Effect of *Evolvulus Alsinoides* L. Ethanolic Extract and Its Fraction In Experimentally Induced Hyperlipidemia In Rats

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Summary

The present study was undertaken to explore the antihyperlipidemic effect of ethanolic extract of aerial parts of *Evolvulus alsinoides* L. and its chloroform fraction in olive oil induced hyperlipidemic rats. The antihyperlipidemic activity of *Evolvulus alsinoides* was compared with a standard drug Atrovastatin (50mg/kg). The study involved phytochemical screening and chromatographic studies of extract and fraction. The ethanolic extract and its fraction were administered orally at doses of 200 and 400 mg/kg body weight in rats. Olive oil (5ml/kg oral dose) was administered 30 min after treatment. Blood was collected by ocular puncture 2 and 4 h after olive oil treatment and centrifuged at 3000 rpm for 15-20 min. Serum samples were further subjected to biochemical analysis. The study dose dependently inhibited the total cholesterol (TC) triglycerides (TG), low density lipoproteins (LDL) level, and significantly increased high density lipoprotein (HDL) level. Phytochemical screening revealed the presence of sterols, flavanoids, tannins and alkaloids. UV λmax was found to be 241 nm with a melting point of 137-138^{0}C for the isolated component. The antihyperlipidemic effect was evaluated in olive oil loaded rats. Acute treatment caused stimulatory effect on HDL level and inhibition in TC and TG elevation induced by olive oil. This drug is highly valued as traditional system of medicine. Allopathic drugs exert various side effects. Therefore it is cleared from the above study that medicinal plants have got potential to be used safely and can control the state of hyperlipidemia upto greater extent.

Keywords: Anti-hyperlipidemic effect; Olive oil; total cholesterols; HDL.

Introduction

Hyperlipidemia is an elevation of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids and triglycerides. They are transported in the blood as part of large molecules called lipoproteins. Hyperlipidemia is a general term, it could be either high cholesterol in the blood (hypercholesterolemia), high triglycerides in the blood (hypertriglyceridemia) or it could be both.

Elevated plasma lipid levels, mainly total cholesterol (TC), triglyerides (TG) and LDL along with decrease in HDL are known to cause hyperlipidemia which is core in initiation and progression of arteriosclerosis impasse. Therefore prime consideration in therapy for hyperlipidemia and arteriosclerosis is to enervate the elevated plasma level of TC, TG and LDL along with increase in HDL lipid levels.

Evolvulus alsinoides L. (Convolvulaceae) is commonly known as Shankhpushpi in Indian traditional medicine. The plant contains alkaloids- shankhapushpine, evolvine, betaine. Fresh plant contains volatile oil and potassium chloride. It also contains a yellow neutral fat, an organic acid and saline substances. The plant is used as a vermifuge and, with oil for promoting growth of hair [1].It is well known for its therapeutic effect on brain disorders in Ayurvedic system of medicine [2], anxiolytic activity [3], antiulcer activity [4], immunomodulatory activity [5], adaptogenic [6] and antioxidant properties [7]. Phytochemically the plant has been reported to contain aliphatic hydrocarbons, fatty acids and alkaloids [8].

Material and methods

Plant material

The aerial parts of *Evolvulus alsinoides* L.were collected From Bhopal (M.P.), India and were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science & Education, Bhopal. A voucher specimen no.175/Bot/Safia/2010 is deposited in the herbarium of botany department.

Extraction and fractionation

The dried drug was coarsely powdered and then exhaustively extracted with 90% ethanol in Soxhlet apparatus. The ethanolic extract so obtained was freed of solvent under vacuum to get 72 g (9.6% yield) of dark green mass. The solvent free ethanolic extract was further dissolved and extracted with chloroform. Ethanolic extract and its chloroform fraction were thus obtained.

Phytochemical profiling

Qualitative chemical test were performed to assess the presence of various phytoconstituents. The preliminary phytochemical screening revealed the presence of tannins, flavanoids and alkaloids in ethanolic extract of *Evolvulus alsinoides* while chloroform fraction revealed the presence of sterols.

Screening for hypolipidemic activity

Screening for hypolipidemic activity was carried out in olive oil-loaded albino rats of either sex weighing 100-120 gm.

