ISSN : 0974 - 7508 Volume 8 Issue 7

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 8(7), 2012 [263-268]

Antidyslipidemic and antioxidant property of piperine

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Received: 21st May, 2012 ; Accepted: 26th September, 2012

ABSTRACT

Antidyslipidemic and antioxidant activity of piperine isolated from the fruits of *Piper longam* extracts have been studied in triton and high fat diet fed hyperlipidemic rats. The piperine lowered the serum lipids at 100 mg/Kg/p.o. in triton WR-1339 induced hyperlipidemia. Chronic feeding of the piperine at the dose level of 50 mg /kg body weight in high fat diet rats for 30 days caused lipid lowering of apoprotein level in experimental animals. It activates lypolytic enzymes in plasma and liver lipids. Piperine also increased faecal bile acid extraction and enhanced plasma lecithin. Piperine also showed potent cholestrol acyltransferase activity. Piperine showed potent antioxidant property and oxygen free radical scavenger activity (*in vitro*) and also induced oxidative stress.

KEYWORDS

Antdyslipidemic;
Antioxidant;
Activity;
Piperine;
Piper longam.

INTRODUCTION

Atherosclerosis and its associated complications is now days a major cause of myocardial morbidity and mortality world wide. Elevated plasma level concentration of the cholesterol specially low density lipoproteins (LDL) and triglycerides along with free radicals and oxidative stress are recognized as leading cause in the development of atherosclerosis and coronary heart desease[1-6]. Generally, oxidative damage takes place in the low density lipoprotein (LDL) of the plasma by the hydroxyl free radicals (OH^-) generated by the metal ions present in the serum due to the oxidative stress. It has been demonstrated that oxidative damaged LDL are more atherogenic than the native LDL[4-6]. Several drugs are being used in the treatment of dyslipidemia. The drugs can intervene by lowering cholesterol (LDL+total cholesterol) or by lowering triglyceride levels in the plasma. Treatment of hyperlipidemia using statins such as atorvastatin, lovastatin, fluvastatin,simvastatin and pravastatin are HMGCoA reductase inhibitors,which act by inhibiting cholesterol synthesis. However common side effects are myositis, arthralgias, gastrointestinal upset and elevated liver function tests. Thus there is a need for the therapeutic benefits of several antidyslipidemic drugs while simultaneously reducing the severe side effects.

The involvement of the hydroxyl free radicals have been found to be major cause for peroxidative damage to lipoproteins which is responsible for inhibition and progression of atherosclerosis in hyperlipidemic subjects[7].

Piper longam (family: Piperaceae) commonly known as Pippali in Hindi or long peeper in English is widely used as an household remedy in treating respiratory disorders. Biological activities have been reported

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in the literature[6-10]. The major chemical constituent of the fruits of *P.longam* extracts is an alkaloid piperine, which has been isolated and characterized from the fruits of this plant for the evaluation of its dyslipidemic and antioxidant properties.

MATERIAL AND METHODS

Collection of plant material

The fruits of *Piper longam* were purchased from local market of Lucknow and were authenticated by the botany division of Central Drug Research Institute, Lucknow

Animals

Male albino rats of Charles foster strain (100-150g) were used for the experiments and were kept under standard hygienic conditions maintaining at 12:12 hours light and dark cycle and fed with standard pellet diet (Liptan's India Ltd.) and water ad libitum. The experiment was approved accordance with the Institutional animal ethics committee.

Triton and high fat diet induced hyperlipidemia

The rats were divided into control, triton treated and triton plus piperine treated group of six rats in each of group. The triton WR-1339 (Sigma chemical company, USA) was administered at 400 mg/Kg. dose by intraperitonially for 18 hrs. Piperine and gemfibrozil (Cipla Ltd. Bombay, India) were macerated with 0.2% aqueous gum acacia suspension and fed daily at 100 mg/Kg dose simultaneously with triton. In the chronic study hyperlipidemia was produced by feeding the high fat diet to the experimental rats once a day for 30 days. Piperine was administered at 100 mg/Kg.dose orally. Control animals received same amount of normal saline. At the end of the experiments, rats were fasted overnight and blood was withdrawn. The animals were killed and liver was excised immediately.

Biochemical analysis of plasma /serum

Plasma lecithin: cholesterol acyl transferase (LCAT) activity[11] and post heparin lypolytic activity (PHLA) were assayed[12], serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyamine precipitation methods[13]. Serum as well as lipoprotein were analyzed for their total cholesterol (TC), phospholipids (PL), triglycerides (TG) and protein by standard procedures reported in our earlier papers[14,15]

Biochemical analysis of liver

Liver was homogenized (10% w/v) in cold 100mM phosphate buffer at pH 7.2 and used for the assay of total lipolytic activity[11]. The lipid extract of each homogenate was used for the estimation of TC,PL,TG and protein.

