Research Article

Hypolipidaemic effect of ethanolic extract of *Ipomoea aquatic*, *Trigonella foenum graecum* and *Bryophyllum pinnatum* in experimental animals

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Abstract

Objective: The aim of present study was to evaluate the hypolipidemic effect of ethanolic extract of *Ipomoea aquatic, Trigonella foenum graecum and Bryophyllum pinnatum* in experimental animals. **Materials and methods:** Hyperlipidemia was induced by daily administration of 1 ml/100 g body weight of a cocktail containing high fat diet. The serum lipid levels were estimated on day 0, 15 and 29 using Rayoto semiauto chemistry analyzer (RT 9600) and assay kits. **Results and conclusion:** Hyperlipidemia is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides, in the blood. Ethanolic extract of *Ipomoea aquatic*, *Trigonella foenum graecum* and *Bryophyllum pinnatum* possessed significant hypolipidemic effect and reduced atherogenic changes in cholesterol rich cocktail diet induced hyperlipidemia in rats. The effects were however lower than the effects shown by the standard drug atorvastatin treated group which has showed the most significant effects compared to all doses of the test drugs.

Keywords: *Ipomoea aquatic, Trigonella foenum graecum, Bryophyllum pinnatum,* hyperlipidemia

Introduction

Hyperlipidemia is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides, in the blood. The two major clinical sequelae of hyperlipidemias are acute pancreatitis and atherosclerosis. The former occurs in patients with marked hypertryglyridemia. Understanding of the pathology and growing research has led to the search for newer drugs. *Ipomoea aquatica* is a common trailing vine with milky sap commonly known as aquatica morning glory found throughout India, Tropical Asia, Africa and Australia, locally known as Kalmi sag (Hindi), Kulmi sag (Bengali), Karemu/ Kalambi (Sanskrit), Kalmoi sag (Assamese) (Westphal, 1992). *Trigonella foenum - graecum (Linn.)* commonly known as Fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated throughout the country, locally known as Methi (Hindi, Bengali, Assamese), Bahuparni (Sanskrit) (Yadav and Baquer, 2014). *Bryophyllum pinnatum* (Lam.) (Crassulaceae) is a perennial herb used in folk medicine in tropical Africa, tropical America, India, China, and Australia, locally known as Patharchatta/Paan-futti (Hindi), Patharkuchi (Bengali), Paate-goja (Assamese), Parnbeej (Sanskrit) (Yadav et al., 2016).

This study was done to evaluate the hypolipidemic effect of ethanolic extract of *Ipomoea aquatic, Trigonella foenum graecum* and *Bryophyllum pinnatum* in experimental animals and to evaluate the effect of ethanolic extract of *Ipomoea aquatic, Trigonella foenum graecum* and *Bryophyllum pinnatum* in prevention of atherosclerosis in experimental animals.

Materials and Methods

Extraction of plant material

The shade dried and finely powdered leaves of *Ipomoea aquatic, Trigonella foenum graecum* and *Bryophyllum pinnatum* were extracted with 70% ethanol using Soxhlet apparatus.

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 this study. The rats were fed rat chaws diet and water ad libitum during the experiment. They were

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maintained under controlled conditions with 12 hour light and 12 hour dark cycles at a temperature of 24 ± 1ºC 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. Animal experimentation protocol is approved by IAEC, Gauhati Medical College and Hospital, Guwahati.

Acute toxicity study

Acute toxicity tests were carried out as per OECD guidelines 425. The test substances were administered in a single dose by gavage using intubation cannula. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation was not fixed rigidly. Different doses were used to obtain a confidence interval that is expected to contain the true LD50 95% of the time.

The acute toxicity study was carried out as per OECD guidelines 425. Administration of EEIA, EETG, and EEBP at 2000 mg/kg did not result in death of any animal. Therefore, 1/40th, 1/20th, 1/10th and 1/5th of 2000 i.e. 50, 100, 200 and 400mg/kg respectively were selected as doses for the study.

Preparation of 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.

