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Therapeutic Potential of Salvia Bucharica for Dyslipidemia and Vascular Dysfunction Induced through Two Different Pathways

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Abstract

Background: The present study was endeavored to validate the antihyperlipidemic potential of *Salvia bucharica* (Lamiaceae) in lipofundin induced hyperlipidemia in rabbits and fructose treated hyperlipidemic rats.

Method: Extract of *Salvia bucharica* and atorvastation (10 mg/kg) were given to the animals through oral route. At the end of study period, blood samples were taken for determination of lipid profile. Furthermore, thoracic aorta and liver were studied for vasorexalation and histopathological changes respectively.

Results: *Salvia bucharica* has shown dose dependent antihyperlipidemic activity with a significant ($p < 0.05$) decrease in the levels of total cholesterol, low density lipoprotein and triglycerides in both models. In addition, Studies performed on the isolated thoracic aorta and histolopathological studies on liver demonstrated that *Salvia bucharica* has endothelial dependent vasoprotective and hepatoprotective activity respectively as compared to fructose fed rats. The toxicity studies of *Salvia bucharica* revealed that plant extract is safer for oral use.

Conclusion Hence, it was suggested that *Salvia bucharica* has the therapeutic potential against dyslipidemia and vascular dysfunction.

Background

Hyperlipidemia is a pathological condition that is characterized by elevation in total cholesterol, rise in LDL cholesterol, increase in triglycerides or decrease HDL cholesterol concentration or combination of these abnormalities [1]. Hyperlipidemia is the key risk factor for cardiovascular disorders (CVDs) which has been reported as the foremost cause of death in developed as well as developing nations [2] . Now a days it is considered that CVDs will turn out to be the most common cause of death and disability around the world by the year 2020 [3]. An increasing body of evidence suggested that oxidative stress is involved in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, and hypertension, diabetes, and heart failure [4].

Hyperlipidemia in general is often associated with endothelial dysfunction which is characterized by reduced vascular relaxation governed by limited NO production. Etiology of oxidative stress induced hyperlipidemia involves reactive oxygen species (ROS). ROS are involved in the oxidation of LDL and oxidized LDL is considered to be a contributor to endothelial dysfunction because it inhibits endothelial NO synthase. It is an independent menace to atherosclerosis which can lead to coronary heart disease and ischemic stroke [4, 5]. Commonly hyperlipidemia is treated with lipid-lowering drugs which are associated with a high risk of side effects such as liver damage, gastrointestinal problems, rhabdomylosis and myopathy. So there is a need of alternative treatment with little adverse effects. The use of plant based medicines has been increasing gradually world-wide in recent years [6] . WHO has recommended the use of indigenous plants as an alternative remedy especially in developing countries. Various medicinal products of herbal origin have been reported to have antihyperlipidemic and antihypercholesteremic properties [2].

So the present study was designed to evaluate the antihyperlipidemic effect of *Salvia bucharica* (Family: Lamiaceae) which is an aromatic plant that grows in Afghanistan, Pakistan and central Asia [7]. *Salvia bucharica* commonly known as Gul-e-Kakar or Sursanda, widely distributed in Baluchistan, Pakistan. The whole plant is soaked in water and the decoction is used for colic pain, kidney pain, jaundice, malaria fever and high blood pressure [8], but the reports on antihyperlipidemic activity are not present. Therefore this study is performed to investigate effects of *Salvia bucharica* in dyslipidemia and vascular dysfunction using two different animal models.

Material And Methods

Chemicals and reagents used

Atorvastatin (Medipak pharmaceutical industry Pvt. Ltd Lahore), Lipofundin (B Braun Melsungen AG Germany), Diphenylpicrylhydrazyl (DPPH) (Merck Germany), Ascorbic acid (Sigma-aldrich Germany), D-Fructose, Acetylcholine, Phenylephrine, Potassium chloride, Ketamine chloride and Sodium phenobarbital (Sigma, St. Louis, USA) were used to carry out experimentation.

