Original Research Article

Hypolipidemic activity of *Phyllanthus acidus* leaves in Hypercholesterolemic diet-induced hyperlipidemia in rats

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Abstract: Dyslipidemia is characterized by alterations in the lipid parameters. Increase in levels of bad cholesterol and triglycerides in dyslipidemia combined with the reduced levels of HDL predispose to development of various pathological conditions. Dyslipidemia is a well-known risk factor for cardiovascular diseases, acute pancreatitis etc. The phyllanthus genus is abundant in phytochemicals that have lipid lowering properties. Various studies have demonstrated hypolipidemic properties of *phyllanthus niruri, Phyllanthus amarus* in animals and humans. This study was conducted to evaluate the hypolipidemic potential of the leaves of *phyllanthus acidus* in hypercholesteromic diet fed rats. Ethanolic extract of leaves of *phyllanthus acidus* was employed in this study. This study demonstrated hypolipidemic activity of the leaves of *phyllanthus acidus* in rats. This was however not comparable to standard hypolipidemic drug atorvastatin.
Keywords: Lipids, Cholesterol, Hypolipidemia, Atherosclerosis, Metabolic syndrome.

INTRODUCTION

Hyperlipidemia or dyslipidemia is characterized by presence of excess lipids, largely cholesterol and triglycerides in blood. These excess lipids travel in blood attached to proteins making them soluble in plasma. Therefore, the term "hyperlipoproteinemia" is also used to refer to this condition [1]. The blood levels of VLDL (very low density lipoprotein), LDL (low-density lipoprotein) and TG (triglycerides) increase, whereas the HDL (high-density lipoprotein) levels fall in dyslipidemia [2]. CVDs (cardiovascular diseases) that also include CADs (coronary artery diseases) are one of the leading causes of death worldwide, accounting for approximately 17.3 million deaths per year [3]. Dyslipidemia along with hypertension are the principle etiological factors behind development of CVDs. Increased levels of LDL-C (LDL- cholesterol) followed by their oxidation and accumulation in vascular cells promote the formation of foam cells. Release of inflammatory mediators like IL (interleukin)-1, IL-6 and TNF (tumor necrosis factor)-α also increase, leading to the development of atherosclerosis [4]. As a result, the risk of development of CAD becomes directly proportional to the rise in LDL-C levels [5]. HDL-C (HDL-cholesterol) is responsible for the reverse transport of cholesterol from the periphery to the liver. Increased levels of HDL-C reduce the risk of development of CAD and vice versa [6]. There are other complications associated with dyslipidemia. Dyslipidemia, especially hypertriglyceridemia has been reported to be the third most common cause of acute pancreatitis after alcohol and gallstones [7]. It also leads to precipitation of cholesterol crystals in biliary ductular system resulting from the super saturation of the micelles. This causes increased formation of cholesterol gall stones [8].

The National Cholesterol Education Program (NCEP) of the NHLBI (National Heart, Lung, and Blood Institute) issued the ATP III (adult treatment panel-the third report) cholesterol management guidelines in May 2001. These had redefined the levels at which blood cholesterol should be treated [9]. The ACC (American College of Cardiology) and AHA (American Heart Association) in collaboration with the NHLBI also released guidelines for management of hyperlipidemia [10]. Management of hyperlipidemia includes pharmacotherapy and life style modification

[10]. A number of hypolipidemic agents are used in its management which includes: statins, fibrates, bile acid sequestrants, cholesterol absorption inhibitors and niacin [11]. These drugs have their own side effect profile [11]. Understanding of the pathology and growing research has led to the search for newer drugs. Thus, there is a need to provide the market with new lipid lowering drugs with high efficacy and low side effects.

Phyllanthus acidus (L) Skeels belongs to the Phyllanthaceae family. It is distributed throughout India. Known as para amlakhi in assumese, it is often used by ethnic people of North-East India in traditional medicine [12]. It has been used in the treatment of painful conditions, inflammatory and oxidative stress related disorders such as rheumatism, bronchitis, asthma, liver diseases, diabetes and gonorrheae. Plants of this family are rich in phytochemicals like flavonoids, saponins, alkaloids, tannins, lignans, phenols and terpenes [13]. Number of studies has demonstrated hypolipidemic activity of flavonoids [14]. This study was therefore undertaken to evaluate the hypolipidemic activity of the leaves of *Phyllanthus acidus* in hypercholesteromic rats.

