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Quality evaluation of *Phyllanthus amarus* (Schumach) leaves extract for its hypolipidemic activity

R P Umbare^{*1}, G S Mate¹, D V Jawalkar², S M Patil¹, S S Dongare¹

¹A.S.P.M's K.T. Patil College Of Pharmacy, Siddharth Nagar, Barshi Road, Osmanabad (M.S.), India- 413501.

²S.G.R.S. College of Pharmacy, Saswad, Pune (M.S.), India.

*Corresponding Author: ramumbare1402@gmail.com, ramumbare1402@rediffmail.com

Abstract

Atherosclerosis, referred to as a "silent killer" is one of the leading causes of death in the developed countries and is on the rise in developing countries like India. Therefore therapists consider the treatment of hyperlipidemia to be one of the major approaches towards decelerating the atherogenic process. Allopathic hypolipidemic drugs are available at large in the market but the side effects and contra- indications of these drugs have marred their popularity. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs. The present study has been carried out to evaluate the antihyperlipidemic effect of plant *Phyllanthus amarus* Schumach against cholesterol diet induced hyperlipidemia in Wister rats. Hydro-alcoholic extract of leaves of *Phyllanthus amarus* Schumach (HAEPAS) was studied for its *in-vivo* anti-hyperlipidemic potential using cholesterol diet induced hyperlipidemia model in rats. The result of study indicated that HAEPAS possess significant hypolipidemic activity at doses 300 and 500 mg/kg.

Keywords: HAEPAS, Nicotinic acid, Cholesterol diet.

Introduction

The plant *Phyllanthus amarus* Schumach (Euphorbiaceae) is commonly known as 'Bhuivali' usually occurs in Asam, Maharashtra, Burma, Nicobar, Islands Malesia and America. *Phyllanthus amarus* Schumach is a native to the Americas (van Holthoon, 1999). The Spanish name of the plant, *Chanca piedra*, means, "Stone breaker". It was named for its effective use by generations of Amazonian indigenous peoples to eliminate gallstones and kidney stones. In India, the plant is used for numerous conditions including colic, diabetes, malaria, dysentery, fever, flu, tumors, jaundice, vaginitis, gonorrhoea, and dyspepsia (Kirtikar and Basu, 1935 And Burkill, 1966.). The juice or extract of its thinner roots and young leaves are taken internally to stimulate the kidney. Heyne recorded its uses in the Dutch Indies (Indonesia) for stomachache, gonorrhoea and children's cough. However, no systemic study on anti-hyperlipidemic activity of the leaves has been reported in the literature. In present investigation, we have screened hydro-alcoholic extract of leaves of *Phyllanthus amarus* Schumach (HAEPAS) for its anti-hyperlipidemic activity (Van Holthoon, 1999.).

Materials and Methods

Plant material

Leaves of *Phyllanthus amarus* Schumach were collected from foothill of Yedshi-ghat, Maharashtra, India. The plant material was identified and authenticated by Head, Department of Botany of BAMU, Aurangabad (M.S.), India.

Preparation of extract

The leaves were dried under shade and then coarsely powdered with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for the extraction. The marc left after Petroleum ether extract was dried and then extracted with ethanol 95% v/v (75-78°C) and distilled water mixture in proportion of 50:50 up to 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark brown color residue (yield-16.35%) was obtained. The residue was then stored in a dessicator. The extract was subjected to phytochemical screening (Trease and Evans 2003., Harboun J B *et al.* 1976 And Pulok K. Mukerjee.2002).

Selection and acclimatization of animals

Wister rats (150-180gm) of either sex and of approximately the same age, procured from listed suppliers of KIM's University Karad, (M.S.), India were used for the study. They were housed in polypropylene cages and fed with

standard rodent pellet diet (Pranav Agro Industries, Sangli (M.S.) and water *ad libitum*. The animals are exposed to alternate cycle of 12hrs of darkness and 12hrs light. Before each test, the animals were fasted for at least 12 hrs and the experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee (P.Col. /14/2006) and were cleared by the same. All experiments were performed in the morning according to current guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals⁷. The standard organistic cannula and syringe were used for drug administration in experimental animals. Animals were housed in plastic bottom cages and were allowed free access to standard laboratory feed and water. All the animals have been divided into five groups and placed in separate cages, each consisting of 6 animals. The animals were acclimatized to the laboratory conditions for one week before the onset of experiment.

Induction of hyperlipidemia

It is a standard model for evaluating anti-hyperlipidemic activity. Cholesterol diet was

used to induce hyperlipidemia in rats (Kutty *et al*, 2004). Cholesterol diet is administrated in rats by oral route. It significantly increases serum total cholesterol, triglycerides and AST and ALT levels.