Selection, collection and authentication of the plant

The plant for this study was selected based upon its traditional repute in various diseases like kidney pain, jaundice particularly in cardiovascular diseases. The aerial parts of *Salvia bucharica* were collected in the month of April and May 2015 from Harboi, Nichara District Kalat, Baluchistan Pakistan. The plant was authenticated by Dr. Muhammad Amin Ullah Shah, Department of Botany, University of Sargodha. For further references the voucher specimen (SB-16-23) of plant was deposited in College of Pharmacy, University of Sargodha.

Preparation of Aqueous methanol extract

The aerial parts of *Salvia bucharica* were dried under shade at room temperature and grounded into a coarse powder. Aqueous methanol (30:70) extract of the plant was prepared using cold maceration process. After 3 days, it was filtered through muslin cloth, the filtrate was collected, and the plant material was again soaked for 3 days, twice. At the end, all of three filtrates were combined, filtered through muslin cloth and Whatmann qualitative Grade 1 filter paper. The filtrate of the extract was evaporated under reduced pressure in rotary evaporator at 50 °C. This aqueous methanol (30:70) extract was then air-dried to obtain a solid mass [9, 10].

Experimental animals used

A local breed of rabbits weighing 1—2 kg, adult Sprague dawlley rats (200—250 g) of both sex and mice (25—35g) were procured from local market. Animals were kept under standard environmental conditions (room temperature 22 ± 2ºC, relative humidity 50 ± 5% and 12 h light and dark cycle) in animal resource center, University of Sargodha. Diet and water were provided *ad libitum.* Experiments were performed in accordance with guidelines for care and use of laboratory animals provided by the National Research council [11]. The experimental protocols were approved from Institutional Animal Ethics Committee, College of Pharmacy, University of Sargodha (Approval No. 43A24 IEC UOS).

Pharmacological studies performed on plant extract

Evaluation of Anti-hyperlipidemic effect of aqueous methanol extract of *Salvia bucharica* against Lipofundin induced dyslipidemia

Lipofundin cause rise in lipid profile thus this experiment was conducted to demarcate effect of *Salvia bucharica* on lipofundin lipid abnormalities. For this purpose, Rabbits weighing 1—2 kg were divided into different groups (n = 5). Group I (Normal control) received normal diet and water ad libitum for 23 days, Group II (negative control) received lipofundin (2 mL/kg i.v) for 8 consecutive days then from 9—23th day normal diet and water were given, Group III (positive control), Group IV (SB250) and group V (SB500) initially received lipofundin (2 mL/kg i.v) for 8 consecutive days then from 9—23th day atorvastatin (10 mg/kg p.o), *Salvia bucharica* aqueous methanol extract 250 mg and 500 mg p.o, administered respectively. Blood samples of animals were drawn on 8th and 23th day through jugular vein of the rabbits. Blood samples were allowed to clot for 20—25 min and centrifuged at 2500 g at 4ºC for 10 min. Serum was collected and aliquots were stored at —70ºC until biochemical analysis [6].

Biochemical parameters measured

Total cholesterol (TC), Triglyceride (TG), high density lipoprotein cholesterol (HDL-C) levels were measured through laboratory testing from the diagnostic laboratory of University Medical and Diagnostic Center of University of Sargodha. VLDL level, Low density lipoprotein (LDL-C), Atherogenic index (AI) and Coronary risk index (CRI) level were assessed by following formulas

VLDL = TG/5

LDL = TC — (HDL + VLDL)

AI = TC — HDL/HDL

CRI = TC/HDL [12-14]

Effect of *Salvia bucharica* aqueous methanol extract on Fructose induced hyperlipidemia in rats

The purpose of this experiment was to investigate the beneficial effect of *Salvia bucharica* on dyslipidemia induced by chronic administration of fructose. Wister albino rats of both sex weighing 100–150g were divided into five following groups: Group I (normal control) received normal diet and water ad libitum for 28 days, Group II (negative control) received fructose (25% w/v) in drinking water and normal diet for 28 days, Group III (positive control) received fructose (25% w/v) for consecutive 14 days then from 15—28th day atorvastatin (10 mg/kg p.o) was administered along with fructose (25% w/v). Group IV and V (treatment groups) received fructose (25% w/v) for consecutive 14 days then from 15—28th day plant extract (250 and 500 mg p.o) was administered along with fructose (25% w/v). On 29th day animals were sacrificed to collect the blood samples and were kept 30 min for coagulation, centrifuged at 3000rpm to get serum and the lipid profile was determined [15]. Aortas of animals were removed to determine the vascular reactivity and liver was dissected for histopathological study [16].