Preparation of test material
Ethanolic extract and chloroform fraction were suspended in distilled water plus Polyoxyethylenesorbiton Mono-oleate (Tween 80).

Animal Model
The Swiss albino rats were selected and housed in polypropylene cages maintained under controlled conditions. The animals were fed with pellet food and water *ad libitum*. The animals fasted for 12-14 h before experimentation but it was allowed free access to water. Rats of either sex, 6 - 8 weeks old and weighing 100-120 g, were taken for the experiments.

Measurement of biochemical parameters
Fasted rats were divided into seven groups of six rats each. Group I served as vehicle control. Group II was kept as hyperlipidemic and administered with Olive oil only. Animals of Group III received atrovastatin at the oral dose of 50 mg/kg. Group IV and V were treated with ethanolic extract at the oral dose of 200mg/kg and 400mg/kg and group VI and VII were treated with chloroform fraction at the oral dose of 200mg/kg and 400mg/kg. Olive oil (5ml/kg oral dose) was administered 30 min after treatment [9].
The blood samples were withdrawn by ocular puncture 2 and 4 h after olive oil treatment and transferred directly into centrifuge tubes and allowed to clot at room temperature for 20-25 min and centrifuged for 15-20 min at 3000 rpm. The supernatant clear serum thus obtained was transferred carefully with the help pf micropipette into small test tubes for estimation. The serum concentration of total cholesterol, HDL and triglyceride were measured by standard procedure using auto- analyzer [10].

Statistical analysis
Statistical evaluation of the data was done by Student‘t’ test. (Graph PAD Instat software, Kyplot).A value of $p<0.05$ was considered to be significant.

Results and Discussion

The preliminary phytochemical screening revealed the presence of tannins, flavanoids and alkaloids in ethanolic extract of *Evolvulus alsinoides* while chloroform fraction revealed the presence of sterols.
Ethanolic extract and its chloroform fraction at dose of 200mg/kg decreased serum level of total cholesterol by 36.11 and 34.77 % respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased serum level of total cholesterol by 40.37 and 38.22% respectively Ethanolic extract and chloroform fraction at dose of 200mg/kg increased the serum HDL cholesterol level by 10.72 and 14.20%. On the other hand ethanolic extract and fraction at dose of 400mg/kg increased the serum HDL cholesterol level by 13.94 and 16.74% respectively.Ethanolic extract and its chloroform fraction at dose of 200mg/kg decreased serum level of triglyceride level by 36.64 and 45.88% respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased serum level of triglyceride level by 40.65 and 53.74% respectively.The reduction in LDL cholesterol level by ethanolic extract and chloroform fraction at dose of 200mg/kg were 69.25 and 72.06%respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased LDL cholesterol by 73.13 and 78.07%.

LCAT plays a key role in the incorporation of free cholesterol into HDL and transferring it back to VLDL and LDL which are taken back later in liver cells [11].The possible mechanism of lipid lowering activity is may be due to enhancement of the activity of lecithin acyl transferase (LCAT) and inhibition of the action of hepatic TG- lipase on HDL [12].

Effect of ethanolic extract and $CHCl_3$ fraction of *Evolvulus alsinoides* on total cholesterol level (mg/dl) in olive oil induced hyperlipidemic model (Table-1)

Group	Initial	Total cholesterol level after 2 h	Total cholesterol level after 4 h
Control	65.32± 1.24	67.21 ± 1.35	68.55±1.68
Hyperlipidemic	68.71 ± 1.16	69.52 ± 1.28	101.85 ±1.22
Atrovastatin (50mg/kg)	70.66 ± 2.70	89.58 ± 2.58	53.22±3.02
E.alsinoides ethanolic extract (200mg/kg)	71.85±1.36	84.94±1.52	65.74±.0.54[b]
E.alsinoides ethanolic extract (400mg/kg)	71.97±1.71	86.85±1.91	57.85±1.35[a]
E.alsinoides $CHCl_3$ fraction (200mg/kg)	72.26± 2.10	87.60± 3.12	62.34±1.58[a]
E.alsinoides $CHCl_3$ fraction (400mg/kg)	71.54±1.50	89.39±2.36	55.43±1.43[a]

Total cholesterol concentrations are estimated by standard method. Values are expressed as mean ± S.E.M for six animals in each group.
[a]: $p<0.01$ [b] : $p<0.05$

