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Efficacy of Stigmast-5-en-3 β -ol Isolated from *Salvadora persica* L. as Antihyperlipidemic and Anti-tumor agent: Evidence from animal studies

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ABSTRACT

Objective: To explore the anti-hyperlipidemic and anti-tumor effect of ethanolic extract, chloroform fraction and stigmast-5-en-3 β -ol from stems of *Salvadora persica* L. **Methods:** The antihyperlipidemic effect of ethanolic extract of *Salvadora persica*, its chloroform fraction and isolated components was evaluated in Triton- induced hyperlipidemic rats. 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 test drugs were administered orally at doses of 200 mg/kg body weight in rats. The ethanolic extract and stigmast-5-en-3 β -ol from *Salvadora persica* were further investigated for the tumor take inhibitory activity in hybrid mice (of C57BL strain + Swiss albino strain). Preventive group animals were injected daily with the extract and Stigmast-5-en-3 β -ol at dose of 50mg/kg body weight i.p. for 10 consecutive days. The animals were observed for the growth of tumor after injection of B16F10 melanoma cells into the dorsal skin of mice. **Results:** Phytochemical investigation of stems of *Salvadora persica* resulted in the isolation of stigmast-5-en-3 β -ol. The structure of the component has been established on the basis of spectral data analysis. UV λ max was found to be 206 nm with a melting point of 138°C for the isolated component. The stigmast-5-en-3 β -ol showed inhibition of total cholesterol, triglycerides, low density lipoproteins level (LDL), and significantly increased high density lipoprotein level (HDL) in Triton- induced hyperlipidemic rats. It suggested marked antihyperlipidemic activity of stigmast-5-en-3 β -ol at the oral dose of 200mg/kg. Pretreatment with the drug showed delay tumor growth by increasing the Volume Doubling Time (VDT) and Growth Delay (GD). The Stigmast-5-en-3 β -ol had shown better mean survival time. **Conclusions:** These observations enabled to conclude that supplementation of antioxidants and phytosterols rich food exerts significant antihyperlipidemic and antitumor activity. The present investigation may be quite useful as this drug is highly valued as traditional system of medicine.

1. Introduction

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. Tumors are caused by mutations in DNA of cells.

An accumulation of mutation is needed for a tumor to emerge. Mutations that activate oncogenes or repress tumor suppressor genes can eventually lead to tumors^[1, 2].

This study provides scientific evidence for the application of the traditional medicinal plant, *Salvadora persica*, in the treatment of hyperlipidemia and tumors.

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^[3], anticonvulsant activity^[4], analgesic activity^[5], antibacterial activity^[6], Oral hygienic activity^[7]. It contains a number of medically beneficial properties including abrasives, astringents and antiseptics. Its main constituents are trimethylamine, an alkaloid which may effectively

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be salvadorine, chlorides, sulphur, terpenes, vitamin C, glycosides, large amounts of fluoride and silica, small amounts of tannins, saponins and flavonoids[8–10]. Three lignin glycosides have been reported from the stem of *S. persica*. The flavonoids rutin and quercetin were detected in the stem of *S. persica*. Salvadoreurea has been reported in the root of *S. persica*. Benzylisothiocyanate was also isolated from the root. Salvadoricine, a new indole alkaloid, was reported in the leaves of *S. persica*.

2. Material 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 drug (800 g) 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. Chloroform fraction 52 g (6.5% yield obtained with 800g dried drug) thus obtained was subjected to further study involving isolation of active component.

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 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: CHCl₃ (25:75) [fraction 44–58] 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 and eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate the active constituent. Elution of the column with CHCl₃: Methanol (24:1) [fraction 51–77] yielded colorless amorphous powder, recrystallised

from methanol. The yield was found to be 133.9 mg (0.180%). TLC of the powdered sample was carried out using various solvent systems. The appropriate one found to be – Petroleum ether: CHCl₃: Methanol (1: 4: 1). This solvent system gave the best resolution.