Total cholesterol (TC), Triglyceride (TG), high density lipoprotein cholesterol (HDL-C) levels were measured through laboratory testing. VLDL level, Low density lipoprotein (LDL-C), Atherogenic index (AI) and Coronary risk index (CRI) level were assessed by above mentioned formulas [12-14].

Evaluation of protective effect of Salvia bucharica on fructose induced vascular dysfunction in rats

As chronic administration of fructose lead to progression of endothelial dysfunction therefore, this study was designed to assess protective effect of *Salvia bucharica* on vascular reactivity. Vascular reactivity was performed to observe the functional integrity of endothelium. Rats were killed by cervical dislocation and dissected .The thoracic aorta was separated and placed in the cold Kreb's solution of the following composition (mM): NaCl 118, KCl 4.73, $KH_2PO_4$1.2, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11 and EDTA 0.026 for 30 seconds. Blood present in the aorta was removed by gently pressing aorta and transferred the aorta to the kreb's solution having temperature 37ºC. Aorta was separated from the surrounding connective tissue and cut into rings of 3 mm long. Special care was taken to avoid any damage to endothelium. Rings were then mounted in organ-baths by means of two stainless steel wires attached with force-displacement transducer. A resting tension of 2 g was applied to facilitate the measurement of isometric force. The organ chamber was filled with 10 mL of Krebs–Henseleit solution at 37ºC and gassed with 95% O_2 and 5% CO_2. Preparations were allowed to equilibrate for approximately 1 h with an exchange of Kreb's solution every 15 min. Following 1h equilibration the active muscle tone of ring segments contraction was then observed by potassium chloride (70—80 mM) and washout was given by changing the Kreb's solution to return back to its initial tension and allowed to stabilize it for 30 min. Phenylephrine (10^{-6} M) was added in the chamber and contraction was noticed and washout period was given by changing the Kreb's solution and allowed to stabilize it at its baseline tension. Again phenylephrine (10^{-6} M) was added in the chamber and contraction was observed. After a stable contraction plateau was reached, relaxation of the aortic rings was measured in response to cumulative additions of acetylcholine (Ach; 10^{-9} to 10^{-4} M) [16-19].

Histopathological examination of liver in fructose treated rats

On 28th day of treatment in fructose induced hyperlipidemia, rats were sacrificed, liver was dissected and washed with normal saline solution in order to clear it from blood and preserved in 10% formalin solution. Fixed liver was sectioned (5 micron thickness), embedded in paraffin and sections stained with Hematoxylin and Eosin (H&E). For estimation of histopathological changes in liver tissues was observed under light microscope [20, 21].

Toxicity profile

Acute toxicity studies performed in present work

This study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines 425 [22].

Study was performed in three phases as following

Phase 1

Mice were randomly divided into four groups of two mice per group. Graded doses (500, 1000, 1500, 2000 mg/kg) of the *Salvia bucharica* was administered to the mice orally. After treatment, Mice were continuously observed during next 24 h (0.25, 0.5, 1, 2, 4, 12 h) for mortality, behavioral changes (restlessness, dullness, agitation, sedation) and signs of toxicity.

Phase 2

After 24 hours based on the findings of phase 1, three groups of mice (n = 2) were given the next doses (2500, 3000, 3500 mg/kg) of *Salvia bucharica* orally. All the animals were continuously observed for general behavioral changes, symptoms of toxicity and mortality during 24 initially after every 15 min, then every 30 min, then after 1, 2, 4 and 12 h.

