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Bioactivity guided fractionation in experimentally induced hyperlipidemia in rats and characterization of phytoconstituent from *Salvadora persica* L.

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ABSTRACT

The present study was undertaken to explore the antihyperlipidemic effect of ethanolic extract of stems of Salvadora persica L. and its chloroform fraction in Triton induced hyperlipidemic rats. Flash chromatography was done for the most active fraction resulting in the isolation of Stigmast-5, 22-dien-3β-ol. Animals were administered with i.p. injection of Triton WR 1339 at dose of 400 mg/kg body weight. After 24 h. of Triton administration the ethanolic extract and its fraction were administered orally at doses of 200 and 400 mg/kg body weight in rats. The study dose dependently inhibited the total cholesterol, triglycerides, LDL level, and significantly increased HDL level. The chloroform fraction was found to be more effective in restoring lipid profile changes in rats treated with Triton probably due to the presence of Stigmast-5, 22-dien-3β-ol .UV λmax was found to be 241 nm with a melting point of 168-170°C for the isolated component.

Keywords: Antihyperlipidemic; Flash chromatography; Stigmast-5, 22-dien-3β-ol.

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), triglycerides (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.

Salvadora persica L. belonging to family- Salvadoraceae is a small tree or shrub with a crooked trunk. It is commonly known as Miswak. It was reported to exhibit antiulcer activity [1], anticonvulsant activity [2], analgesic activity [3], antibacterial activity [4], anti-inflammatory activity [5] .It contains a number of medically beneficial properties including abrasives, astringents and antiseptics. Its main constituents are trimethylamine, an alkaloid which may effectively be salvadorine, chlorides, sulphur, terpenes, vitamin C, glycosides, large amounts of fluoride and silica, small amounts of tannins, saponins and flavonoids [6-8].

MATERIALS AND METHODS

2.1 Plant material

The stems of *Salvadora persica* 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.

2.2 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 74 g (9.25% yield) of dark brown mass. The solvent free ethanolic extract was further dissolved and extracted with chloroform. Ethanolic extract and its chloroform fraction were thus obtained.

2.3 Phytochemical profiling

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

2.4 Screening for hypolipidemic activity

Screening for hypolipidemic activity was carried out in Triton loaded albino rats of either sex weighing 100-120 g.

2.5 Preparation of test material

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

2.6 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. The usage of animals were approved by the ethical committee of the Research Centre having following CPCSEA Reg. No.-778/03/c/CPCSEA.

2.7 Measurement of biochemical parameters

Albino 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 Triton only. Group II-VII were given i.p. injection of Triton WR 1339 at dose of 400 mg/kg body weight. After 24 h. of Triton administration, 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. Group VI and VII were treated with chloroform fraction, at the oral dose of 200mg/kg and 400mg/kg. The treatment was continued for 5 days with a view to see the effect on lipid profile [9].

The blood samples were withdrawn by ocular puncture and transferred directly into centrifuge tubes and allowed to clot at room temperature for 20-25 min and centrifuged for 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.

2.8 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.

2.9 Chromatographic studies and isolation of compound

Flash chromatography (Buchi controller C-610) was done for the chloroform fraction. Elution of the column with n-hexane: $CHCl_3$ (20:80) [fraction 31-47] yielded amorphous powder.

The amorphous powder was further adsorbed on silica gel (60-120 mesh). It was dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate the active constituent.

Elution of the column with $CHCl_3$: Methanol (99:1) [fraction 90-114] yielded colorless amorphous powder, recrystallised from methanol. The yield was found to be 216 mg (0.015% yield).

TLC of the powdered sample was carried out using various solvent systems. The appropriate one found to be - Petroleum ether: $CHCl_3$: Methanol (7: 1: 2). This solvent system gave the best resolution.

Melting point of 168-170° C was recorded for the component and UV λmax (MeOH) was recorded as 241 nm.

2.10 Characterization of compound

The compound I has R_f: 0.43 ; m.p: 168-170 ^{0} C; λmax in EtOH: 210 nm; IR bands (KBr): 3419, 2922, 2852, 1636, 1463, 1378, 1164, 965 cm^{-1} ; Positive ion FAB MS m/z: 412$[M]^+$ ($C_{29}H_{48}O$), 397, 394, 382, 253, 229, 213; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR($CDCl_3$) data have shown in the Table-1.

