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Research Article

Evaluation of Phytochemical and *in-vivo* Antihyperlipidemic Activity of *Solanum spirale* Roxb. Leaves

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Abstract: The objective of the study was to evaluate the phytochemical and *in-vivo* antihyperlipidemic activity of *Solanum spirale* Roxb. leaves. The physiochemical standardization of the dried leaves powder was done with respect to ash values, foaming index, extractive values and moisture content. The dried leaves were extracted with petroleum ether, chloroform and water. The phytochemical analyses were carried out and the antihyperlipidemic activity of the chloroform and aqueous extracts were evaluated. The antihyperlipidemic study was carried out by inducing hyperlipidemia in rats by means of triton. The serum collected was analyzed for total cholesterol, triglyceride, low density lipoprotein, and high density lipoprotein. The result of the present study revealed that both the aqueous and chloroform extracts of leaves of *Solanum spirale* Roxb. possess antihyperlipidemic activity.

Keywords: Hyperlipidemia, *Solanum spirale* Roxb., Antihyperlipidemic activity, Atherogenic index, Standardization, Phytochemical

INTRODUCTION

The term hyperlipidemia can be defined as increased level of lipid in blood. Hyperlipidemia is the major risk factor for many complications in human being. It is the first and foremost factor that leads to diseases like atherosclerosis, coronary heart disease, ischemic cerebro vascular disease, hypertension, obesity and diabetes mellitus (Type-II) etc. [1]. Although human being has developed many Allopathic drugs to combat the hyperlipidemia and these drugs to certain extent have shown promising lipid lowering activity. But these drugs have been found to be associated with side effects. As a result of side effects that are mostly associated with Allopathic system of medicine, the world population today is turning back to traditional system of medicine as these medicines are free from side effects, easily available with less cost. Thus, in present scenario more research activities are required for development of drug from the natural sources to meet the demand of world population.

Solanum spirale Roxb. is commonly known as brush night shade is a shrub that belong to Solnaceace family. The plant is mostly found in a height of 500-1,900m [2]. The plant grows in China, India, Thailand, Vietnam, Indonesia, Laos etc. [3]

Traditionally the roots of the plant are used as narcotic, diuretic; while bark macerate is used as febrifuge [4]. Leaves are used for the treatment of malaria and high blood pressure [5]. The leaves are used in Akha's traditional medicine in Thailand and China for prevention of fever and cold [6]. Leaves and berry are used as vegetable in Arunachal Pradesh [7].

The isolated compounds from the leaves have been reported to possess antibacterial and anticancer activities [8]. The antioxidant, antibacterial, and anticancer activities of the flowers and unripe fruits were investigated [9].

The present study was conducted to investigate the antihyperlipidemic activity of both aqueous and chloroform extracts of *Solanum spirale* Roxb. leaves in Wistar rats. The phytochemical analysis of the extracts and physiochemical evaluation of the dried leaves powder was planned.

MATERIALS AND METHODS

Collection of Plant Material

Plant leaves were collected on the month of August 2014 from the Rasam, Arunachal Pradesh and the plant was identified by the local villagers as they commonly used this plant for preparing local dishes.

Drying of Plant Material

The collected plant leaves were dried under shade. The drying of the plant material was continued for 20 days. After drying the leaves were grinded to coarse power.

Standardization of Plant Material

The ash values such as Total ash, Acid-insoluble ash, Water soluble ash, Sulphated ash, Extractive Values (Alcohol soluble extractives), Foaming Index and Moisture Content (Gravimetric method) [10] were determined for the dried plant powder of the leaves.

Extraction Procedure for Plant Material

Successive solvent extraction method was used for extraction of the leaves. Initially a weighed quantity of plant material was introduced into the Soxhlet apparatus and extracted with low polar solvent (petroleum ether) in order to remove the fatty material. After extracting with petroleum ether, the plant material was removed and left out for air drying. After drying, the plant material was re-weighed and was introduced again into the Soxhlet apparatus and extracted with medium polar solvent (chloroform). After completion of the extraction with chloroform, the plant material was taken out, dried. The dried material was re-weighed and finally extracted with high polar solvent (water). Finally three extracts were obtained namely petroleum ether extract, chloroform extract and aqueous extract.

Drying of the Extract

The extracts were concentrated. After that complete drying was achieved by air drying.

