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Research Article

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Antihyperlipidemic activity of seed extract of *Piper attenuatum* in triton X-100 induced hyperlipidemia in rats

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ABSTRACT

Piper attenuatum is a potent medicinal plant in the Indian systems of medicine. Piper attenuatum having Piperine, Piperlonguminine and other active constituents which are used as muscle relaxant, central nervous system depressant, in headache and as insecticide agent against Musca domestica. Piperine reduced the total body temperature and display analgesic, and anti-inflammatory activities. The aim of present study is to determine the Anti-Hyperlipidemic activity of Ethanolic seed extract of plant Piper attenuatum (Linn.) B. Ham. (Fam. Piperaceae). Piperine was the first amide to be isolated from Piper species. Ethanolic extracts of Piper attenuatum with a dose of 200 mg/kg exhibited significant Anti-Hyperlipidemic activity in Triton X-100 Induced Hyperlipidemia in rats (P<.05). It was found that Piperine & Piperlonguminine the active constituent of the plant was responsible for the Anti-Hyperlipidemic activity because this constituent have the ability to reduce the Total Cholesterol & Total Triglyceride level in rats. Fenofibrate was used as standard drug (65 mg/kg). The total time period of this study was one week.

Keywords: *Piper attenuatum*, Anti-Hyperlipidemic activity, Triton-X 100, Fenofibrate, Cholesterol, Triglyceride Kits.

INTRODUCTION

Piper attenuatum is an Indian medicinal plant Linn. (B. Ham.), (Fam. Piperaceae) growing widely throughout India and Tropical countries of the world & Eastern tropical Himalayas, Assam, khasi hills, & nilgiris & also found in southern region of India like Kerala & Tamilnadu. Each and every part of the plant is claimed to possess some therapeutic property. In Ayurveda the seeds, leaves, root, stem, bark, herb, shrub are used. Plant is also known as kattumulaku (Malayalam Name), Oval leaved peeper plant (English Name). Plant contains about eight or nine genera, 2000 - 3000 species which are present in tropical and sub tropical region. Piper genus has a lot of different species like *Piper nigrum, Piper chaba Piper cubeba, Piper auranticum* etc. *Piper nigrum, Piper longum, Piper bettle* shows anti diabetic activity. Root of *Piper attenuatum* contain diuretic activity.[1] Plant is used as rubfacient, poultice, muscular pain & in headache.[2] Leaves of *Piper attenuatum* having anti-Trypanosomal activity[3]. Crotepoxide present in whole plant of *Piper attenuatum* having antitumor activity for Lewis lung carcinoma[4]. Aristolactams have been reported from the seed of the plant showing antitumor activity[5]. Taking into account the fact that there are 700 species belonging to this genus spread throughout the World and only 12% of them have been Phytochemical investigated (only 84 species have been examined thus far), this genus still remains a potent one to work on. Phytochemistry of the plant shows presence of alkaloids, amides, lignans, neolignans & steroids. *Piper attenuatum* contain Piperine, Piperlonguminine, guineesine, piperadione, galbelgin etc[6]. Piperlonguminine present in the plant having insecticidal activity against Musca domestica[7]. Piperine present in plant showed strong potentiating effect on hexobarbital- induced hypnosis in mice. Piperine decreased passivity & ptotic symptoms & lowered body temperature. Piperine exhibited central nervous depressant activity as well as muscle relaxant activity[8].

This research work is novel and work on *Piper attenuatum* on Anti-Hyperlipidemic activity is not carried out by anyone previously. There are no scientific studies in support of this traditional claim. Hence in the present study, an attempt has been made to investigate Anti-Hyperlipidemic effects of *Piper attenuatum.*

EXPERIMENTAL SECTION

Plant material

The Plant *Piper attenuatum* was collected from Tropical Botanic Garden & Research Institute, Palode, Kerala, India. The Plant is authenticated by Dr. Dan Mathew, Scientist, PGR, Division, Tropical Botanic Garden & Research Institute. Seeds were shade dried and chopped into small pieces separately at NIMS institute of pharmacy, Shobha nagar, Jaipur, Rajasthan, India.

Test Animals

Male wistar albino rats (b.w.150-200 gm.) either sex, maintained in the Animal Experimental Laboratory of NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India at room temperature of 25 ± 2°C, relative humidity of 75±5% and 12 h dark-light cycle. Food and water were given ad libitum. The project was approved by Institutional animal Ethical Committee (Registration No. NU/PH/M/COL/12/72). Each experimental group consisted of four animals housed in separate cages. The animals had access to standard laboratory feed.

