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HYPOLIPIDEMIC ACTIVITY OF *PIPER LONGUM* IN EXPERIMENTAL HYPERLIPIDEMIA

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ABSTRACT: In the present study, the hypolipidemic activity of *Piper longum* (Family: Piperaceae) fruit extract was studied in two experimental models of hyperlipidemia. In an acute model of hyperlipidemia, induced by injecting a single dose of triton WR-1339 (400 mg/kg, b.w.) intraperitonially in rats, administration of *Piper longum* extract at the dose of 500 mg/ kg, b.w. resulted in significant lipid lowering effect of total cholesterol (TC), phospholipids (PL), triglyceride (TG) and reactivation of post heparin lipolytic activity (PHLA). We observed significant decrease in TC, PL and TG levels by 32%, 19%, 39%, respectively, and increase in PHLA activity by 29% as compared to hyperlipidemic animals. In the chronic model, hyperlipidemia induced by cholesterol rich-high fat diet, we recorded significant decrease in TC, PL and TG levels by 27%, 15%, 34%, respectively, and increase in PHLA activity by 20 % as compared to hyperlipidemic animals. The treatment with fruit extract of *Piper longum* (500 mg/ kg b.w) simultaneously for 15 days also caused lowering of lipid levels in liver homogenate, following reactivation of plasma post heparin lipolytic activity in animals. This hypolipidemic activity of *Piper longum* was comparable to standard drug guggulipid in both models.

INTRODUCTION: The word pepper is derived from the Sanskrit word '*pippali'*. Long pepper (*Piper longum*), is a flowering vine of the family Piperaceae, cultivated for its fruit. *P. longum* is a small shrub with a large woody root and numerous creeping, jointed stems that are thickened at the nodes. The plant grows in evergreen forests and is cultivated in Assam, Tamil Nadu, and Andhra Pradesh etc. Dried fruits are commonly used as a spice in Indian kitchen. Long pepper fruit is black in color, and has hotter flavor. The plant and its parts such as the fruits are used traditionally, but detailed information regarding its medicinal use is poorly documented. In Ayurvedic medicine, it is said to be a good rejuvenator. This plant is inexpensive and readily available. When applied topically, it soothes and relieves muscular pains and inflammation [1]. Despite its wide use as a folk remedy over a long period of time, the biochemical details of its action as physiological/ pathophysiological functions have not been

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systematically investigated. There are several research studies demonstrating remarkable effects of *P. longum* against various disease including cancer, inflammation, depression, diabetes, obesity and hepatotoxicity [2]. The plant has also been reported in improving microbial infections, cardiac disease, and protection against the effects of radiation [3]. The specific effects of the plant make it more useful for animals and human beings. Furthermore, the plant appears to be nontoxic, as no deaths have been reported with the use of high doses of the plant extracts [4]. Therefore, we planned to investigate the hypolipidemic potential of *P. longum* fruit extract in experimental models of hyperlipidemia.

MATERIAL AND METHODS:

Preparation of *Piper longum* fruit extract: *Piper longum* fruits were collected from local area of Lucknow and identified taxonomically by Department of Pharmacology, Era's Lucknow Medical College Lucknow. A voucher specimen (AV-006/10) was also submitted. Fruits were crushed and dried under shade. *Piper longum* powder (500 g) was extracted with 95 % ethanol in a soxhlet apparatus for 72 h, the extract was concentrated to dryness under reduced pressure and controlled temperature (50-60 °C), yielding 26 g of black brown solid (crude extract). This was stored in refrigerator and used to investigate hypolipidemic activity in rats.

Chemicals: Triton WR-1339, deoxycholic acid, cholesterol and heparin were procured from Sigma Chemical Company, USA. Guggulipid, a potent lipid lowering agent from gum resin of *Commephora mukul* (guggul) developed in Central Drug Research Institute, Lucknow, was used as a standard drug [5].

Preparation of Cholesterol rich- high fat diet: Deoxycholic acid (5 g) was mixed thoroughly with 700g of powdered rat chow diet supplied by Ashirvad Industries, Chandigarh, India. Simultaneously cholesterol (5 g) was dissolved in 300 g warm coconut oil. This oil solution of cholesterol was added slowly into powdered mixture to obtain homogeneous soft cake. This cholesterol rich -high fat diet (HFD) was molded in shape of pellet of about 3 g each [6].