Selection of dosage

The dose of leaves extract of HAEPAS was arbitrarily chosen as 300 mg/kg. Dose of nicotinic acid (270 mg/kg) was calculated based on a human dose of 3 gm per day. The drugs were suspended in 4% gum acacia for oral administration.

Treatment protocol

Rats were made hyperlipidemic by the oral administration (P.O) of cholesterol (400 mg/kg) along with cholic acid (50 mg/kg) in coconut oil for 20 days, once daily. The rats with elevated cholesterol level were divided into 4 groups of 8 animals each and given drug/ vehicle treatment for 10 days. During these 10 days all groups also received cholesterol in same dose as earlier. In each group 8 animals were taken. Animals were kept fasted throughout the experimental period, but were provided water *ad libitum*.

- Group – I: Hypercholesterolemic Control (HC- C)**
 - Animals received vehicle (Acacia 4%) 5 ml/kg P.O
- Group – II: Standard group (HC- NIC)**
 - Animals received Nicotinic acid 270 mg/kg; P.O
- Group- III: Test Group – I (HC- HAEPAS - I)**
 - Animals received HAEPAS (300 mg/kg; P.O)
- Group- IV: Test Group – II (HC- HAEPAS - II)**
 - Animals received HAEPAS (500 mg/kg; P.O)

Blood samples for biochemical estimation were withdrawn from rats, after overnight fast, by decapitation after inducing anesthesia. Serum cholesterol and triglyceride levels were determined on day 1 (before treatment) & on day 10 (after treatment). Four

groups of normal rats, each with 6 animals weighing 150- 180 gm were selected & given 10-day drug/vehicle treatment as above. However, no cholesterol was administered to them, neither as pre-treatment nor during treatment.

- Group – V: Normal control (N- C)**
 - Animals received vehicle (Acacia 4%) 5 ml/kg P.O
- Group – VI: Standard group (N - NIC)**
 - Animals received Nicotinic acid 270 mg/kg; P.O
- Group- VII: Test Group – I (N- HAEPAS - I)**
 - Animals received HAEPAS (300 mg/kg; P.O)
- Group-VIII: Test Group – II (N- HAEPAS - II)**
 - Animals received HAEPAS (500 mg/kg; P.O)

In each group 6 animals were taken. Animals were kept fasted throughout the experimental period, but were provided water *ad libitum*. Their serum total cholesterol and triglyceride levels were determined on day 1 and day 10 as for hypercholesterolemic rats. The lipid profiles of cholesterol diet induced hyperlipidemia model were summarized in Table No.1.

Biochemical estimation

After the experimental period, animals in different groups were sacrificed. The rats were killed by decapitation after inducing anesthesia (pentobarbitone sodium, 60mg/kg) and blood samples were collected into dry clean tubes. After centrifugation for 10 min, the serum samples were taken for biochemical assay. Serum levels of AST, ALT (Kutty *et al*, 2004), total cholesterol, triglyceride, were determined by standard biochemical kit obtained from Merck, Germany.

Preparation of tissue homogenate

For estimation of lipids from the tissues, sample of liver were rinsed in ice-cold saline and blotted carefully. Each liver sample placed in ice-cold glass homogenizer containing phosphate buffer at pH 7.4. The wet weight of the added sample was determined. After homogenization and centrifugation the supernatant was collected for determination of total cholesterol and triglyceride by standard biochemical kit obtained from Merck, Germany.

Histopathological examination

A small portion of the liver tissues from all the groups was excised immediately after sacrifice. Tissues were fixed in 10% formalin in phosphate buffer (pH 7.0) for 24 hr at room temperature for histopathology. Tissues were embedded in

paraffin was and sections were cut 3-5 μm slices and were stained with haematoxylin and eosin (H&E) and observed under light microscope (Galigher *et al*, 1971).

Statistical analysis

The data were statistically analyzed by Student's t-test and all values were expressed as mean \pm SEM. The data were also analyzed by one-way ANOVA followed by Dunnet's t-test (S.K. Kulkarni, 1999).