Induction of hyperlipidaemia

Hyperlipidemia was induced by daily administration by gavage of 1 ml/100 g body weight of a cocktail containing in 1l peanut oil: 100g cholesterol, 30g propylthio-uracil and 100g cholic acid over a period of 14 days. The serum lipid levels were estimated on day 0, 15, and 29.

Table 1. Different animal grouping and protocol

Groups		Day 1 – Day 14	Day 15 – Day 28
Group I	Normal Control	Normal saline 10ml/kg	Normal saline 10ml/kg
Group II	Hyperlipidemic control	Cocktail diet	Normal saline 10ml/kg
Group III	Hypolipidemic standard	Cocktail diet	Atorvastatin 20mg/kg
Group IV	*I. aquatica* 50mg/kg	Cocktail diet	EEIA 50mg/kg
Group V	*I. aquatica* 100mg/kg	Cocktail diet	EEIA 100mg/kg
Group VI	*T. Foenum graecum* 200mg/kg	Cocktail diet	EETG 200mg/kg
Group VII	*T. Foenum graecum* 400mg/kg	Cocktail diet	EETG 400mg/kg
Group VIII	*B. pinnatum* 100 mg/kg	Cocktail diet	EEBP 100mg/kg
Group IX	*B. pinnatum* 400 mg/kg	Cocktail diet	EEBP 400mg/kg

Standard hypolipidemic drug

Atorvastatin is the inhibitor of the enzyme HMG-coA reductase (3-hydroxy-3-methyl-glutarylcoenzyme A reductase).HMG-coA reductase is the rate limiting enzyme in the synthesis of cholesterol. The study drugs and atorvastatin were suspended in 1% carboxy methycellulose and administered orally.

Study design

The rats were divided into nine groups each containing six animals. The detail of groups is given in table 1.

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) (Singha et al., 2016).

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.

Results

Baseline Lipid Levels: The mean serum levels of TC (Table 2), TG (Table 3), LDL (Table 4), HDL (Table 5) and VLDL (Table 6) 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 2), TG (Table 3), LDL (Table 4) and VLDL (Table 6) increased in all the groups except normal control group. This increase was found to be statistically significant when compared to normal control group (p value < 0.05). HDL levels (Table 5) showed a decreasing trend in all the groups (except normal control group). 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 2), TG (Table 3), LDL (Table 4), and VLDL (Table 6) in all groups. The lipid levels (except HDL) in all groups were significantly lower than disease control group ($p<0.05$). HDL levels in all groups were found to be significantly higher than disease control group ($p<0.05$).

Table 2. Effects on total cholesterol levels

Groups	Baseline	Day 15	Day 29
Normal control	86.667 ± 1.367	85.166 ± 1.442	84.333 ± 2.329
Disease control	86 ± 1.856	205.5 ± 3.717[a]	202.666 ± 3.347
Standard	87 ± 1.795	204 ± 3.44[a]	102.333 ± 2.117[b]
EEIA 50mg/kg	84.666 ± 2.269	196.333 ± 4.032 [a]	159.666667 ± 4.868 [b]
EEIA 100mg/kg	88.666 ± 3.141	202.5 ± 4.019 [a]	115.5 ± 4.299 [b]
EETG 200mg/kg	79 ± 4.422	191.5 ± 5.777 [a]	169 ± 4.865 [b]
EETG 400mg/kg	88 ± 1.986	206.166 ± 3.0132 [a]	147.666 ± 1.283 [b]
EEBP 100mg/kg	86.833 ± 1.673	205.333 ± 2.599 [a]	174.667 ± 1.805 [b]
EEBP 400mg/kg	86.833 ± 1.673	204.1667 ± 3.077 [a]	117.5 ± 2.22 [b]

Value expressed as mean ± SEM; [a]denotes $p<0.05$ when compared to normal control (group I), [b]denotes $p<0.05$ when compared to disease control (group II)