MATERIALS AND METHODS
Study Setting:
This study was conducted in the Department of Pharmacology, Gauhati Medical College and Hospital, Guwahati, Assam, India.

Ethical Approval:
The study was conducted after receiving due approval from the Institutional Animal Ethics Committee, Gauhati Medical College.

Drugs and chemicals used in the study:
EPA (Ethanolic extract of leaves of *Phyllanthus acidus*), Cholesterol, Peanut oil, Propyl thio-uracil, Cholic acid and Atorvastatin obtained from Sigma Aldrich (Bangalore, India), Alcohol obtained from Helix India (Guwahati, India).

Plant material:
Leaves of *Phyllanthus acidus* (L.) Skeels were collected from Karchowa, Sivas agar, Assam. The plant was identified and confirmed after consulting the Gauhati University Herbarium. The identified voucher

specimen was deposited at the Gauhati University Herbarium for future reference.

Preparation of EPA (Ethanolic extract of leaves of *Phyllanthus acidus*):
The shade dried leaves of *Phyllanthus acidus* were pulverized into fine powder. The Soxhlet method using the soxhlet apparatus was employed for extraction.

Experimental animals:
Healthy albino rats of Sprague dawley variety of either sex weighing between 200-250 gm procured from the Animal House of Department of Pharmacology, Gauhati Medical College were used in the study. The rats were fed rat chaws diet and water *ad libitum* during the experiment. They were maintained under controlled conditions with 12 hour light and 12 hour dark cycles at a temperature of $24 \pm 1°$ C and humidity of 55 ± 5 %. All the animals were acclimatized to laboratory condition for 7 days before conducting the experiment. The animals were housed in separate polypropylene cages inside a well-ventilated room and their bedding changed at regular intervals.

Acute toxicity tests:
The acute toxicity study was carried out as per OECD guidelines 425. Administration of EPA at 2000 mg/kg did not result in death of any animal. Therefore, $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of 2000 i.e. 100, 200 and 400mg/kg respectively were selected as doses for the study.

Preparation of HC (Hyperlipidemic Cocktail):
The hyperlipidemic cocktail solution was prepared dissolving 100g of cholesterol, 30g of propyl thiouracil and 100g of cholic acid in 1litre of peanut oil.

Standard Hypolipidemic drug:
Atorvastatin is the inhibitor of the enzyme HMG-coA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A *reductase*).HMG-coA reductase is the rate limiting enzyme in the synthesis of cholesterol.

Study design:
The rats were divided into six groups each containing six animals. The groups were:

Groups	Drug	Dose	Route	Duration
I (Normal Control)	Normal Saline	10ml/kg	Oral	28 days
II (Disease Control)	HC (Hyperlipidemic Cocktail)		Oral	First 14 days
III (Hypolipidemic Standard)	i. HC		Oral	i. First 14 days
	ii. Atorvastatin	20 mg/kg	Oral	ii. Last 14 days

IV (EPA dose A)	i. HC		Oral	i. First 14 days
	ii. EPA	100mg/ kg	Oral	ii. Last 14 days
V (EPA dose B)	i. HC		Oral	i. First 14 days
	ii. EPA	200mg/ kg	Oral	ii. Last 14 days
VI (EPA dose C)	i. HC		Oral	i. First 14 days
	ii.EPA	400mg/ kg	Oral	ii. Last 14 days

The EPA and atorvastatin were suspended in 1% carboxy methycellulose and administered orally.

Study Duration: 28 days.

Collection of blood:
Blood was collected before the start of the experiment to determine the baseline serum lipid levels. Next, blood was collected on 15th day to determine the induction of hyperlipidemia and on 29th day to assess the effect of the test drugs.

Site for Collection of Blood:
Day 0: Tail vein.
Day 15: Tail vein.
Day 29: Cardiac puncture.

Estimation of serum lipid levels:
The blood collected was allowed to clot for approximately 1 hour at room temperature and then centrifuged at 12,000 rpm to obtain the serum. Serum was collected in the Eppendorf tubes and stored at -20 °C until analysis. The serum lipid levels were estimated using the commercial biochemical assay kits. They were analyzed in the Rayoto semi auto chemistry analyzer (RT 9600).

STATISTICAL ANALYSIS:
Results were expressed as mean ± SEM. The significance of difference among the groups were assessed using one way analysis of variance (ANOVA) followed by Bonferroni's test using Graph pad prism software 5.0. $P<0.05$ was considered to be significant.