2.5 Characterization of compound

The compound has Rf : 0.42 ; m.p: 137–138°C; λ max in EtOH: 206 nm; IR bands (KBr): 3465, 2935, 2867, 1637, 1475, 1365, 1210, 1105 cm⁻¹ ; Positive ion FAB MS m/z: 414[M]⁺ (C₂₉H₅₀O), 397, 396, 381, 365, 339, 371, 255, 239, 213, 199, 159, 145, 119,105; 1 H–NMR (CDCl₃) and 13 C–NMR (CDCl₃) data have shown in the table–1.

Table 1

δ H = Chemical shift values in 1H–NMR spectrum; δ C = Chemical shift values in 13C–NMR spectrum.

Position	δ H	δ C
1		37.33
2		31.63
3	3.51 (1H, brs)	71.8
4		72.3
5		141.7
6	5.30 (1H,d)	121.4
7		31.6
8		31.81
9		49.57
10		36.74
11		20.9
12		31.80
13		40.4
14		56.04
15		24.19
16		28.60
17		50.1
18	0.67 (3H,brs)	11.36
19	1.01 (3H, brs)	19.30
20		36.74
21	0.97 (3H,d)	18.15
22		33.30
23		25.73
24		42.3
25		30.5
26	0.82 (3H,t)	20.37
27	0.85 (3H,d)	19.30
28		23.56
29	0.86 (3H,d)	11.5

2.6 Screening for hypolipidemic activity

Screening for hypolipidemic activity was carried out in albino rats either sex weighing 100–120 g. Triton WR 1339 (Tyloxapol) was purchased from Sigma Chemicals Co., USA.

2.6.1 Preparation of test material

Ethanolic extract, chloroform fraction and stigmast–5–en–3 β –ol were suspended in distilled water plus

Polyoxyethylenesorbiton Mono-oleate (Tween 80– 0.771g). Triton was dissolved in normal saline to give a 7% solution.

2.6.2 Animal

The Albino rats were selected and housed in polypropylene cages maintained under controlled conditions. The animals were fed standard mice feed and acidified water *ad libitum*. 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.

2.6.3 Group treatment and measurements

Albino rats were divided into six groups of six rats each. Group I served as vehicle control. Group II was kept as hyperlipidemic and administered with Triton only. Triton administration was done only once. Group II–VI 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 standard (beta-sitosterol). Group III, IV, V and VI were treated with standard (beta-sitosterol), ethanolic extract, chloroform fraction and stigmast-5-en-3 β -ol at the oral dose of 200mg/kg. The treatment was continued for 5 days with a view to see the effect on lipid profile.

The blood samples were withdrawn from the eye vein and transferred directly into centrifuge tubes and allowed to clot at room temperature for 20–25 min and centrifuged for 15–20 min at 2000 r/min. The supernatant clear serum thus obtained was transferred carefully with the help of micropipette into small test tubes for estimation. The serum concentration of total cholesterol, HDL and triglycerides were measured by Span diagnostic kit using auto-analyzer^[11].

2.6.4 Histopathological study

Histopathological study of rat liver of five groups was also performed. Tissue sections of 3 μ m thickness were stained with Ehrlich's hematoxylin and eosin and examined by light microscopy.

2.7 Screening for anti-tumor activity

2.7.1 Animal

The hybrid mice (of C57BL strain + Swiss albino strain) was selected, from a random breed colony maintain in the animal house of Research Department of Jawaharlal Nehru Cancer Hospital, Bhopal, M.P., India. The mice were housed in polypropylene cages maintained under controlled conditions. The animals were fed standard feed (formula obtained from Cancer Research Institute, Mumbai, India) and acidified water *ad libitum*. Mice of either sex, 6 – 8 weeks old and weighing 25–30 g, were selected from the above colony for the experiments.