Results

Serum and tissue lipid profile

There were significant elevation noted in the levels of total cholesterol, triglyceride in the serum, liver tissue of HC diet group in compared to normal diet group (Table 1, 2). In the present study HAEPAS significantly prevented the high cholesterol diet induced rise in the levels of total cholesterol, triglyceride in serum and liver tissue of Group IV (HC+ HAEPAS) rats compared to group I rats (HC diet). Serum and tissue transaminases (ALT & AST) levels were also significantly increased in the nicotinic acid group compared to cholesterol diet induced group (Table No. 1, 2). This increase in the transaminase levels in liver tissue indicates the propensity to cause hepatotoxicity. This manifested as an elevation of AST & ALT in liver tissue and serum of patients receiving nicotinic acid. This is one of the major limitations of nicotinic acid. However, HAEPAS (300 mg/kg) and HAEPAS (500 mg/kg) did not shown any significant elevation of ALT & AST levels in the serum as well as liver tissues, compared to the nicotinic group. The results are depicted in Table No. 1 and 2.

Table 1: Effect of HAEPAS on lipid profiles in serum against cholesterol diet induced hyperlipidemia model.

Gp No	Groups	Total cholesterol (mg/dl)		Triglyceride (mg/dl)		Transaminases (U/L)	
		Pre Treatment	Post Treatment	Pre Treatment	Post Treatment	AST	ALT
I	HC- C	90.16 \pm 0.82	82.35 \pm 1.09	134.06 \pm 1.73	163 \pm 1.57	195.93 \pm 2.43	178 \pm 1.15
II	HC- NIC	83 \pm 2.85	65.78 \pm 2.99*	163.63 \pm 4.12	110.55 \pm 1.28*	334.21 \pm 4.2*	282 \pm 1.70*
III	HC- HAEPAS I	94.54 \pm 2.45	75.09 \pm 1.97*	176.10 \pm 3.32	121 \pm 1.56*	239.93 \pm 2.99*	230 \pm 2.88*
IV	HC- HAEPAS II	94.83 \pm 0.83	70.33 \pm 1.80*	162.38 \pm 3.31	117 \pm 2.12*	235 \pm 2.3*	219 \pm 4.85*
V	N- C	64.56 \pm 4.30	71.10 \pm 2.12	115.30 \pm 1.81	111.09 \pm 2.01	-	-
VI	N- NIC	61.54 \pm 2.92	55.63 \pm 1.28	137.17 \pm 2.17	98.56 \pm 4.98	-	-
VII	N- HAEPAS I	66 \pm 2.13	77.16 \pm 2.12	144 \pm 2.53	110.57 \pm 2.32	-	-
VIII	N- HAEPAS II	66.16 \pm 1.43	79.50 \pm 1.68	152 \pm 3.20	110.54 \pm 2.65	-	-

*P<0.05 vs before treatment

The data were statistically analyzed by Paired t-test and all values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values p<0.05 were considered significant.

Table 2: Effect of HAEPAS on lipid profile in liver tissue against cholesterol diet induced hyperlipidemia model.

Group	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Transaminase (U/L)	
			ALT	AST
N – C	4.28±0.43	7.11±0.32	-	-
HC – C	8.21±0.31	14.95±1.97	20.39±1.521	16.25±1.543
HC – NIC	5.74±0.260*	5.96±0.491*	33.60±1.97	29.40±2.04
HC – HAEPAS I	7.17±0.649*	8.41±0.90*	24.83±1.23*	20.98±1.42*
HC – HAEPAS II	4.98±0.418*	7.89±0.56*	22.13±1.64*	21.16±0.95*

*P<0.05 vs control

The data were statistically analyzed by Student's t-test and all values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values p<0.05 were considered significant.

Morphological and histopathological observation
 The liver of the high cholesterol (HC) diet group was significantly enlarged and the color of liver becomes pale. The physical appearance, i.e. color, size and smoothness of the liver of HAEPAS with HC diet group remain unaltered comparing those of the normal control group. Microscopical examination of liver section of control group (Fig. No. 1) showed normal arrangement of hepatocytes with clear broad of central vein at portal layer. Microscopical examination of liver section of cholesterol diet treated group (Fig. No. 2) showed various degrees of pathological changes such as centrilobular fatty degeneration, cloudy swelling and necrosis of hepatic cells. The

histopathological study showed recovery of the damaged liver cells in the drug treated group. The ruptured cells of the intoxicated liver were reformed. The degree of vascularization was also reduced as compare to hyperlipidemic group. (Fig. No. 4, 5). But, situation is somewhat different in case of cholesterol diet group. There is degeneration of hepatocytes, swelling observed in the standard treatment group (Fig. No. 3). It is due to increase in the levels of AST and ALT in the liver tissue. Microscopical examination of liver section of HAEPAS (300 mg/kg & 500 mg/kg) treated group (Fig. No. 4, 5) clearly showed normal hepatic cells and central vein, which are comparable with nicotinic acid, treated group (Fig. No. 3).