Baseline Lipid Levels:
The mean serum levels of TC (Table 1), TG(Table 2), LDL(Table 3), HDL(Table 4) and VLDL(Table 5) before the start of the experiment were found to be comparable in all the groups.

Lipid Levels on Day 15:
The serum levels of TC (Table 1), TG (Table 2), LDL (Table 3) and VLDL (Table 5) increased in all the groups except group I. This increase was found to be statistically significant when compared to group I (p value < 0.05). HDL levels (Table 4) showed a decreasing trend in all the groups (except group I). There was however no significant difference in HDL levels amongst all the groups.

Lipid Levels on Day 29:
There was a decrease in the serum levels of TC (Table 1), TG (Table 2), LDL (Table 3), and VLDL (Table 5) in groups III, IV, V and VI. The lipid levels (except HDL) in III, IV, V & VI were significantly lower than II ($p<0.05$). HDL levels in III, IV, V & VI were found to be significantly higher than II ($p<0.05$).

All the parameters (except HDL) in group III were found to be significantly lower in comparison to groups IV, V and VI. HDL level in group III was found to be significantly higher in comparison to group IV, V and VI. However, a dose dependent decrease in all the parameters (except HDL) was seen in the EPA administered groups (IV, V, VI). A dose dependent increase in the serum HDL levels in the EPA administered groups (IV, V, VI) was also seen.

RESULTS

Table 1: Effect of EPA on Serum TC (Total Cholesterol)

Groups	Serum Total Cholesterol Levels		
	Baseline	Day 15	Day 29
I	87.667±2.489	83. 333±1.306	86.5±2.102
II	85.65±1.754	212.5±2.081 [a]	202.45±2.654
III	87±3.563	209.667±2.002 [a]	115.833±3.544 [b]
IV	85.333±3.008	217.5±3.561 [a]	170.733±2.093 [b,d]
V	87.833±2.875	211.05±2.036 [a]	150.667±2.857 [b,d]
VI	88.667±1.003	215.333±3.067 [a]	131.333±1.765 [b,c]

[a] denotes $p<0.05$ when compared to group I,   [b] denotes $p<0.05$ when compared to group II
[c] denotes $p<0.05$ when compared to group III, [d] denotes $p<0.05$ when compared to group VI

Table 2: Effect of EPA on Serum TG (Triglycerides)

Groups	Serum Triglycerides Levels		
	Baseline	Day 15	Day 29
I	95.667±3.256	94.5±1.306	91.333±2.507
II	93.333±2.171	177.5±2.287 [a]	169.667±1.643
III	96±2.811	175.326±2.675 [a]	87.65±2.333 [b]
IV	96.45±3.484	179.333±1.032 [a]	149.833±2.002 [b,d]
V	93.65±1.692	180.667±2.303 [a]	118.667±3.385 [b,d]
VI	95.333±2.974	179.333±1.383 [a]	107.333±1.433 [b,c]

[a] denotes $p<0.05$ when compared to group I,   [b] denotes $p<0.05$ when compared to group II
[c] denotes $p<0.05$ when compared to group III, [d] denotes $p<0.05$ when compared to group VI

Table 3: Effect of EPA on Serum LDL (Low density lipoprotein)

Groups	Serum Low density lipoprotein Levels		
	Baseline	Day 15	Day 29
I	42.233 ±2.667	40.667±2.832	39.533±1.648
II	41.3±3.634	141.25±2.12 [a]	136.333±2.742
III	39.367±1.033	139.05±1.723 [a]	37.211±2.196 [b]
IV	41.25±2.603	139.233±2.034 [a]	101.667±1.932 [b,d]
V	40.85±1.114	137.67±1.333 [a]	79.003±2.566 [b,d]
VI	42.333±1.532	138.67±2.033 [a]	57.333±3.085 [b,c]

[a] denotes $p<0.05$ when compared to group I,   [b] denotes $p<0.05$ when compared to group II
[c] denotes $p<0.05$ when compared to group III, [d] denotes $p<0.05$ when compared to group VI

Table 4: Effect of EPA on Serum HDL (High density lipoprotein)