2.7.2 Tumor cell lines: B16F10 Melanoma

B16F10 melanoma cell lines were originally obtained from National Cell Centre, Pune, India. These cells were then transplanted in C57BL mouse, which further led to the development of tumor in this mouse.

2.7.3 Tumor propagation

Tumor bearing mouse (C57BL mouse, as discussed in previous section) was sacrificed by cervical dislocation and the whole animal was dipped in 70% alcohol and the tumor was excised to single cell suspension by mechanical dispersion. The cell suspension was filtered through a 45 μ nylon mesh. The single cell suspension was then passed through different gauze size needle. The cell suspension was again passed through nylon mesh so as to remove the clumps of cells.

2.7.4 Group treatment

The animals were divided into four groups. Group I served as control and was injected with double distilled water. Group II, III and IV were injected daily with the ethanolic extract, chloroform fraction and stigmast-5-en-3 β -ol 50 mg/kg body wt. i.p. for 10 consecutive days. Three weeks after the last injection of the test compounds, the animals were injected subcutaneously with 5 x 10⁵ viable B16F10 melanoma cells into the dorsal skin. The animals were observed for the growth of tumor. Volume doubling time and Growth delay were calculated^[12–13].

2.7.5 Tumor Growth Kinetics

The tumor size was measured every alternate day and the tumor volume was calculated.

Tumor diameters are measured with digital callipers, and the tumor volume in mm³ is calculated by the formula:

$$\text{Volume} = (\text{width})^2 \times \text{length}/2$$

Tumor growth response was assessed from the following parameters. (Fig. 1)

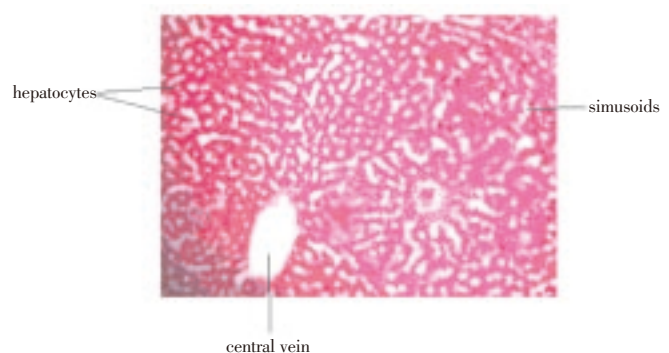


Fig. 1 –Control Group: Normal rat liver

Volume doubling time (VDT): The time, in days for the tumors size to reach double the treatment volume. Growth delay (GD): Difference in the time, in days, needed for the treated and untreated tumor to reach five times the treatment volume.

2.8 Statistical analysis

Statistical evaluation of the data was done by One way variance analysis (ANOVA) test. A value of $p < 0.05$ was considered to be significant.

3. Results

The preliminary phytochemical screening revealed the presence of tannins, coumarins, alkaloids and glycosides in *S. persica* ethanolic extract while chloroform fraction showed the presence of sterols.

The structure of the compound, obtained from chloroform fraction of *S. persica*, was elucidated as stigmast-5-en-3 β -ol on the basis of spectral data analysis. The compound was obtained as colourless amorphous powder from chloroform: methanol (24:1) eluent. Its IR spectrum displayed characteristic absorption bands for 3465 cm^{-1} (O-H), 2935 cm^{-1} (CH₂), 2867 cm^{-1} (C-H), 1637 cm^{-1} (C=C), 1105 cm^{-1} (C-O). The +ve FAB mass spectrum of the compound exhibited a molecular ion peak at m/z 414 consistent with the molecular formula (C₂₉H₅₀O). The important peaks appearing are m/z 397, 396, 381, 365, 339, 371, 255, 239, 213, 199, 159, 145, 119, 105. The ¹H NMR spectrum of the compound suggested H-6 olefinic proton displayed as doublet at δ 5.30 (J=5.5 Hz). 1H proton appeared as broad singlet at δ 3.51. Moreover six methyl protons appeared at δ 1.01 (3H, brs, Me-19), δ 0.97 (3H, d, J=6.5 Hz, Me-21), δ 0.86 (3H, d, J=6.0 Hz, Me-29),