Faecal bile acid estimation

The rats faeces were collected from the groups throughout 30 days and processed for the estimation of cholic acid and deoxycholic acid[15].

Antioxidant activity (generation of free radicals)

Superoxide anions (O_2^-) were generated enzymatically by xanthine (160mM) xanthine oxidase (0.044) and nitrobluetetrazolium (320µM) in absence or in presence of piperine (100 to 200µg/ml) in 100mM phosphate buffer before use. The reaction mixture was incubated at 37^0C and after 30 minutesthe reaction was stopped by adding 0.5 ml glacial acetic acid. The amount of formazine formed was calculated spectrophotometrically. In another set of experiment, effect of piperine on generation of hydroxyl radical was studied by nonenzymatic reactants. Briefly hydroxyl free radicals were generated in a nonenzymatic system (2.0 mM) and H_2O_2 (2.8 mM) In 50mM KH_2PO_4 buffer pH 7.4 to a final volume of 2.5 ml. The above reaction mixture in the absence or in the presence of piperine (100 to 200µg/ml) was incubated at 37^0C for 90 minutes. The Piperine was also studied for its inhibiting action against microsomal lipid peroxidation in vitro by nonenzymatic inducer. Reference tubes and reagent blankwere also run simultaneously.malondialdehyde (MDA) contents in experimental and reference both the tubes were estimated spectrophotometrically.thiobarbuturic acid as mentioned above. Allopurinol, mannitol and á-tocopherol were used as a standard compounds for superoxide anions, hydroxyl radicals and microsomal lipid peroxidations.

Statistical analysis

Data were analysed using students 't' test, hypolipidemic groups were compared with control. Hyperlipidemic

and piperine treated with hyperlipidemic. Experimental group compared with reference groups $\rho < 0.05$ was considered as significant.

RESULTS

Effect of piperine in triton induced hyperlipidemia

The acute administration of triton R-1339 caused a marked hyperlipidemia in serum levels of TC (+ 3.07 F), PL (+2.34 F), TG (+2.83F) and protein (+2.10F). Treatment with piperine caused reversal of these levels of TC(-24%),PL (-25%), TG (-20%)and protein (-23%) repectively.(TABLE-1). The lipid lowering activity of piperine in hyperlipidemic ratswas comparable to that of gemfribrozil.

Effect of piperine on lipid composition in serum lipoproteins and liver

The data in TABLE 2 shows that administration of high fat diet in rats increased their serum levels of TC(+3.0 F),PL (+2.66F), TG (+2.22F) and protein (+2.03F) respectively. Feeding of piperine with gemfibrozil recovered the levels of these serum lipids of TC(-26%), PL (-24%), TG (-25%) and protein (-30%) respectively. The analysis of lipid serum showed a marked increase with levels of lipids and apoproteins constituting â-lipoproteins and there treatment with the piperine and gemfibrozil significantly reduced these levels of VLDL lipids (22, 33, 27& 20%) as well as LDL-TG (-33%) LDL-PL (-27%) LDL-TG (-33%) and apo-LDL (-32%) respectively in hyperlipidemic rats. At the some time decreased level of HDL-lipids and apo-HDL in these animals particularly recovered (TABLE-2). The increased the levels of lipids ie TC (+22%),PL (+5%), TG (+26%) and protein (+17%) of high fed rats were observed and treatment of piperine by lowering of lipids i.e.TC (-23%), PL (-22%), TC (24%) and protein (+ 20%) respectively.

Effect of the lipolytic enzymes

HFD feeding caused the inhibition of plasma LCAT (-47%) and PHLA (-40%) respectively.(TABLE-2)and total lipolytic activity (-43%) in liver.(TABLE-3). Treatment with piperine and gemfibrozil partially reactivated these lypolytic activities in plasma and liver of hyperlipidemic rats.

Effect of faecal excretion of the bile acid

Feeding with HFD caused a significant decrease in faecal excretion of bile acids (-31%) and deoxycholic acid (-50%) and these levels were shown to be recovered by the treatment of piperine (17+21%) and gemfibrozil (24+28%) in HFD and piperine fed rats.

Antioxidant activity of piperine

The antioxidant activities of piperine was evaluated by generating free radicals at a dose of 100µg to 200 µg/ml against formation of O_2^-, OH^- andmirosomal lipid peroxidation was studied (TABLE -4). The significant decrease in superoxide anions (-26+36%), hydroxyl radicals (28=37%) and microsomal lipid peroxidation (22+38%) respectively.