Table-1 δ_H = Chemical shift values in ^{1}H-NMR spectrum; δ_C = Chemical shift values in ^{13}C-NMR spectrum of isolated component

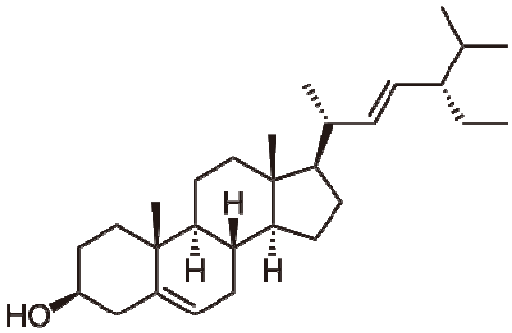
Position	δ_H	δ_C
1		37.33
2		31.09
3	3.51(1H,brs)	69.74
4		41.92
5		141.08
6	5.16(1H,brs)	119.83
7		33.23
8		34.97
9		50.18
10		36.68
11		21.09
12		39.78
13		41.92
14		55.98
15		24.34
16		27.29
17		55.33
18		11.48
19		20.31
20		35.09
21		18.65
22	5.14 (1H,m)	137.38
23	5.07 (1H,m)	128.69
24		45.08
25		28.52
26		25.73
27		18.69
28		23.50
29		18.71

RESULTS AND DISCUSSION

The preliminary phytochemical screening revealed the presence of tannins, coumarins, alkaloids and glycosides in ethanolic extract of *S. persica* while chloroform fraction revealed the presence of sterols. Melting point of 168-170°C was recorded for the component and UV λmax (MeOH) was recorded as 241 nm for the component.

The structure of the compound obtained from chloroform fraction of *S. persica*, was elucidated as Stigmast-5, 22-dien-3β-ol on the basis of spectral data analysis. The compound was obtained as colourless amorphous powder from chloroform: methanol (99:1) eluent. Its IR spectrum displayed characteristic absorption bands for 3419 cm $^{-1}$ (O-H), 2922 cm $^{-1}$ (C-H), 2852 cm $^{-1}$ (C-H), 1636 cm $^{-1}$ (C=C), 1164 cm $^{-1}$ (C-O). The +ve FAB mass spectrum of the compound exhibited a molecular ion peak at m/z 412 consistent with the molecular formula ($C_{29}H_{48}O$). The important peaks appearing are m/z 397, 394, 382, 253, 229, 213. The ^{1}H NMR spectrum of the compound suggested H-6 olefinic proton displayed at δ 5.16 (1H, brs, H-6). 1H proton appeared as broad singlet at δ 3.51. Two olefinic protons appeared downfield at δ5.14 (1H, m, H-22) and

δ5.07 (1H, m, H-23). Moreover six methyl protons appeared at δ1.12 (3H, brs, Me-19), δ 0.97 (3H, d, J=7.8 Hz, Me-21), δ 0.88 (3H, d, J=6.5 Hz, Me-26), δ 0.85 (3H, d, J=6.3 Hz, Me-27), δ 0.81 (3H, d, J=6.0 Hz, Me-29) and δ 0.68 (3H, brs, Me-18). The carbons of alkenes conjugated are at 141.08 ppm (C_5) and 119.83 ppm (C_6) which was confirmed from the ^{13}CNMR.These assignments are in good agreement for the structure of Stigmast-5, 22-dien-3β-ol.



Stigmast-5, 22-dien-3β-ol

IUPAC name: 17-(4-ethyl-1,5-dimethyl-hex-2-enyl)-10,13-dimethyl- 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol

Ethanolic extract and its chloroform fraction at dose of 200mg/kg decreased serum level of total cholesterol by 32.54 and 37.09 % respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased serum level of total cholesterol by 36.18 and 39.35% respectively (Table-2).

Table-2 Effect of ethanolic extract and $CHCl_3$ fraction of *Salvadora persica* on total cholesterol level (mg/dl) in Triton induced hyperlipidemic model

Group	Total cholesterol level after the administration of triton	Total cholesterol level after the vehicle/drug treatment
Control	66.19 ± 0.35	67.54±0.68
Hyperlipidemic	68.50 ± 0.28	98.83 ±0.22
Atrovastatin (50mg/kg)	88.56 ± 1.58	52.19 ±1.02 [a]
S.persica ethanolic extract (200mg/kg)	83.92± 0.52	64.72±.0.54[b]
S.persica ethanolic extract (400mg/kg)	85.83± 0.91	56.83±0.35[a]
S.persica $CHCl_3$ fraction (200mg/kg)	86.58± 1.12	61.32±0.58[a]
S.persica $CHCl_3$ fraction (400mg/kg)	88.37±1.36	54.41±0.43[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 compared to hyperlipidemic group

Ethanolic extract and its chloroform fraction at dose of 200mg/kg decreased serum level of triglyceride level by 34.58 and 41.73% respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased serum level of triglyceride level by 43.88 and 50.25% respectively (Table-3).