δ 0.85 (3H, d, J=6.0 Hz, Me-27), δ 0.82 (3H, t, J=6.2 Hz, Me-26) and δ 0.67 (3H, brs, Me-18). The carbons of alkenes conjugated are at 141.7ppm (C5) and 121.4 ppm (C6) which was confirmed from the ¹³CNMR. These assignments are in good agreement for the structure of stigmast-5-en-3 β -ol (Table-1).

The rats treated with triton showed increase in serum cholesterol level as compared to initial level. In *S. persica* ethanolic extract treated group the initial level of total cholesterol was found to be 56.51 \pm 0.95 which was increased upto 75.02 \pm 1.03 by triton. After the treatment with plant extract the total cholesterol level was reduced upto 66.8 \pm 0.29. In *S. persica* CHCl₃ fraction treated group the initial level of total cholesterol was 58.92 \pm 0.88 which was increased upto 73.24 \pm 0.96 and after the treatment with fraction the total cholesterol level was reduced upto 65.03 \pm 0.62. In *S. persica* isolated compound treated group the initial level of total cholesterol was found to be 60.31 \pm 0.61 which was increased upto 72.46 \pm 1.15 by triton. After the treatment with compound the total cholesterol level was reduced upto 64.79 \pm 0.92 (Table-2).

In *S. persica* ethanolic extract treated group the initial level of triglyceride was 54.79 \pm 0.79 which was increased upto 89.46 \pm 1.59 and after the treatment with the plant extract it was decreased upto 80.27 \pm 0.27. In *S. persica* CHCl₃ fraction treated group the initial level of triglyceride was found to be 55.52 \pm 1.15 which was increased upto 82.75 \pm 1.07 by triton. After the treatment with fraction the triglyceride level was reduced upto 78.41 \pm 0.54. In *S. persica*

Table 2

Effect of ethanolic extract, CHCl₃ fraction and Stigmast-5-en-3 β -ol from *Salvadora persica* on total cholesterol level (mg/dl) in triton induced hyperlipidemic model

Group	Initial	Total cholesterol level after the administration of Triton	Total cholesterol level after the vehicle/drug treatment
Control	59.57 \pm 0.58	71.52 \pm 0.93	73.60 \pm 0.54
Hyperlipidemic	59.74 \pm 0.76	74.11 \pm 0.54	85.97 \pm 0.77
Standard(beta-sitosterol) (200 mg/kg)	57.60 \pm 0.63	71.75 \pm 0.33	58.80 \pm 1.13
<i>S. persica</i> ethanolic extract (200 mg/kg)	56.51 \pm 0.95	75.02 \pm 1.03	66.80 \pm 0.29 ^a
<i>S. persica</i> CHCl ₃ fraction(200 mg/kg)	58.92 \pm 0.88	73.24 \pm 0.96	65.03 \pm 0.62 ^a
Stigmast-5-en-3 β -ol (200 mg/kg)	60.31 \pm 0.61 ^a	72.46 \pm 1.15	64.79 \pm 0.92

Total cholesterol concentrations are estimated by standard method. Values are expressed as mean \pm S.E.M for six animals in each group.

a: $P < 0.01$ compared with hyperlipidemic group

Table 3

Effect of ethanolic extract, CHCl₃ fraction and Stigmast-5-en-3 β -ol from *Salvadora persica* on triglyceride level (mg/dl) in triton induced hyperlipidemic model