Effect of ethanolic extract and $CHCl_3$ fraction of *Evolvulus alsinoides*

on triglyceride level (mg/dl) in olive oil induced hyperlipidemic model (Table-2)

Group	Initial	Triglyceride level after 2 h	Triglyceride level after 4 h
Control	59.53± 1.48	59.25 ± 1.65	59.42±1.70
Hyperlipidemic	58.85 ± 1.64	59.70 ± 1.58	113.77 ±1.72
Atrovastatin (50mg/kg)	59.13 ± 1.70	74.58 ± 2.26	60.72±2.52
E.alsinoides ethanolic extract (200mg/kg)	54.43±2.36	74.48±2.42	66.72±.1.41[a]
E.alsinoides ethanolic extract (400mg/kg)	56.13±1.84	75.85±2.27	64.95±1.32[b]
E.alsinoides $CHCl_3$ fraction (200mg/kg)	55.76± 2.40	75.60± 2.85	62.26±1.39[b]
E.alsinoides $CHCl_3$ fraction (400mg/kg)	57.46±2.50	76.93±2.02	60.44±1.96[a]

Triglyceride concentrations are estimated by standard method. Values are expressed as mean ± S.E.M. for six animals in each group.

[a]: $p<0.01$ [b] : $p<0.05$

Effect of ethanolic extract and $CHCl_3$ fraction of *Evolvulus alsinoides*

on HDL level (mg/dl) in olive oil induced hyperlipidemic model (Table-3)

Group	Initial	HDL level after 2 h	HDL level after 4 h
Control	42.34± 1.36	42.36 ± 1.11	42.86±1.34
Hyperlipidemic	42.20 ± 1.58	42.53 ± 1.24	43.02 ±1.49
Atrovastatin (50mg/kg)	43.45±1.63	54.43 ± 1.43	64.96.± 1.01
E.alsinoides ethanolic extract (200mg/kg)	44.20± 1.98	53.58±1.57	55.40±.1.71[b]
E.alsinoides ethanolic extract (400mg/kg)	43.69±2.11	52.36±1.49	57.35±1.52[b]
E.alsinoides $CHCl_3$ fraction (200mg/kg)	43.30± 1.74	56.46± 2.09	59.83±1.42[a]
E.alsinoides $CHCl_3$ fraction (400mg/kg)	43.46± 1.72	54.35± 1.92	60.44± 1.96[a]

HDL concentrations are estimated by standard method. Values are expressed as mean ± S.E.M. for six animals in each group.

[a]: $p<0.01$ [b] : $p<0.05$

Effect of ethanolic extract and $CHCl_3$ fraction of *Evolvulus alsinoides on* LDL level (mg/dl) in olive oil induced hyperlipidemic model (Table-4)

Group	Initial	LDL level after 2 h	LDL level after 4 h
Control	43.20± 1.36	43.31 ± 1.25	42.97±1.49
Hyperlipidemic	43.85 ± 1.85	43.88 ± 1.66	68.73 ±1.74
Atrovastatin (50mg/kg)	44.62±1.56	66.22 ± 1.42	45.23± 1.20
E.alsinoides ethanolic extract (200mg/kg)	44.31±2.13	61.16±1.72	54.75±.1.73[b]
E.alsinoides ethanolic extract (400mg/kg)	43.42±1.85	62.44±1.60	51.38±1.58[b]
E.alsinoides $CHCl_3$ fraction (200mg/kg)	42.30± 1.64	64.33± 2.32	52.35±1.63[b]
E.alsinoides $CHCl_3$ fraction (400mg/kg)	42.56± 1.77	65.23±2.15	49.44±2.05[a]

LDL concentrations are estimated by standard method. Values are expressed as mean ± S.E.M. for six animals in each group.
[a]: $p<0.01$ [b] : $p<0.05$

Conclusion

The acute treatment with *E. alsinoides* ethanolic extract and its chloroform fraction caused inhibitory effects both on total cholesterol (TC) and triglyceride level (TG) after olive oil administration. The maximum inhibitory effect on serum TG and TC level was observed with 400 mg/kg chloroform fraction. The drug and its fraction showed protective action as it slightly increased the HDL cholesterol level. The present investigation may be quite useful as this drug is highly valued as traditional system of medicine and can be used to control the state of hyperlipidemia.

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