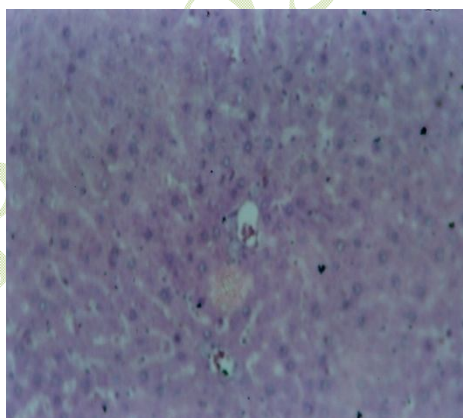


Fig 1: Liver section of control (H&E, x 400)

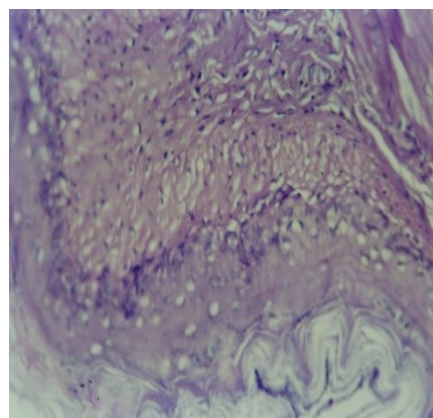


Fig 2: Liver section of high cholesterol (HC) diet treated rats showing marked vascular congestion fatty deposition and foamy degeneration of hepatocytes (H&E, x 400)

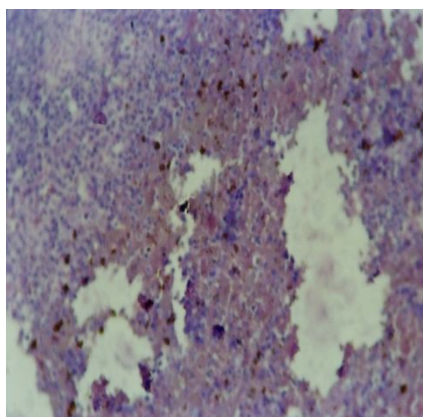


Fig 3: Liver section of HC+ standard drug showing normal hepatocytes and central vein but some degree of swelling (H&E, x 400)

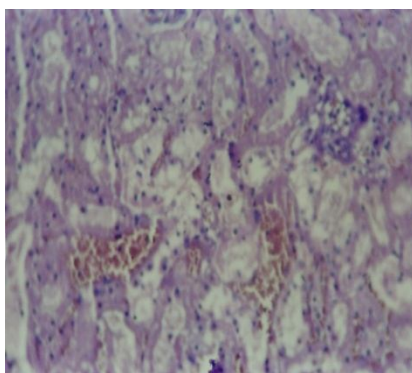


Fig 4: Liver section of HC+ HAEPAS (300 mg/kg) showing recovered normal hepatocytes (H&E, x 400)



Fig 5: Liver section of HC+ HAEPAS (500mg/kg) showing recovered normal hepatocytes (H&E, x 400)

Discussion

The phytochemical and pharmacological studies on the leaves of the plant *Phyllanthus amarus* Schumach was done. The phytochemical constituents were extracted by successive solvent extraction. Hydro- alcoholic extract of *Phyllanthus amarus* Schumach (HAEPAS) shows the presence of various phytoconstituent (alkaloids, flavonoids, saponins and tannins). Previous studies have reported some of these phytoconstituents to elicit a wide range of biological activities, which include hypolipidemic, hypoglycemic, hypoazotemia among others (Olapade *et al.*, 1995). Specifically, saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile

acids (Topping *et al.*, 1980). In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia (Kritchevsky, 1977; Potter *et al.*, 1979). Hence, it was selected for the pharmacological studies. In the pharmacological studies, HAEPAS shows significant antihyperlipidemic activity in dose dependent manner. The anti-hyperlipidemic activity was evaluated by using cholesterol diet induced hyperlipidemia. It was found that HAEPAS was more effective in higher dose as compared to lower dose as an anti-hyperlipidemic agent against cholesterol diet induced hyperlipidemia model. Present studies reveal that HAEPAS can be used as effective antihyperlipidemic. Equally literature has reported the hypolipidemic effects

of flavonoids, alkaloids, saponin and tannins (Olagunju *et al.*, 1995). The presence of these phytochemicals in the extract in high concentrations could account for these observed biological effects, particularly hypoglycemic and hypolipidemic effects. Again, this hypothesis would require experimental validation.

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