Group	Initial	Triglyceride level after the administration of Triton	Triglyceride level after the vehicle/drug treatment
Control	58.85 \pm 0.53	92.67 \pm 0.35	101.30 \pm 1.61
Hyperlipidemic	57.60 \pm 0.31	84.10 \pm 0.88	135.80 \pm 1.95
Standard(beta-sitosterol) (200 mg/kg)	55.34 \pm 0.78	83.58 \pm 0.53	72.82 \pm 0.82 ^a
<i>S. persica</i> ethanolic extract (200 mg/kg)	54.79 \pm 0.79	89.46 \pm 1.59	80.27 \pm 0.27 ^a
<i>S. persica</i> CHCl ₃ fraction(200 mg/kg)	55.52 \pm 1.15	82.75 \pm 1.07	78.41 \pm 0.54 ^a
Stigmast-5-en-3 β -ol (200 mg/kg)	58.88 \pm 0.23	90.05 \pm 0.90	77.69 \pm 0.42 ^a

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

a: $P < 0.01$ compared with hyperlipidemic group

isolated component treated group the initial levels of triglyceride was 58.88±0.23 which was increased upto 90.05±0.90 and after the treatment with the compound the triglyceride was decreased upto 77.69±0.42 (Table-3).

In *S. persica* ethanolic extract treated group the initial level of HDL was 51.79±0.58 which was increased upto 52.03±0.64 and after the treatment with the plant extract it was increased upto 54.46±0.51. In *S. persica* CHCl₃ fraction treated group the initial level of HDL was found to be 52.97±1.28 which was increased upto 54.92±0.92 by triton. After the treatment with fraction the HDL level was raised upto 57.20±1.11. In *S. persica* isolated component treated group the initial levels of HDL was 51.88±0.23 which was increased upto 53.6±1.05 and after the treatment with the compound the HDL was increased upto 57.54±0.26 (Table-4).

In *S. persica* ethanolic extract treated group the initial level of LDL was found to be 15.97±0.21 which was increased upto 42.79±0.69 by triton. After the treatment with plant extract the LDL level was reduced upto 40.53±0.81. In *S. persica* CHCl₃ fraction treated group the initial level of LDL was 14.54±0.73 which was increased upto 38.42±1.02 and after the treatment with fraction the LDL level was reduced upto 36.03±1.39. In *S. persica* isolated component treated group the initial level of LDL was found to be 13.20±0.36 which was increased upto 39.78±0.59 by triton. After the treatment with the compound the LDL was reduced upto 34.22±0.80 (Table-5).

As is evident from Fig. 1, the histopathological alterations viz. steatosis, sinusoidal dilatation and congestion, Kupfer cell hyperplasia or portal triaditis were not present in normal rat liver. Histopathology of liver in hyperlipidemic rats (Fig. 2) showed

microvesicular steatosis along with sinusoidal dilatation, congestion and Kupfer cell hyperplasia. Liver histopathology (Fig. 3) of treated with Triton and Standard showed only mild sinusoidal congestion. Portal tracts and overall hepatic architecture were within normal limits. Rats treated with *S. persica* ethanolic extract and stigmast-5-en-3^β-ol (Fig 4 and 5) showed mild feathery degenerative changes and mild vascular congestion but no steatosis. The overall hepatic architecture was maintained.



Fig. 2 –Hyperlipidemic Group– Triton alone treated rat liver

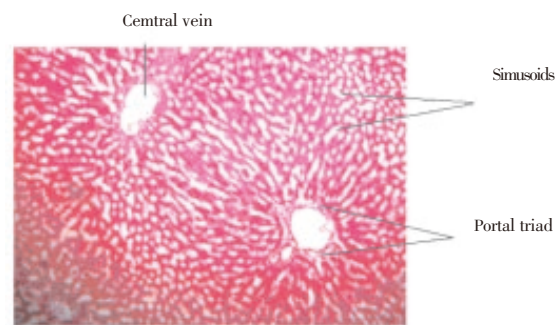


Fig. 3 – Standard (beta-sitosterol) treated rat liver

Table 4

Effect of ethanolic extract, CHCl₃ fraction and Stigmast-5-en-3^β-ol from *Salvadora persica* on HDL level (mg/dl) in triton induced hyperlipidemic model