DISCUSSION

Hyperlipidemia is one of the important risk factors involved in the development of cardiovascular diseases. Atherosclerosis and congestive heart failure are strongly associated with disorders of lipid metabolism and plasma lipoproteins. Triton WR-1339 treated rats are considered to be useful acute hyperlipidemic model associated with inactive lipoprotein lipase. triton WR-1339 acts as a surfactant to block the uptake of lipoproteins from the circulation by the extra tissues resulting in an increase in the level of circulatory lipoproteins Piperine and gemfibrazil both caused a significant decrease in serum levels of lipids in triton induced hypolipidemic rats and this model has been successfully used for the evaluation of lipid lowering drugs In the present investigation,the high fat diet fed hyperlipidemic rats, piperine could increase the level of HDL by increasing the activity of lecithin, cholesterol, acyltransferase activities, which may contribute to the regulation of blood lipids. LCAT play a key role in lipoprotein metabolism and most of the lipoprotein changes are the out come of primary abnormality owing to the liver diseases Piperine facilitate the lipid catabolism of the LDL through the hepatic receptors in hyperlipidemic situation. This property may also overcome to hepatitis C virus (HCV) infections because LDL receptors have been proposed as a candidate receptors for HCV antigen.

Treatment with piperine provokes a rapid binding of β-lipoproteins, which compitively inhibits the involve-

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ment of HCV intigents with above receptors.

Piperine enhances the extraction of bile acids through faeces and this contributes to regress,the cholestesteosis in liver damage. Disorders of lipid metabolism are associated with peroxidative degradation of membrane lipids like picrolive

The potentially reactive derivatives of oxygen ascribed as Ros such as superoxide anions hydroxyl radicals and hydrogen peroxide are continuously generated inside the human body, as a consequence exposure to oxygen, chemicals/a number of metabolites as a result of metabolic process involving redox enzymes and bioenergetic electron transfer owing to the Ros over production or inadequate antioxidant the piperine which is a natural product has shown antioxidant property can be developed as lipid lowering diffuse, there is upsurge of Ros and this culminates in oxidative stress. It is quite interesting to note that with antioxidant drug.

TABLE 1 : Lipid lowering activity of piperine in triton treated hyperlipedemic rats

Experimental Schedule	Total Cholesterol [a]	Phospholipid [a]	Triglyceride [a]	Protein [b]
Control	90.32±7.89	85.33±6.70	88.19±5.82	7.50±0.37
Triton treated	277.77±22.84***	220.44±16.14***	245.81±20.23***	15.18±1.10***
	(+3.07F)	(+2.34F)	(2.83F)	(2.10F)
Triton + Piperine	210.11±16.79***	150.17±13.20***	199.12±12.78***	12.10±0.77***
	(-24)	(-25)	(-20)	(-23)
Triton+Gemfibrozil	179.39±12.22***	130.79±10.27***	170.02±14.00***	11.00±0.67***
(standard Drug)	(-38)	(-34)	(-32)	(-30)

Unit : (a) mg/dl (b) g/dL

Values are mean ± SD from 6 rats ***P< 0.001, **P< 0.01. Triton group compared with control, triton plus piperine treated compared with triton

TABLE 2 : Effect of piperine and gemfibrozil on blood lipids and lipolytic enzymes in hyperlipidemic rats

Parameters	Control	High fat diet treated	High fat diet and piperine treated	High fat diet and Gemfibrozil treated
A. Serum				
Total Cholesterol [a]	88.23±6.14	265.30±21.17***	194.44±13.80***	174.22±16.00***
		(+3.0 F)	(-26)	(-34)
Phospholipid [a]	84.17±5.88	240.88±20.30***	183.03±17.00***	170.70±14.10***
		(+2.86F)	(-24)	(-29)
Trigliyceride [a]	90.39±7.10	200.77±17.39***	150.29±12.88***	134.44±10.77***
		(+2.22F)	(-25)	(-36)
Protein [b]	6.40±0.30	13.00±0.77***	9.00±0.66***	8.88±0.37***
		(+2.03F)	(-30)	(-31)
B.VLDL				
Total Cholesterol [a]	8.50±0.62	30.81±2.14***	24.14±2.00***	22.84±2.00***
		(+3.62F)	(-22)	(-25)
Phospholipid [a]	16.10±1.01	33.33±3.00***	22.16±1.66***	21.70±2.00***
		(2.07F)	(-33)	(-25)
Triglyceride [a]	41.88±3.17	92.84±6.37***	67.33±5.37***	65.77±5.31***
		(+2.21F)	(-27)	(-29)
Apoprotein [b]	6.50±0.27	13.00±1.10***	9.60±0.77***	9.77±0.33***
		(+2.0 F)	(-26)	(-25)
C. LDL				
T Cholesterol [a]	13.58±0.66	65.10±5.17***	47.37±4.00***	45.50±3.10***
		(+4.79F)	(-27)	(-30)