Preliminary Phytochemical Screening

Preliminary phytochemical investigations were carried out by following standard procedures [11].

***In-vivo* Acute Oral Toxicity Studies**

In the present study the acute oral toxicity of the extracts were performed according to OECD guideline 423 [12]. In this method, the toxicity of the extracts was planned to evaluate using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b.w. Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days.

Evaluation of *in-vivo* Anti-Hyperlipidemic Activity

In the presented study, the *in-vivo* anti-hyperlipidemic activity was evaluated by inducing hyperlipidemia in rats by mean of triton. The blood was collected from retro-orbital route. The biochemical tests were performed to evaluate the anti-hyperlipidemic potential of the extracts.

Experimental animals

White male albino rats weighing about 250-500g were used. They were obtained from the animal house of Anurag Pharmacy College, Kodad after getting the ethical committee approval. The animals were kept under normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5°C) and normal 12- hour light/dark cycle under hygienic conditions.

Dose Preparation of Standard and Leaves Extracts

Atorvastatin at a dose of 10 mg Kg^{-1} was prepared by suspending Atorvastatin in aqueous 0.5 % tween-40. The aqueous leaves extract was dissolved in water and chloroform extract was prepared as emulsion in olive oil using 0.5 % tween 40 as the emulsifying agent. Doses of 250 mg/Kg b.w. and 500 mg/Kg b.w. of both the extracts were administered by oral route to the rats.

Procedure (Triton induced)

Animals were kept for fasting for 24 hours and injected a solution of Triton X-100 at a dose of 250 mg/kg body weight intraperitoneallly. The plant extracts, at a dose of 250 mg/kg and 500 mg/kg body weight were administered orally though gastric intubation.

The first dose was given immediately after triton injection and second dose 20 hrs later. After 4 hrs of the second dose, the animals were used for the study of various biochemical parameters. Blood were collected through retro orbital plexus route on anesthesia and centrifused at 2000 rpm for 30 minute [13, 14].

The experimental animals were divided into the following groups consisting of six animals in each group randomly.

Group-1: Normal [standard diet + 0.5 % tween-40 (p.o.)]
Group-2: Control [standard diet + triton (i.p.) + 0.5 % tween-40 (p.o.)]
Group-3: Standard drug (10 mg/Kg b.w., p.o.) + triton +diet.
Group-4: High dose (500 mg/ Kg b.w., p.o.) of chloroform extract of *Solanum spirale* Roxb. (HDCESS) + triton+ standard diet
Group-5: Low dose (250 mg/ Kg b.w., p.o.) of chloroform extract of *Solanum spirale* Roxb. (LDCESS) + triton+ standard diet
Group-6: High dose (500 mg/ Kg b.w., p.o.) of aqueous extract of *Solanum spirale* Roxb. (HDAESS) + triton + standard diet

Group-7: Low dose (250 mg/ Kg b.w., p.o.) of aqueous extract of *Solanum spirale* Roxb. (LDCESS) + triton + standard diet

Biochemical analysis

Serum samples were analyzed for

- Total serum cholesterol (TC),
- Triglyceride (TG)
- High-density lipoprotein cholesterol (HDL-C)
- Low density lipoprotein (LDL-C)
- Atherogenic Index (AI) were calculated out using the following formula
    - AI = (Total cholesterol – HDL-C)/HDL-C
- LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C levels.

RESULTS AND DISCUSSION

Results of Physiochemical evaluation

The results obtained for different physiochemical tests for standardization of the leaves of *Solanum spirale* Roxb. are given below.

Ash value

Table 1: Results of ash values of *Solanum spirale* Roxb. leaves

Sl. No.	Type	Amount
a.	Total ash	1.147mg/g
b.	Water soluble ash	0.217 mg/g
c.	Acid insoluble ash	0.015 mg/g
d.	Sulphated ash	0.194 mg/g

Foaming Index: It was found to be < 100

Moisture content: 0.27%

Extractive values: The extractive values for Petroleum ether, chloroform and water were found to be 4, 10 and 15% respectively.

Results of Preliminary Phytochemical Analysis

The aqueous and chloroform extracts of *Solanum spirale* Roxb. leaves were found to possess different phytoconstituents like alkaloids, glycosides, saponins, tannins, carbohydrates, flavonoids, steroids.