Table 3. Effects on total triglyceride levels

Groups	Baseline	Day 15	Day 29
Normal control	89.833 ±1.479	87.666 ± 1.407	86.333 ± 0.769
Disease control	89 ±1.944	184 ± 2.748 [a]	179.333 ± 2.704
Standard	88.166 ± 1.978	185 ± 1.9 [a]	92.333 ± 2.388 [b]
EEIA 50mg/kg	85.333 ± 3.033	169.5 ± 5.399 [a]	141.1666 ± 4.238 [b]
EEIA 100mg/kg	94.333 ± 2.805	182.666 ± 3.724 [a]	105.5 ± 5.162 [b]
EETG 200mg/kg	86.166 ± 3.982	183.333 ± 4.22 [a]	142.5 ± 3.991 [b]
EETG 400mg/kg	89.833 ± 1.991	186 ± 2.582 [a]	124.333 ± 2.036 [b]
EEBP 100mg/kg	88.833 ± 1.801	184 ± 2.357 [a]	157.833 ± 2.019 [b]
EEBP 400mg/kg	89.333 ± 1.367	186.666 ± 2.117 [a]	104.333 ± 2.490 [b]

Value expressed as mean ± SEM; [a]denotes $p<0.05$ when compared to normal control (group I), [b]denotes $p<0.05$ when compared to disease control (group II)

Table 4. Effects on total serum LDL levels

Groups	Baseline	Day 15	Day 29
Normal control	47.033 ± 0.988	46.8 ± 1.580	47.566 ± 2.245
Disease control	46.866 ± 0.536	133.866 ± 3.517 [a]	123.8 ± 6.445
Standard	48.033 ± 0.817	132.833 ± 1.801 [a]	28.533 ± 0.582 [b]
EEIA 50mg/kg	46.933 ± 1.602	131.1 ± 2.551 [a]	94.1 ± 3.053 [b]
EEIA 100mg/kg	44.966 ± 2.188	130.3 ± 2.663 [a]	46.566 ± 2.895 [b]
EETG 200mg/kg	43.6 ± 4.159	123.5 ± 2.882 [a]	96.333 ± 0.698 [b]
EETG 400mg/kg	46.866 ± 0.532	132.3 ± 0.787 [a]	74.966 ± 0.3603 [b]
EEBP 100mg/kg	40.4 ± 3.382	137.366 ± 3.237 [a]	109.266 ± 3.911 [b]
EEBP 400mg/kg	37.8 ± 3.234	134 ± 3.926 [a]	60.466 ± 3.752 [b]

Value expressed as mean ± SEM; [a]denotes $p<0.05$ when compared to normal control (group I), [b]denotes $p<0.05$ when compared to disease control (group II)

All the parameters (except HDL) in standard control group were found to be significantly lower in comparison to other groups. HDL level in standard control group was found to be significantly higher in comparison to other groups. However, a dose dependent decrease in all the parameters (except HDL) was seen in groups. A dose dependent increase in the serum HDL levels was also seen in groups.

Table 5. Effects on total serum HDL levels

Groups	Baseline	Day 15	Day 29
Normal control	21.667 ± 1.122	20.833 ± 0.925	19.5 ± 0.8416
Disease control	21.333 ± 1.593	34.833 ± 1.977 [a]	33.166 ± 1.656
Standard	21.333 ± 1.593	34.167 ± 1.801 [a]	55.333 ± 1.835 [b]
EEIA 50mg/kg	20.666 ± 1.122	31.333 ± 3.79 [a]	37.333 ± 2.902 [b]
EEIA 100mg/kg	24.833 ± 2.152	35.666 ± 2.501 [a]	47.833 ± 1.188 [b]
EETG 200mg/kg	18.166 ± 1.382	31.333 ± 3.22 [a]	44.166 ± 4.591 [b]
EETG 400mg/kg	23.166 ± 1.816	36.666 ± 2.036 [a]	47.833 ± 1.188 [b]
EEBP 100mg/kg	28.666 ± 3.177	31.166 ± 2.216 [a]	33.833 ± 3.832 [b]
EEBP 400mg/kg	31.166 ± 2.373	32.833 ± 2.385 [a]	36.166 ± 4.406 [b]

Value expressed as mean ± SEM; [a]denotes $p<0.05$ when compared to normal control (group I), [b]denotes $p<0.05$ when compared to disease control (group II)