Group	Initial	HDL level after the administration of triton	HDL level after the vehicle/drug treatment
Control	53.12±0.47	53.87±0.64	53.92±0.56
Hyperlipidemic	52.24±0.31	52.60±0.38	53.63±0.93
Standard(beta-sitosterol) (200 mg/kg)	55.34±0.78	56.22±0.46	58.83±0.38 ^a
<i>S.persica</i> ethanolic extract(200 mg/kg)	51.79±0.58	52.03±0.64	54.46±0.51
<i>S. persica</i> CHCl ₃ fraction(200 mg/kg)	52.97±1.28	54.92±0.92	57.20±1.11 ^a
Stigmast-5-en-3 ^β -ol (200 mg/kg)	51.88±0.23	53.68±1.05	57.54±0.26 ^a

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

a: P<0.01 compared with hyperlipidemic group

Table 5

Effect of ethanolic extract, CHCl₃ fraction and Stigmast-5-en-3^β-ol from *Salvadora persica* on LDL level (mg/dl) in triton induced hyperlipidemic model

Group	Initial	LDL level after the administration of triton	LDL level after the vehicle/drug treatment
Control	18.22±0.29	36.18±0.53	39.95±0.85
Hyperlipidemic	13.66±0.25	33.69±0.50	58.5±0.47
Standard(beta-sitosterol) (200 mg/kg)	13.32±0.16	30.24±0.59	27.56±0.41 ^a
<i>S.persica</i> ethanolic extract(200 mg/kg)	15.97±0.21	42.79±0.69	40.53±0.81 ^a
<i>S. persica</i> CHCl ₃ fraction(200 mg/kg)	14.54±0.73	38.42±1.02	36.03±1.39 ^a
Stigmast-5-en-3 ^β -ol (200 mg/kg)	13.20±0.36	39.78±0.59	34.22±0.80 ^a

LDL concentrations are estimated by standard method. Values are expressed as mean±SEM. for six animals in each group.

a: P<0.01

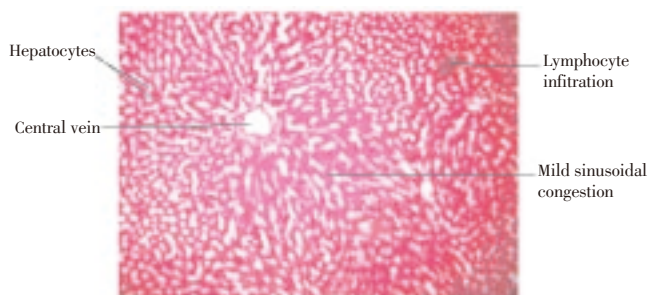


Fig. 4 – *S. Persica* ethanolic extract treated group

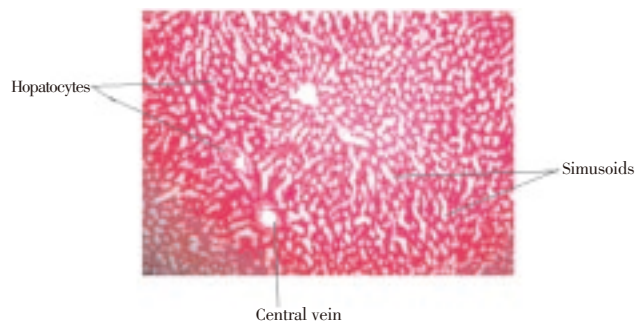


Fig. 5 – Stigmast-5-en-3 β -ol treated group

From the growth curves of tumor, it is clear that control tumors showed an exponential growth. The stigmast-5-en-3 β -ol treated group produced slow growth response when compared to control. The volume doubling time and growth delay were calculated from the growth curves of individual tumor bearing mice.