Results of Acute Oral Toxicity Studies

Acute oral toxicity studies of *Solanum spirale* Roxb. leaves extracts were carried out according to OECD-423 guidelines. The study was carried out in Wister rats at a dose of 2000 mg/kg, p.o. The animals were observed for 14 days for mortality and acute toxicities. They exhibited normal behaviour, without any signs of toxicity. Their motor activity and secretary signs were also normal and no sign of depression was observed.

Results of Antihyperlipidemic Activity

The antihyperlipidemic activity of the *Solanum spirale* Roxb. leaves extracts was evaluated by using triton induced hyperlipidemic model in rats. The results obtained are given in the Table 2.

Table 2: Results of Antihyperlipidemic Activity

Groups	Total Cholesterol (mg/dL) (Mean ± SEM)	Triglyceride (mg/dL) (Mean ± SEM)	HDL (mg/dL) (Mean ± SEM)	LDL (mg/dL) (Mean ± SEM)	VLDL (mg/dL) (Mean ± SEM)
Normal	73.33 ± 4.19	74.00±6.18	40.17±2.38	18.37±1.948	14.80±1.236
Control	116.0 ± 5.43***	130.5±7.58***	23.00±2.55***	66.90±4.627***	26.10±1.518***
Standard	79.50 ± 4.48***	82.50±6.83***	39.67±1.78**	23.43±4.209***	16.40±1.331***
HDCESS	89.67 ± 5.82*	89.67±8.21**	35.17±2.85*	36.57±5.893***	17.93±1.643**
LDCESS	97.17± 6.69^{ns}	109.0±7.43^{ns}	29.67±3.58^{ns}	45.70±5.723*	21.80±1.488^{ns}
HDAESS	87.67 ± 5.53**	93.00±4.71**	37.17±2.91**	31.90±6.215***	18.60±0.9423**
LDAESS	96.67 ± 6.60^{ns}	100.8±8.97*	28.33±3.38**	48.17±4.263*	20.83±1.565^{ns}

N.B: Significant differences with respect to control was evaluated by ANOVA using Dunnet's t test [*P<0.05, **P<0.01, ***P<0.001, ns (Non significant)]

Table 3: Atherogenic Index

Group	Atherogenic Index
Control	4.04
Standard	1.00
HDCESS	1.54
LDCESS	2.27
HDAESS	1.35
LDAESS	2.43