Determination of acute oral Toxicity (LD_{50})

The acute oral toxicity study was done by 'Up-and- Down' method in healthy adult female albino rats according to CPCSEA recommended 'OECD' guideline 425. There were no changes from dose level of 175 mg/kg. p.o, to 2000 mg/kg, p.o. Drug extract did not cause any death up to 2000 mg/kg. The LD_{50} calculated is 2000 mg/kg for the Ethanolic extract, so one tenth of the maximum tested dose (i.e. 200 mg/kg, p.o.) was selected for the evaluation of the Anti-Hyperlipidemic effect and we take one dose for evaluating Anti-Hyperlipidemic activity 200 mg/kg.

Chemicals

All the chemicals like Fenofibrate, Triton X-100, Cholesterol & Triglyceride Kits were brought from the Jaipur, Rajasthan, India.

Preparation of extract

Seeds of *Piper attenuatum* were shade dried and powdered. The total quantity of powdered material was about 270 gm. This powdered material was subjected to defat with petroleum Ether for 72 hours in a Soxhlet apparatus. Then after 72 hours this defatted material is subjected to extraction with ethanol (99.99%) in a Soxhlet apparatus for 48 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50^{0}C) using flash evaporator. Preliminary Phytochemical screening of the seed extract extracts of the plant *Piper attenuatum* showed the presence of steroids, alkaloids, saponins, flavonoids, tannins and carbohydrates.

Procedure

Hyperlipidemia was induced in Wistar albino rats by single intraperitonial injection of freshly prepared solution of Triton X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 hour's. The animals were divided into four groups of four rats each.

The Group I was marked as normal received standard pellet diet water and orally administered with 5% CMC. GROUP II, III, IV were made Hyperlipidemic by Triton X-100 at a single dose of 100 mg/kg, i.p. After 72 hours of Triton X-100 injection, these groups were received a daily dose of 5% CMC (p.o) for 7 days. Group II was marked as diseased group while GROUP III was administering a daily dose of *Piper attenuatum* 200 mg/kg/ day p.o for 7 days, after inducing hyperlipidemia and GROUP IV was administering with the standard drug Fenofibrate (65mg/ kg body weight) p.o for 7 days.

Treatment was given daily for 7 days. At the 8th day the blood sample was collected by the heart puncture. Serum sample was analyze for, total cholesterol and total triglyceride by colorimeter. Body weight was also recorded. The animals were weight at the beginning and end of the experimental period.

Collection of blood sample and analysis

On the 8thday, blood was collect by heart puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collect and used for various biochemical experiments.

Biochemical analysis

The serum extract was assayed for total cholesterol & triglycerides, using standard protocol methods.

Parameters

After a daily treatment for 7 days, blood was collected from heart puncture & blood was analyzed for Total cholesterol (mg/dl), total triglyceride (mg/dl), body weight on daily basis.

Statistical analysis:

The result was expressed as mean ± S.E.M. (standard error mean). Data was analyzed by one way anova followed by Dunnett's multiple comparison tests against Hyperlipidemic control. The statistical analysis was done using the trial version of Graph Pad 5.0 software[9,10].

RESULTS

Ethanolic extract of *Piper attenuatum* showed significant Anti-Hyperlipidemic activity in Triton X-100 induced Hyperlipidemia in rats. Single dose (200 mg/kg) was taken for this study. Time period for this study was one week. Rats showed increase in body weight, Total Cholesterol & Total Triglycerides in Triton-X 100 treated rats & body weight, Total Cholesterol, Total Triglycerides significantly decrease in Ethanolic extract treated & Fenofibrate treated rats.

Table 1 Table shows the effect of *piper attenuatum* extract on Triton X-100 induced Hyperlipidemia in rats

*Values are in mean ±SEM; Number of animals in each group =4 * p < 0.05, ** p< .01, ***p<.001*

Sr. No.	Groups	Body Weight Initial	Body Weight after one week	Cholesterol (mg/dl)	Triglyceride (mg/dl)
1.	Normal	138.8 ± 4.2	148.8 ± 4.2	252 ± 1.7	155.0 ± 1.4
2.	Untreated	137.5 ± 6.2	208.8 ± 4.2	316 ± 1.6	197.0 ± 1.2
3.	Standard 65mg/kg	138.8 ± 4.2	188.8 ± 4.2***	264.5 ± 2.0***	164.0 ± 2.3***
4.	Extract 200mg/kg	125 ± 6.4	173.8 ± 2.3**	256.3 ± 1.4***	157.3 ± 1.2***