Silent period: The silent period (*i.e.* time taken for palpable growth) for the control group was found to be 7 days while in case of stigmast-5-en-3 β -ol treated group, it was found to be 10 days, which was very significant. ($P < 0.0001$) (Table-6)

Volume Doubling Time: The volume doubling time observed for stigmast-5-en-3 β -ol treated group was found to be one day that was significant ($p = 0.0062$) compared to control. (Table-6)

Mean survival time: The maximum survival time was observed to be 26 days for control group. The MST observed for stigmast-5-en-3 β -ol treated group was 31 days, which was 5 days more than the control group (Table-6). Comparison in between MST of control group with stigmast-5-en-3 β -ol treated group was found to be very significant. ($p = 0.0004$) (Fig. 6)

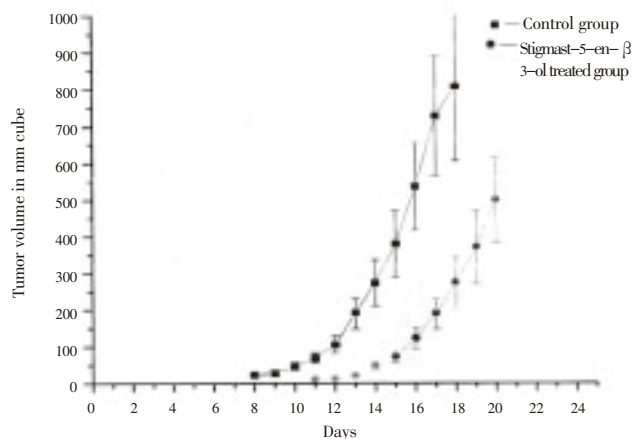


Fig. 6 Growth curve– Tumor volume

Growth Delay: The GD was 0.804 days in stigmast-5-en-3 β -ol treated group, which was significant ($P = 0.003$) compared to control. (Table-6)

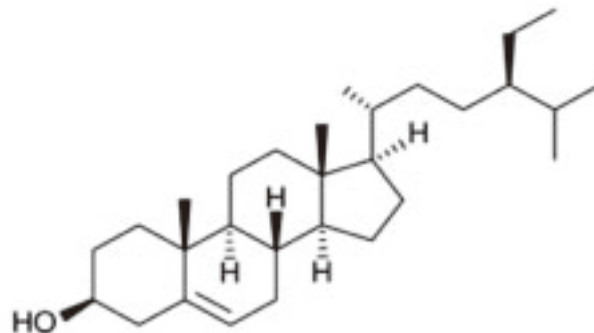


Fig. 7 Structure of Stigmast-5-en-3 β -ol

4. Discussion

Triton WR – 1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting into increased blood lipid concentration[11]. The possible mechanism of activity may be due to enhancement of the activity of lecithin acyl transferase (LCAT) and inhibition of the action of hepatic TG- lipase on HDL[14].

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[15].

Table 6

Response of B16F10 mouse melanoma to the treatment with ethanolic extract, chloroform fraction and Stigmast-5-en-3 β -ol at the dose of 50 mg / kg: Silent period, VDT, GD and MST

Parameter	Control	Ethanolic extract	Chloroform fraction	Stigmast-5-en-3 β -ol
Silent period days	04 \pm 0.24	06 \pm 0.33	07 \pm 0.45	10 \pm 0.24 ^a
Volume doubling time (VDT) days	01 \pm 0.16	02 \pm 0.25	02 \pm 0.31	03 \pm 0.28 ^b
Growth delay (GD) days	–	01 \pm 0.19	01 \pm 0.22	02 \pm 0.24
Mean Survival Time (MST) days	23 \pm 0.70	25 \pm 0.61	28 \pm 0.47	31 \pm 0.58 ^c

a : $P < 0.0001$ b : $P = 0.0062$ c : $P < 0.01$ compared to control

It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of cholesterol and LDL levels achieved by administration of test samples, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about. The extract, fraction and the active phytoconstituent, inhibited the total cholesterol; triglycerides, low density lipoproteins level (LDL), and significantly increased high density lipoprotein level (HDL). Tumor regression studies showed a regression response for tumor growth in vivo of a murine mouse melanoma. The treatment produced a delay in tumor growth, as demonstrated by increasing the VDT and GD. Indications are available that this plant has got antioxidant properties. Oxidative stress has been implicated in numerous pathophysiological conditions including cancer. Prevention of oxidative damage can be employed as one of the ways in tumor regression.

