has a plasma LDL-C, cholesterol, triglyceride lowering effect and has a plasma HDL-C raising effect. We think that it is interesting and meaningful to report that dietary intake of Capsaicinoids can improve plasma lipid levels.

AUTHOR CONTRIBUTION

L. T. designed all the experiments, carried out the main experimental work and wrote the manuscript. J. V. K supported the idea of the manuscript and provided a number of suggestions.

CONFLICT OF INTERESTS

Declare none

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