Animals: *In vivo* experiments were conducted as per guidelines of Animal Ethics Committee of Institute. Male adult rats of Charles Foster strain (200 – 225 g) were used for study. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25- 26°C, relative humidity 50-60% and 12/12 h light/dark cycle (light from 8:00 am to 8:00 pm) and provided with standard rat pellet diet and water *ad libitum*.

Triton and Cholesterol rich-HFD induced hyperlipidemia: The rats were divided into four groups having six animals in each as follows: control, triton treated hyperlipidemic, hyperlipidemic treated with *Piper longum* and hyperlipidemic treated with guggulipid. In the acute experiment to induce hyperlipidemia, triton WR-1339 was administered (400 mg/ kg b.w., p.o.) by intraperitonial injection to induce hyperlipidemia. *Piper longum* extract and guggulipid were macerated with aqueous gum acacia (1% w/v) suspension and fed orally at the doses of 500 and 200 mg/kg, b.w., respectively. Control animals received same amount of vehicle. The diet was withdrawn and blood from fasted rats was collected after 18 hours.

Animals were anaesthetized with thiopentone solution (50 mg/kg b.w., i.p.), prepared in normal saline. Heparin (10mg/ml) in normal saline was prepared and injected to each rat (1mg/kg, b.w.) through tail vein. After 15 min, blood was withdrawn from retro-orbital plexus using glass capillary in EDTA coated tubes.

In the chronic experiment, hyperlipidemia was produced by feeding with cholesterol rich-HFD for 15 days. Drugs were administered orally once daily at the same doses as above, simultaneously, with cholesterol rich-HFD in the drug treated groups. Control animals, kept over normal rat pellet diet, received the same amount of vehicle. At the end of experiment, rats were fasted overnight and anaesthetized. Blood was drawn after 15 minutes of heparin treatment. Blood was centrifuged and plasma was taken. Thereafter, animals were sacrificed and liver was excised promptly, washed with cold 0.15 M KCl and kept at - 40 °C till analyses.

Biochemical Analysis of Plasma and Liver: Post heparin lipolytic activity (PHLA) was assayed in plasma spectrophotometrically using intralipid as artificial substrate [7]. Plasma was diluted with normal saline in a ratio of 1:3 and used for the analysis of total cholesterol (TC), phospholipids (PL) and triglyceride (TG) using standard enzymatic kits supplied by Merck India Ltd. Mumbai India [8, 9, 10]. Technical bulletin supplied with the kit was followed for the estimations. Liver was homogenized (10% w/v) in cold 100mM phosphate buffer pH 7.2 and used for the estimation of PHLA activity. The lipid extract of each homogenate prepared in a mixture of $CHCl_3$: CH_3OH (2:1, v/v) was used for estimation of TC, PL and TG.

Statistical Analysis: One way analysis of variance (ANOVA) was performed by comparison of values for hyperlipidemic groups with control, hyperlipidemic and drug treated groups with hyperlipidemic All hypothesis testing were two-tailed. P<0.05 was considered statistically significant and results were expressed as mean ± SD of six rats. The graph pad INSTAT 3.0 software carried out the statistically analysis [11].

RESULTS:

Effect of *Piper longum* extract in triton induced hyperlipidemia: The acute administration of triton WR-1339 in rats caused marked increase in their plasma levels of TC (159%), PL (179%) and TG (176%) following inhibition of PHLA by 32%. Thus, hyperlipidemia was confirmed. Treatment with *Piper longum* extract at the dose of 500mg/kg, b.w., resulted in decrease in TC, PL and TG levels by 32, 19 and 39% respectively, simultaneously with reactivation of PHLA by 29%. However, lipid lowering action of guggulipid even at a lower dose of 200mg/kg b.w., was comparatively higher (35-41%) to that of *Piper longum* extract (**Table 1**).