The present work characterized active phytoconstituent exhibiting anti-hyperlipidemic and anti-tumor potential from the stems of *Salvadora persica*. The observations enabled to conclude that supplementation of antioxidants and phytosterols rich food exerts significant antihyperlipidemic and antitumor activity. The present investigation may be quite useful as this drug is highly valued as traditional system of medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Amar M, Waihay W, Celeste S. Hypoxia-Inducible Factors and the Response to Hypoxic Stress. *Mol Cell* 2010; **40**: 294–309.
- [2] Fredika R. Inflammatory Breast Cancer: The Disease, the biology, the treatment. *Cancer J Clin* 2010; **60**: 351.
- [3] Monforte MT, Miceli N, Mondello MR, Sanogo R, Rossitto A, Galati EM. Antiulcer Activity of *Salvadora persica* on Experimental ASA-Induced Ulcer in Rats: Ultrastructural Modifications. *Ind J Expl Biol* 2001; **39**: 289–292.
- [4] Monforte MT, Trayoto A, Foresteieri AM, Aquino DA, Rossitto A, Galati EM. Anticonvulsant and sedative effects of *Salvadora persica* L. stem extract. *Phyto Res* 2002; **16**: 395–397.
- [5] Talea H, Mansoor A, Mohammad S, Asif R. Analgesic activity of *Salvadora persica* in mice. *Med Channel* 2011; **4**: 22–24.
- [6] Sofrata AH, Claesson RL, Lingstram PK, Gustafsson AK. Strong antibacterial effect of *miswak* against oral microorganisms associated with periodontitis and caries. *J Periodontol* 2008; **79**: 1474–9.
- [7] M., Bergstrom J, Batwa S, Meshari F, Al-Otaibi M. Significance of chewing sticks (*miswak*) in oral hygiene from a pharmacological view-point. *Saudi Dental Journal* 2006; **18**: 125–33.
- [8] Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement to Glossary of India Medicinal Plants with Active Constituents, NISCAIR, India, 1992, pp. 1965–1985.
- [9] Bungorn S, Varima W, Pisamai L, Jamsai S, Durit J. Diuretic effects of selected Thai indigenous medicinal plants in rats. *J Ethnopharmacol* 2001; **75**: 185–190.
- [10] Chatterjee A. Treatise of Indian Medicinal Plants. Council for Scientific and Industrial Research, India, 1990, pp. 327.
- [11] Hicham H, Nour B, Mohammed A, Hana S, Noreddine G, Souliman A. The Triton WR-1339 induced hyperlipidemic rats: A comparison with fenofibrate. *J Ethnopharmacol* 2007; **109**: 156–160.
- [12] Uma Devi P, Guruprasad K. Influence of clamping-induced ischemia and reperfusion on the response of a mouse melanoma to radiation and hyperthermia. *Int J Hyper* 2001; **17**: 357–367.
- [13] Uma Devi P, Rao AV, Kamath R. In vivo radioprotective effect of *Moringa oleifera* leaves. *Ind J Exp Biol* 2001; **39**: 858–63.
- [14] Patil UK, Dixit VK. Hypolipidemic activity of *Cassia tora* L. seeds. *J Ethnopharmacol* 2004; **90**: 249–252.
- [15] Ghule BV, Ghante MH, Saoji AN, Yeole PG. Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* Mol. fruit extracts. *Ind J Exp Biol* 2006; **44**: 905–909.