Table-3 Effect of ethanolic extract and $CHCl_3$ fraction of *Salvadora persica* on triglyceride level (mg/dl) in Triton induced hyperlipidemic model

Group	Triglyceride level after the administration of triton	Triglyceride level after the vehicle /drug treatment
Control	51.25 ± 1.65	53.43±1.70
Hyperlipidemic	50.70 ± 1.58	87.77 ±1.72
Atrovastatin (50mg/kg)	66.58 ± 2.26	52.72±2.52 [a]
S.persica ethanolic extract (200mg/kg)	57.48±2.42	59.72±.1.41[a]
S.persica ethanolic extract (400mg/kg)	57.85±2.27	56.95±1.32[b]
S.persica $CHCl_3$ fraction (200mg/kg)	57.60± 2.85	54.26±1.39[b]
S.persica $CHCl_3$ fraction (400mg/kg)	58.93±2.02	52.44±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 compared to hyperlipidemic group

Ethanolic extract and chloroform fraction at dose of 200mg/kg increased the serum HDL cholesterol level by 8.47 and 11.2%. On the other hand ethanolic extract and fraction at dose of 400mg/kg increased the serum HDL cholesterol level by 12.66 and 14.71% respectively (Table-4).

Table-4 Effect of ethanolic extract and $CHCl_3$ fraction of *Salvadora persica* on HDL level (mg/dl) in Triton induced hyperlipidemic model

Group	HDL level after the administration of triton	HDL level after the vehicle/drug treatment
Control	33.36 ± 1.11	34.86±1.34
Hyperlipidemic	32.53 ± 1.24	33.02 ±1.49
Atrovastatin (50mg/kg)	45.43 ± 1.43	56.96.± 1.01 [a]
S.persica ethanolic extract (200mg/kg)	44.58±1.57	46.40±.1.71[b]
S.persica ethanolic extract (400mg/kg)	43.36±1.49	48.35±1.52[b]
S.persica $CHCl_3$ fraction (200mg/kg)	47.46± 2.09	50.83±1.42[a]
S.persica $CHCl_3$ fraction (400mg/kg)	45.35± 1.92	51.44± 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 compared to hyperlipidemic group

Table-5 Effect of ethanolic extract and $CHCl_3$ fraction of *Salvadora persica* on LDL level (mg/dl) in Triton induced hyperlipidemic model

Group	LDL level after the administration of triton	LDL level after the vehicle/drug treatment
Control	34.31 ± 1.25	33.97±1.49
Hyperlipidemic	34.88 ± 1.66	59.73 ±1.74
Atrovastatin (50mg/kg)	57.22 ± 1.42	36.23± 1.20 [a]
S.persica ethanolic extract (200mg/kg)	52.16±1.72	45.75±.1.73[b]
S.persica ethanolic extract (400mg/kg)	53.44±1.60	42.38±1.58[b]
S.persica $CHCl_3$ fraction (200mg/kg)	55.33± 2.32	43.35±1.63[b]
S.persica $CHCl_3$ fraction (400mg/kg)	56.23±2.15	40.44±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 compared to hyperlipidemic group

The reduction in LDL cholesterol level by ethanolic extract and chloroform fraction at dose of 200mg/kg were 67.41 and 70.04%respectively. On the other hand ethanolic extract and its chloroform fraction at dose of 400mg/kg decreased LDL cholesterol by 71.93 and 75.48% (Table-5).

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 [10].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 [11].

The acute treatment with *S. persica* ethanolic extract and its chloroform fraction caused inhibitory effects both on total cholesterol (TC) and triglyceride level (TG) after Triton 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.

CONCLUSION

The present work characterized an isolated component from active fraction of *S. persica*. It may be concluded that the lowering of lipid level from active fraction is due to the presence of isolated component. Complete treatment of various types of hyperlipidemia is possible by herbal drugs. Various herbal remedies including one or more herbal drugs not only decrease the serum levels of TC, TG and LDL cholesterol but also increase the serum level of beneficial HDL cholesterol.

REFERENCES

[1] MT Monforte, N Miceli, MR Mondello, R Sanogo, A Rossitto and EM Galati, *Indian J Exp Biol*, **2001**, 39, 289-292.
[2] MT Monforte, A Trayoto, AM Foresteieri, DA Aquino, A Rossitto and EM Galati, *Phyto Res*, **2002**, 16, 395-397.
[3] MI Mansour, TL Khateeb and AA Mazraoo, *The Saudi Den J*, **1996**, 08, 87-91.
[4] F Al- Ali and T Al- Lafi, *Nat Pro Res*, **2003**, 17, 189-194.
[5] MK Al- Hindwai, IH Al- Deen, MH Nabi and MA Ismail, *J Ethnopharmacol*, **1989**, 26, 163-168.
[6] LV Asolkar, KK Kakkar and OJ Chakre, NISCAIR, India, **1992**, pp. 1965-1985.
[7] S Bungorn, W Varima, L Pisamai, S Jamsai and J Durit, *J Ethnopharmacol*, **2001**, 75, 185-190.
[8] A Chatterjee, Council for Scientific and Industrial Research, India, **1990**, pp. 327.
[9] M Friedman and SO Bayer, *Am J Physiol*, **1957**, 190, 439- 445.
[10] UK Patil and VK Dixit, *J Ethnopharmacol* , **2004**, 90, 249- 252.
[11] BV Ghule, MH Ghante, AN Saoji and PG Yeole, *Ind J Exp Biol*, **2006**, 44, 905-909.