Groups	Serum High density lipoprotein Levels		
	Baseline	Day 15	Day 29
I	27.667±3.585	27.25±2.105	29.667±2.473
II	26.833±3.323	24±1.934	21.333±1.667
III	28.166±1.247	23.667±2.342	57.25±2.381 [b]
IV	29.833±2.667	22.667±2.911	30.25±1.98 [b,d]
V	26.333±2.347	23.25±1.585	37.333±2.632 [b,d]
VI	28.25±1.272	21.333±2.546	46.25±2.756 [b,c]

[a] denotes $p<0.05$ when compared to group I,   [b] denotes $p<0.05$ when compared to group II
[c] denotes $p<0.05$ when compared to group III, [d] denotes $p<0.05$ when compared to group VI

Table 5: Effect of EPA on Serum VLDL (Very low density lipoprotein)

Groups	Serum Very low density lipoprotein Levels		
	Baseline	Day 15	Day 29
I	17.166±1.247	17.25±2.746	19.333±2.218
II	16.333±2.333	14±1.754 [a]	44.25±2.682
III	18.667±3.585	16.667±2.911 [a]	15.25±2.101 [b]
IV	19.833±2.667	17.667±2.343 [a]	36.333±265 [b,d]
V	16.833±3.933	17.25±2.461 [a]	29.333±1.035 [b,d]
VI	18.25±1.272	16.333± 2.305 [a]	22.667±1.811 [b,c]

[a] denotes $p<0.05$ when compared to group I,   [b] denotes $p<0.05$ when compared to group II
[c] denotes $p<0.05$ when compared to group III, [d] denotes $p<0.05$ when compared to group VI

DISCUSSIONS

Dyslipidemia continues to be a major health problem in India and other developing countries. It is an important risk factor for atherosclerosis, cardiovascular diseases, stroke etc. Hyperlipidemia damages the endothelial lining of the blood vessels and deregulates the cellular functions leading to development of various pathological conditions. In this study, the ethanolic extract of the leaves of *phyllanthus acidus* showed dose dependent hypolipidemic activity, which was however inferior to the standard hypolipidemic agent atorvastatin.

Various studies have reported lipid lowering properties of plants of phyllanthus genus. In a study conducted by Khanna *et al.;* in 2002, Phyllanthus niruri was shown to possess hypolipidemic effect in rats. The lipid lowering activity was proposed to be mediated through inhibition of hepatic cholesterol biosynthesis, increased fecal bile acids excretion and enhanced activity of plasma lecithin cholesterol acyltransferase [15]. Joseph *et al.;* 2012 reported hypolipidemic activity of *phyllanthus emblica* in a prospective, randomized, parallel, open-label, positive controlled study [16]. Usharani *et al.;* 2014 also demonstrated lipid lowering properties of *Phyllanthus emblica* in type II diabetes mellitus patients. The study also demonstrated improved endothelial function, reduction in biomarkers of oxidative stress and systemic inflammation in those patients [17]. *Phyllanthus*

amarus was studied for its in-vivo anti-hyperlipidemic potential using cholesterol diet induced hyperlipidemia model in rats. The results of study showed significant hypolipidemic activity of *Phyllanthus amarus* [18].

Many phytoconstituents have been shown to possess hypolipidemic activity. Olagunju *et al.;* 1995 reported the hypolipidemic effects of flavonoids, alkaloids, saponins and tannins [19]. Flavonoids have been reported to increase HDL-C concentration and decrease LDL and VLDL levels in hypercholesteromic rats [14]. Flavonoids from lotus were shown to reduce hyperglycemia and hyperlipidemia in alloxan-induced diabetic mice [20]. Total flavonoids of *P. frutescens* leaves showed lipid lowering properties in hyperlipidemic rats [21]. Saponins have been shown to possess blood cholesterol lowering activity by inhibiting the intestinal reabsorption of cholesterol. Saponin also possesses resin like action. It reduces the enter hepatic circulation of cholesterol [22, 23].

The mechanism by which *phyllanthus acidus* causes hypolipidemia cannot be exactly pointed out in this study. The probable mechanism can be one or more of the following:
1. Increased activity of plasma lecithin cholesterol acyl transferase.
2. Decreased enter hepatic circulation of cholesterol.
3. Inhibition of hepatic cholesterol biosynthesis.
4. Increased fecal bile acids excretion.

However, further studies have to be carried out to find the precise mechanism of hypolipidemia.

CONCLUSION
Ethanolic extract of leaves of *phyllanthus acidus* have hypolipidemic effect in rats.

Conflict of interest: None.

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