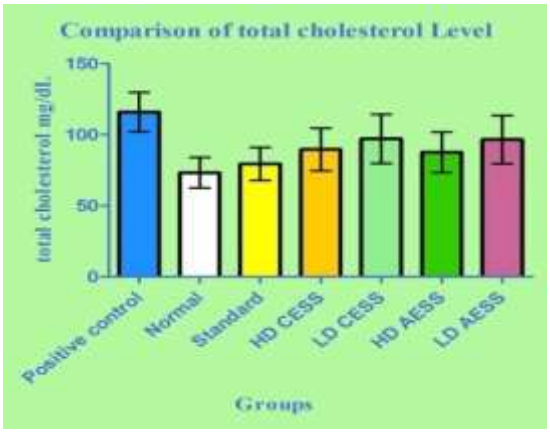

Fig. 1: Effect of test drugs on total cholesterol profile in rats

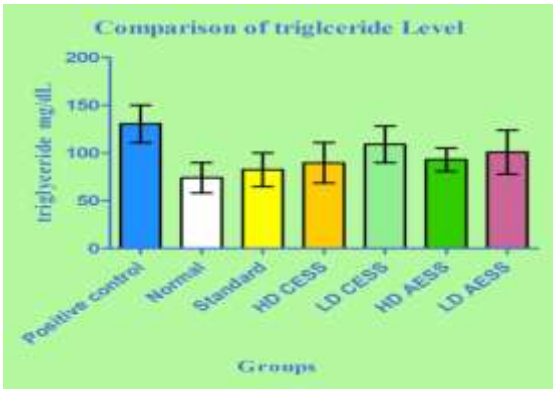

Fig. 2: Effect of test drugs on TG profile in rats

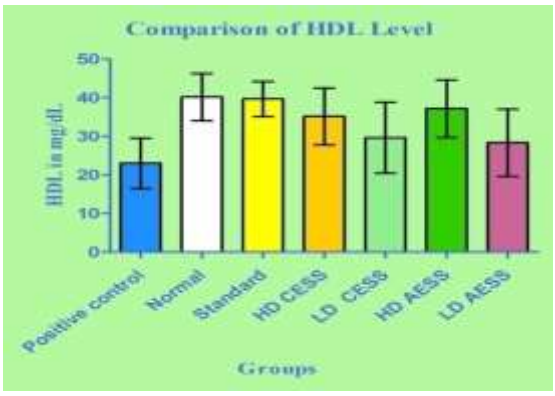

Fig. 3: Effect of test drugs on HDL profile in rats

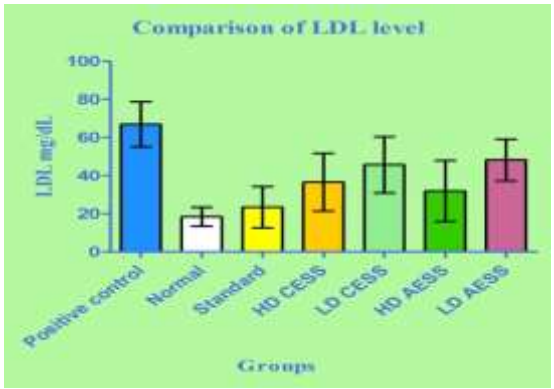

Fig. 4: Effect of test drugs on LDL profile of rat

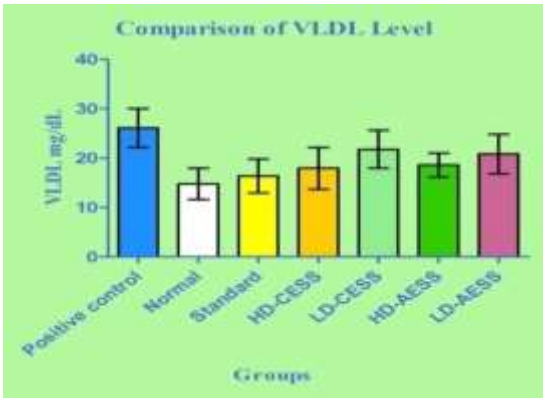

Fig. 5: Effects of test drug on VLDL profile in rats

DISCUSSION

Physiochemical evaluation

The physiochemical parameters for the *Solanum spirale* Roxb. leaves had been evaluated. The physiochemical parameter like total ash, water soluble ash, acid insoluble ash, sulphated ash were found to be 1.147mg/g, 0.217 mg/g, 0.015 mg/g, 0.194 mg/g respectively. The foaming index of the *Solanum spirale* Roxb. leaves was found to be less than 100.

The physiochemical parameter like moisture content and extractive value of the leaves has also been determined, found to be 0.27% and the extractive value of the leave using three solvents namely Petroleum ether, chloroform and water were found to be 4, 10 and 15% respectively.

Acute Oral Toxicity Studies

Acute oral toxicity studies for the aqueous and chloroform extracts of the leaves of *Solanum spirale* Roxb. revealed that all the extracts were non-toxic at tested dose levels and well tolerated by the experimental animals.

Antihyperlipidemic Activity

The present study was performed to evaluate the antihyperlipidemic activity of *Solanum spirale* Roxb. leaves. Triton was used as a hyperlipidemia inducing agent in the rats. The treatment groups received standard drug atorvastatin (10 mg/kg b.w.), aqueous and chloroform extracts of the leaves (250 mg/kg b.w. and 500 mg/kg b.w. of both the extracts), carried out simultaneously. The collected blood serum suggested increase in the level of TC, TG, LDL and decrease in HDL level in control i.e.; the group that received only triton. The estimation of these parameters in the standard group (triton+ Atorvastatin) and test group (triton + *Solanum spirale* leaves extract) revealed that the level of TC, TG, LDL were decreased and level of HDL was increased than the control group in a significant manner when evaluated statistically by using the Graph Pad prism 5. ANOVA studies were done using Dunnet t-test.

The standard drug decreased the level of TC, TG, LDL and VLDL to 79.50, 82.50, 23.43 and 16.40 mg/dL respectively and increased the level of HDL to 39.67 mg/dL than the control.

In comparison to the control the test drug namely high dose aqueous, low dose aqueous, high dose chloroform and low dose chloroform decreased the level of TC, TG and LDL and increased the level of HDL. The activity was found to be higher in HDAESS group in terms of TC, LDL and HDL; while activity was more in HDCESS group in terms of TG and VLDL.