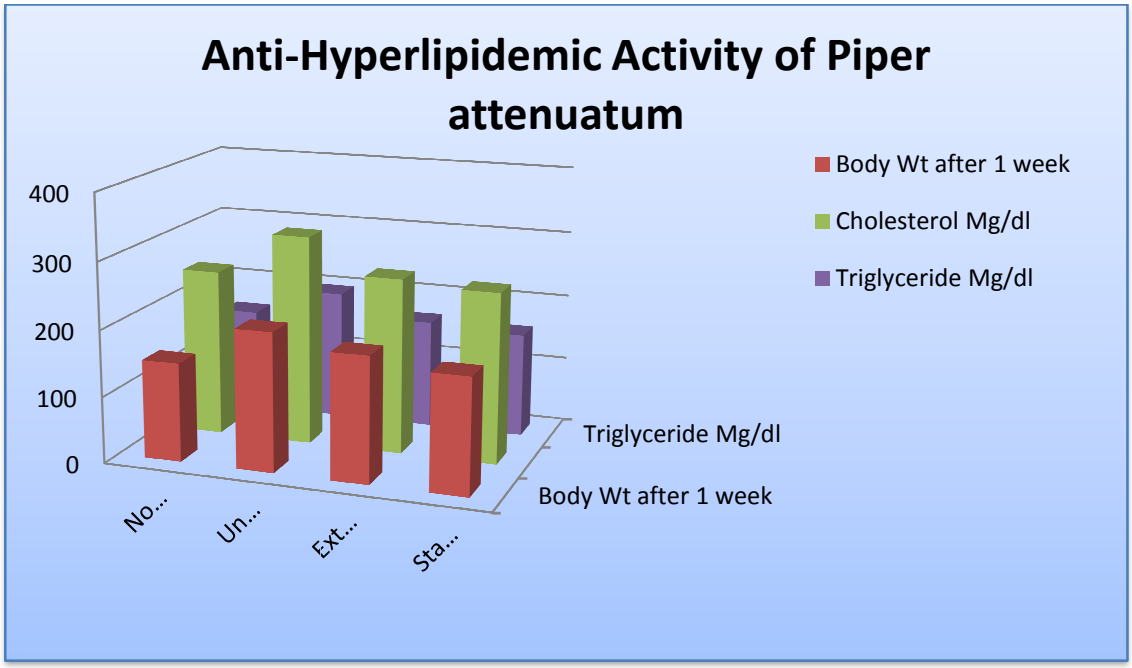

Figure 1 Show's Anti-Hyperlipidemic Activity in Rats

DISCUSSION

Elevated levels of total cholesterol are associated with increased risk of atherosclerosis. High level of triglycerides and LDL are associated with coronary artery disease, which is seen in untreated Group which include Triton-X100 induced rats. Ethanolic extract treated rats significantly reduced (P<0.001) Total Cholesterol and Triglycerides concentration at the administered dose when compared with experimental controls animals. Thus, the reduction in serum Total Cholesterol concentration effected by the Ethanolic extract of *Piper attenuatum* is beneficial and may reduce the risk of cardiovascular disease.

After reviewing the literature it was found that Piperlonguminine which is the active constituent of the plant give Anti-Hyperlipidemic effect in rats[11].

Furthermore there are no reports demonstrated the Hyperlipidemic potential of the Ethanolic extract of the plant *Piper attenuatum* in triton-X induced Hyperlipidemia in rats.

Hence, in the present study we demonstrated that Ethanolic extract of the plant shows the anti Hyperlipidemic effect on the Triton-X100 induced hyperlipidemia in rats.

CONCLUSION

The Ethanolic extract of *Piper attenuatum* showed significant Anti-Hyperlipidemic activity, it should be possible that Piperlonguminine & Piperine the active constituent of Piper attenuatum is responsible for the Anti-Hyperlipidemic activity. This study has established the Anti-Hyperlipidemic activity of *Piper attenuatum* and thus, justifies the Anti-Hyperlipidemic and ethno medical uses of this plant for Hyperlipidemia. Piperine & Piperlonguminine is isolated from the extract by using coloumn chromatography & structural identification of Piperine is done by using HR-MS at Indian Institute of Integrative Medicine, Jammu.

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