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Parameters	Control	High fat diet treated	High fat diet and piperine treated	High fat diet and Gemfibrozil treated
Phospholipid [a]	13.00±1.00	45.00±4.17*** (+3.46F)	32.89±2.17** (-27)	32.22±2.14*** (-28)
Triglyceride [a]	15.39±0.58	37.22±3.18*** (+2.41F)	24.80±2.00*** (-33)	26.66±1.67*** (-28)
Apoprotein[b]	17.39±1.18	32.15±2.18*** (+1.84F)	21.66±1.17*** (-32)	22.18±1.44*** (-31)
D. HDL				
T Cholesterol [a]	47.00±3.60	35.38±2.14*** (- 24)	45.44±4.08* (+22)	46.16±3.61*** (+23)
Phospholipid [a]	40.17±4.00	30.10±1.88*** (- 25)	31.77±2.10NS (+5)	35.88±3.00* (+16)
Triglyceride [a]	17.30±0.50	11.77±1.00*** (- 29)	16.00±1.18*** (+26)	16.18±0.77*** (+25)
Apoprotein[b]	171.18±13.19	120.14±10.77*** (- 29)	145.33±11.18* (+17)	154.44±13.10** (+22)
Plasma LCAT activity[c]	70.70±5.10	37.37±2.18*** (- 47)	48.78±4.12*** (+23)	50.18±3.00*** (+25)
PHLA[d]	18.60±1.19	11.17±0.69*** (-40)	14.84±1.00*** (+24)	15.17±1.10*** (+26)

Units: (a) mg/dl serum, (b) g/dL serum, (c) n mol cholesterol released /h/l plasma,(d) n mol free fatty acid formed /h/ml plasma. Values are mean ± SD from six animals; ***P<0.001, **P<0.01, *P<0.05; Cholesterol treated compared with control, cholesterol and drug treated with triton only.

TABLE 3 : Effect of piperine and gemfibrozil on hepatic lipids and faecal bile acid excretion in hyperlipemic rats

Parameters	Control	Cholesterol treated	Cholesterol and piperine treated	Cholesterol and Gemfibrozil treated
A Liver				
LPL activity[a]	134.44±12.15	77.12 ±4.30*** (-43)	100.37 ±7.39*** (+23)	112.88 ±9.23*** (+31)
Total cholesterol[b]	7.33±0.55	12.88 ±0.69*** (+1.75F)	9.83 ±0.62*** (-23)	9.13 ±0.82*** (-29)
Phospholipid[b]	25.17±2.80	42.22 ±3.18*** (+1.65F)40%	32.77 ±3.00*** (-22)	29.99 ±1.84*** (-28)
Triglyceride[b]	11.66±0.88	17.39 ±1.12*** (+1.49F)	13.13 ±1.10*** (-24)	12.78 ±1.00*** (-26)
Protein[b]	154.00±12.77	225.33±20.14*** (+1.46F)	180.11 ±16.23*** (-20)	170.00 ±13.58*** (-24)
B Faecal bile acids				
Cholic acid[c]	87.17±6.12	60.13 ±5.31*** (-31)	72.88 ±6.11* (+17)	79.82±5.87*** (+24)
Deoxycholic acid[c]	57.33±3.18	28.81 ±2.30*** (-50)	36.44 ±2.00*** (+21)	39.09 ±3.00*** (+28)

Unit : (a) n mole free fatty acid formed/h/mg protein, (b) mg/g, (c) µg/g.

Values are mean ±SD of six animals; ***P<0.001, **P<0.01, *P<0.05. Cholesterol treated group compared with control and cholesterol plus drug treated group compared with cholesterol treated.

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TABLE 4: Effect of piperine on the generation of superoxide anions, hydroxyl radicals and lipid peroxidation in microsomes

Test compound	Conc of compounds (µg/ml)	Superoxide anions[a] (O_2^-)	Hydroxyl radicals[b](OH')	Microsomal lipid peroxidation[b]
	Control	192.34±14.72	109.24±9.76	90.27±5.77
Piperine	100	142.84±12.37***	77.80 ±5.39***	70.29±7.00***
		(-26)	(-20)	(-22)
	200	122.22±11.78***	59.88±3.87***	56.14±4.32***
		(-36)	(-37)	(-30)
Standard drug	200	110.00±8.27***	50.32±4.10***	47.20±3.14***
		(-43)	(-54)	(-48)
		(Allopurinol)	(Mannitol)	(α-tocopherol)

Units: (a) n mol formazone formed/min., (b) n mol MDA formed/h/mg protein.
Each value is mean ± SD of six values ***P< 0.001, **P< 0.01. Experimental data compared with control experiment.

ACKNOWLEDGEMENT

We are grateful to the HRDG,Council of Scientific and industrial Research, New Delhi for providing to VL emeritus scientist ship to complete the work and to publish the data. We are also thankful to the Council of Scientific and Industrial Research, Government of India New Delhi for providing us excellent research facilities at Central Drug Research Institute Lucknow.

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