Table 6. Effects on total serum VLDL levels

Groups	Baseline	Day 15	Day 29
Normal control	17.966 ± 0.295	17.533 ± 0.281	17.266 ± 0.154
Disease control	17.8 ± 0.388	36.8 ± 0.549 [a]	35.866 ± 0.541
Standard	17.633 ± 0.395	37 ± 0.38 [a]	18.466 ± 0.477 [b]
EEIA 50mg/kg	17.066 ± 0.606	33.9 ± 1.079 [a]	28.233 ± 0.847 [b]
EEIA 100mg/kg	18.866 ± 0.561	36.533 ± 0.744 [a]	21.1 ± 1.032 [b]
EETG 200mg/kg	17.233 ± 0.796	36.666 ± 0.844 [a]	28.5 ± 0.798 [b]
EETG 400mg/kg	17.966 ± 0.398	37.2 ± 0.516 [a]	24.866 ± 0.407 [b]
EEBP 100mg/kg	17.766 ± 0.36	36.8 ± 0.471 [a]	31.566 ± 0.403 [b]
EEBP 400mg/kg	17.866 ± 0.273	37.333 ± 0.423 [a]	20.866 ± 0.498 [b]

Value expressed as mean ± SEM; [a]denotes $p<0.05$ when compared to normal control (group I), [b]denotes $p<0.05$ when compared to disease control (group II)

Discussion

Induction of hyperlipidemia in albino rats by an experimental hyperlipidemic diet consisting of well pulverized mixture of cholesterol (2%), cholic acid (1%), peanut oil (10%), sucrose (40%) and normal laboratory diet (47%) (Rajasekaran et al., 2003). In another study, Sikarwar et al. (2012) has induced hyperlipidemia in albino rats by both cholesterol rich mixture diet and by triton, a hyperlipidemic agent. They suggested that the synergistic

interaction of polyphenols, steroids and tannins contents in the extract may impart hypolipidemic property to the extract (Sikarwar et al., 2012).

In the study done by Sivaraman et al. (2010) reported that methanolic leaf extract of *Ipomea aquatica* showed significant hypolipidemic activity in hyperlipidemic rats. Hypolipidemic effects were evaluated with the single, daily oral dosing of 200 and 400 mg/kg of methanol leaf extract of *Ipomea aquatica* in Swiss albino rats for 30 days (Sivaraman et al., 2010). Hamid et al. have showed that the methanol extracts of the leaves of *Ipomea aquatica* showed potent free radical scavenging activity (Hamid et al., 2011).

Preethy et al. (2012) showed that the *Trigonella foenum graecum* had potent hypolipidaemic effect (Preethy et al., 2012). The atherogenic index plays as predictive value for atherosclerosis and can be used as an available index of highest sensitivity for assessing cardiovascular risk factors, and for predicting the acute coronary events (Niroumand et al., 2015). All extracts of *Trigonella foenum graecum* exhibited antioxidant activity highest by the ethanolic extract by modulating the activity of SOD, catalase and GST (Billaud et al., 2001).

Reports by Plosch et al. (2006) have suggested that several plant sterols reduce serum cholesterol by the inhibition of intestinal cholesterol absorption (Plösch et al., 2006). Thus, the hypolipidemic effect of *Ipomoea aquatic, Trigonella foenum graecum* and *Bryophyllum pinnatum* can therefore be attributed to its phytochemical constituents. The flavonoids, steroids, alkaloids, glycosides, tanins etc present in these plants can be considered to be the active constituents responsible for hypolipidemic activities demonstrated in the present study. Moreover, prevention of the development of atherosclerosis by these plants can be considered by decreasing the hyperlipidemic changes as well as by its antioxidant property.

In conclusion, ethanolic extract of *Ipomoea aquatic, Trigonella foenum graecum* and *Bryophyllum pinnatum* possessed significant hypolipidemic effect and reduced atherogenic changes in cholesterol rich cocktail diet induced hyperlipidemia in rats. The effects were however lower than the effects shown by the standard drug, atorvastatin treated group which showed the most significant effects compared to all doses of the test drugs.

Conflicts of interest: None

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