TABLE 1: EFFECT OF *PIPER LONGUM* EXTRACT AND GUGGULIPID ON PLASMA LIPIDS IN TRITON INDUCED HYPERLIPIDEMIA IN RATS

Groups	Total Cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	Post Heparin Lipolytic Activity (nmol FFA released/h/L)
Control	90.52 ± 6.11	84.46 ± 3.33	90.66 ± 8.69	16.89 ± 0.95
Triton treated hyperlipidemic	234.60*** ± 12.09 (+159)	235.89*** ± 12.09 (+179)	250.57*** ± 24.86 (+176)	11.56** ± 1.34 (-32)
Triton + *Piper longum* (500mg/kg, b.w.)	158.82** ± 10.39 (-32)	192.22** ± 3.09 (-19)	152.41** ± 14.80 (-39)	14.89* ± 0.80 (+29)
Triton + Guggulipid (200mg/kg, b.w.)	153.13*** ± 8.65 (-35)	159.14*** ± 7.85 (-33)	149.14*** ± 8.23 (-41)	14.36* ± 0.68 (+24)

Values are expressed as mean ± SD. Triton treated hyperlipidemic group is compared with control; drug treated group with triton hyperlipidemic treated group. Values in parenthesis indicate percent change. *P<0.05; **P<0.01; ***P<0.001.

Effect of *Piper longum* extract in cholesterol rich-HFD induced hyperlipidemia: In this model of hyperlipidemia, feeding with cholesterol rich-HFD in rats caused significant increase in their plasma levels of TC (135 %), PL (71 %) and TG (118 %) following inhibition of PHLA by 32%. Thus, hyperlipidemia was confirmed. Treatment with *Piper longum* extract for 15 days, reversed these plasma levels of TC, PL and TG by 27, 15 and 34%, respectively, simultaneously with reactivation of PHLA by 20%. The hypolipidemic action of *Piper longum* at the dose of 500 mg/kg, b.w., was comparable to that of guggulipid at the dose of 200mg/kg b.w. (**Table 2**).

TABLE 2: EFFECT OF *PIPER LONGUM* EXTRACT AND GUGGULIPID ON PLASMA LIPIDS IN CHOLESTEROL RICH-HFD INDUCED HYPERLIPIDEMIA IN RATS

Groups	Total Cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	Post Heparin Lipolytic Activity (nmol FFA released/h/L)
Control	88.59 ± 6.98	80.62 ± 4.29	114.80 ± 11.30	19.31 ± 1.30
Cholesterol rich-HFD treated	208.18*** ± 22.10 (+135)	138.27*** ± 24.86 (+71)	251.35*** ± 24.59 (+118)	13.11** ± 1.18 (-32)
Cholesterol rich - HFD + *Piper longum*	152.17** ± 14.13	118.86* ± 11.80	167.77** ± 13.22	15.73* ± 1.64

(500mg/kg, b.w.)	(-27)	(-15)	(-34)	(+20)
Cholesterol rich-HFD+ Guggulipid (200mg/kg, b.w.)	153.39** ± 8.83 (-27)	100.30** ± 8.77 (-28)	169.92** ± 6.09 (-33)	16.33 * ± 1.64 (+24)

Values are expressed as mean ± SD. Cholesterol rich-HFD treated group is compared with control, cholesterol rich-HFD and drug treated groups with cholesterol rich-HFD. Values in parenthesis indicate percent change. *P<0.05; **P<0.01; ***P<0.001.

Effect of *Piper longum* extract in cholesterol rich-HFD induced steosis in liver: Feeding with cholesterol rich- HFD in rats also caused accumulation of TC (53%), PL (72%) and TG (87%) following diminution of PHLA activity by 37% in liver. However, treatment with *Piper longum* extract showed a decrease in TC, PL and TG levels by 31, 23 and 33%, respectively, following reactivation of PHLA activity (39%) in hyperlipidemic animals. Guggulipid was more effective hypolipidemic than *Piper longum*, as it could decrease the level of lipids by 29 to 41%, following reactivation of PHLA activity (29%) in the liver of hyperlipidemic rats (**Table 3**).