The atherogenic index was found to be reduced by the leaves extracts. The standard drug reduced atherogenic index and was found to be 1.00. The atherogenic index of high dose aqueous extract was found to be 1.35 while in case of low dose aqueous extract it was found to be 2.27. The values for both high dose and low dose chloroform extract were found to be 1.54, 2.27 respectively.

LDL/ HDL ratio has also been found to be reduced by the test drug. The LDL/HDL ratio for control, standard, HDAESS, LDAESS, HDCESS and LDCESS were found to be 2.90, 0.59, 0.85, 1.70, 1.03 and 1.54 respectively.

Thus it can be reported that both aqueous and chloroform extract of *Solanum spirale* Roxb. possess significant anti-hyperlipidemic activity.

CONCLUSION

The results obtained from the pharmacological screening led us to the conclude that, aqueous and chloroform leaves extract of *Solanum spirale* Roxb. have significant anti hyperlipidemic activity. The observed activity might be due to the different phytochemicals present in the extracts. Further systematic investigations could be done to isolate the important phytoconstiturnts.

REFERENCES

1. Hyperlipidemia. Available from http://en.wikipedia.org/wiki/Hyperlipidemia
2. Solanum spirale Roxburgh, Flora of China. Available from http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200020606
3. Solanum spirale. Available from http://treeflower.la.coocan.jp/Solanaceae/Solanum%20spirale/Solanum%20spirale.htm
4. Solanum spirale Roxb., Global Biodiversity Information Facility. Available from http://www.gbif.org/species/2930705
5. Baruah, S., Borthakur, S. K., Gogoi, P., & Ahmed, M. (2013). Ethnomedicinal plants used by Adi–Minyong tribe of Arunachal Pradesh, Eastern Himalaya. *Indian Journal of Natural Products and Resources*, *4*(3), 278-282.
6. Inta, A., Shengji, P., Balslev, H., Wangpakapattanawong, P., & Trisonthi, C. (2008). A comparative study on medicinal plants used in Akha's traditional medicine in China and Thailand, cultural coherence or ecological divergence?.*Journal of ethnopharmacology*, *116*(3), 508-517.
7. Payum, T., Das, A.K., & Shankar, R. (2014). Nutraceutical folk food plants used among indigenous people of east Siang district of Arunachal Pradesh, *American Journal of PharmTech Research*, 4(4), 696-704.
8. Keawsa-ard, S., Natakankitkul, S., Liawruangrath, S., Teerawutkulrag, A., Trisuwan, K., Charoenying, P., & Liawruangrath, B. (2012). Anticancer and antibacterial activities of the isolated compounds from Solanum spirale Roxb. leaves. *Chiang Mai J. Sci.*, 39(3), 445-454.
9. Keawsa-ard, S., Liawruangrath, B., Natakankitkul, S., Chowwanapoonpohn, S., Liawruangrath, S., Teerawutgulrag, A., & Pyne, S. G. (2012). In vitro evaluation of the antioxidant, antibacterial and anticancer activities of Solanum spirale Roxb.(Solanaceae). *Asian Journal of Traditional Medicines*, *7*(2), 66-72.
10. Khandelwal, K.R., (2013). Practical Pharmacognosy, 22nd edition, Nirali Prakashan, Pune, India, 23.8-23.12.
11. Khandelwal, K.R., (2013). Practical Pharmacognosy, 22nd edition, Nirali Prakashan, Pune, India, 25.1-25.6.
12. Guideline, O. T. (2001). 423. *Acute Oral Toxicity–Acute Toxic Class Method, adopted*, *17*.
13. Sikarwar, M. S., & Patil, M. B. (2015). Anti-hyperlipidemic activity of Crataeva nurvala Buch-Hum ethanolic extract fractions. *International Medical Journal of Sifa University*, *2*(2), 31-36.
14. Jadeja, R. N., Thounaojam, M. C., Patel, V., Devkar, R. V., & Ramachandran, A. V. (2009). Antihyperlipidemic potential of a polyherbal preparation on triton WR 1339 (Tyloxapol) induced hyperlipidemia: A comparison with lovastatin.*International Journal of Green Pharmacy*, *3*(2), 119-124.