TABLE 3: EFFECT OF *PIPER LONGUM* EXTRACT AND GUGGULIPID ON LIVER LIPID IN CHOLESTEROL RICH-HFD INDUCED HYPERLIPIDEMIA IN RATS

Groups	Total Cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	Post Heparin Lipolytic Activity (nmol FFA released/h/L)
Control	6.75 ± 0.30	22.43 ± 2.71	4.54 ± 0.38	79.85 ± 4.82
Cholesterol rich- HFD treated	10.33*** ± 0.62 (+53)	38.58*** ± 5.33 (+72)	8.56*** ± 1.02 (+87)	50.26*** ± 2.72 (-37)
Cholesterol rich- HFD + *Piper longum* (500mg/kg, b.w.)	7.10** ± 0.19 (-31)	29.80** ± 2.34 (-23)	5.77*** ± 0.39 (-33)	70.09* ± 7.15 (+39)
Cholesterol rich-HFD + Guggulipid (200mg/kg, b.w.)	7.36** ± 0.90 (-29)	28.47** ± 5.04 (-27)	5.11*** ± 0.62 (-41)	64.99** ± 4.66 (+29)

Values are expressed as mean ± SD. Cholesterol rich-HFD treated group is compared with control, cholesterol rich-HFD and drug treated groups with cholesterol rich-HFD. Values in parenthesis indicate percent change. *P<0.05; **P<0.01; ***P<0.001.

DISCUSSION: Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting in increased blood lipid concentration [12]. The lipid-lowering effect caused by feeding with *Piper longum* extract, as in the case of guggulipid, may be due to an early clearance of lipids from circulation in triton model and it may be due to reactivation of lipolytic enzymes as evidenced by increased plasma PHLA [13].

We have successfully used this model for the evaluation of the lipid-lowering activity of some natural products [14, 15].

The present investigation with cholesterol rich–HFD fed hyperlipidemic animals showed that *Piper longum* extract could stimulate PHLA and hepatic LPL activity, both of which play a key role in lipid catabolism and their utilization in body [16]. This situation imposed by feeding with the test sample, may be responsible for the decrease in the level of plasma and liver lipids in this model. We have reported that hypolipidemic action of guggulsterone, the active principle of guggulipid, is mediated through activation of PHLA, LPL, and lecithin cholesterol acyl transferase activities, inhibition of hepatic cholesterol biosynthesis, and increased faecal bile acid excretion. The same mechanisms may also interplay to cause the hypolipidemic effect of *P. longum* extract.

Hypolipidemic activity of *P. longum* can be attributed towards the active principal ingredients present in the fruit. The *P. longum* fruit contains a many alkaloids and related compounds such as piperine, methyl piperine, pipernonaline, piperettine, asarinine, piperundecalidine, piper longumine, piperlonguminine, brachystamide-B, N-isobutyl decadienamide, brachyamide-A, brachystine, pipercide, piperderidine, longamide, dehydropipernonaline piperidine etc [17, 18].

Piperine, piperlongumine, tetrahydropiper long umine, tri methoxy cinnamoyl-piperidine, and piper longuminine have been found in the root.

Several new active chemical constituents have also been identified in *P. longum* fruits, for *e.g*. 1-(3', 4'-methylenedioxyphenyl)-1E-tetradecene, piperoic acid, 3',4'di-hydroxy-biabola-1, 10-diene, eudesm-4(15)-ene-1beta, 6-alpha-diol, 6beta-diol, guinea sine, (2E, 4E, 8E) -Nisobutylhenicosa- 2,4,8-tri enamide, Piperoctadecalidine, 3', 4', 5' tri methoxy cinnamate etc [19, 20].

Limited research has been conducted using the *P. longum* and its various parts for their medicinal use and therapeutic potential [21].

However, studies have been available on the other species of *Piper* such as *Piper nigrum, Piper methysticum, Piper umbellatum, Piper galeatum etc.* for their anti-inflammatory, anti-oxidant properties and ethnopharmacological evaluation [22, 23].

CONCLUSION: In present study, we have tested crude extract of *Piper longum* which however, upon research and development, may produce a more potential lipid lowering natural product. Further, research on crude drug metabolism and assessment of biological activity *in vivo* and *in vitro* of *Piper longum* are required to substantiate the present findings.

LIMITATIONS OF THE STUDY: Restricted use of animals was allowed for experiment following CPCSEA guidelines.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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