Phase 3

After 24 hours, based on the observations of phase 2, Two groups of mice (n = 2) were given the next higher doses (4000, 5000 mg/kg) of *Salvia bucharica* orally. Mice were observed for 24 h post-treatment for mortality, behavioral changes (restlessness, dullness, agitation, sedation) and signs of toxicity [23].

LD_{50} determination

Based on the findings of the acute toxicity study, the lowest dose that killed one mice and the maximum dose that had not killed any mice was recorded. The geographic mean of these doses gave the LD_{50} of the *Salvia bucharica* aqueous methanol extract [23].

Sub-acute Toxicity Test

Sub-acute toxicity study was performed as per the OECD guidelines 407 (OECD, 2008) with slight modifications. On the bases of findings of acute toxicity test,1/5th of the highest dose of acute toxicity study protocol of *Salvia bucharica* extract was selected and administered orally on daily basis for 14 days to a group of mice (n = 6). Control group of mice (n = 6) was received only vehicle for the same duration. All the experimental animals were observed after treatment daily for any abnormal clinical signs and mortality for 14 days. At the end of 14 day's observation period, body weight of mice was measured. The mice were anaesthetized, dissected and their blood samples were collected immediately through cardiac puncture with and without anticoagulant (EDTA), for hematological and biochemical studies respectively[24] . Heart, liver and kidneys of each mouse were individually weighed [25].

Determination of anti—oxidant activity

Free radical scavenging by use of DPPH (1,1—Diphenyl—2—picryl hydrazyl) radical

DPPH radical scavenging capacity of aqueous methanol extract *Salvia bucharica* was determined according to the method of Brand- Williams modified by Miliauskas [26] DPPH radicals have an absorption maximum at 515 nm, which disappears with reduction by an antioxidant compound. DPPH solution (6×10^{-5} M) in methanol was prepared, and 3 mL of this solution was mixed with 100 µL of each methanol solutions of *salvia bucharica* extracts and standard solution of Ascorbic acid. Sample and the standard Ascorbic acid

solution were incubated for 20 min at 37 °C in a water bath, and then decrease in absorbance at 515 nm was measured (AE). A blank sample containing100µL of methanol in the DPPH solution was prepared, and its absorbance was also measured (AB). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

$$(\% \text{ inhibition}) = [(AB-AE)/AB] \times 100$$

Where AB absorbance of the blank sample, and AE absorbance of the plant extract [27].

Phytochemical analysis of Salvia bucharica

Bioactive constituents in crude extract were analyzed through using RP UHPLC-MS by adopting the method of [28] . In brief, UHPLC of Agilent 1290 Infinity liquid chromatography system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source was used. Column used in this experiment was Agilent Zorbax Eclipse XDB-C18, narrow-bore 2.1×150 mm, 3.5 µm (P/N: 930990-902). The Column was kept at 25°C while 4°C was set for auto-sampler. Flow rate was maintained at 0.5ml/min and sample was injected as 1.0 µL. 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) were used as mobile phase. Sample was run for 25 min and an extra of 5 min were given. Full scan mass spectrometry analysis was conducted on m/z 100–1000 employing electro-spray ion source in negative fashion. Whereas, obtained results were processed with Agilent Mass Hunter Qualitative Analysis B.05.00 (Metabolomics-2017- 00004.m). Search Database: METLIN_AM_PCDL-Ne 170502.cdb, with parameters as: Match tolerance: 5 ppm, Positive Ions: +H, +Na, +NH4, Negative Ions: H. was used to identify the compounds.

Statistical analysis

Data obtained from all experiments in current study was expressed as means ± S.E.M. For estimating the statistical significance, data was analyzed using, one way or two way analysis of variance (ANOVA) followed by an appropriate posttest either Dunnet or Bonfferoni. All statistical analysis were performed and graphs drawn using GraphPad Prism version 5.0 and 6.0 for windows (GraphPad Software, San Diego, CA, USA). The results showing a probability of < 0.05 were considered as statistically significant.

Results

Anti-hyperlipidemic effect of Salvia bucharica against lipofundin induced dyslipidemia

Effect on lipid profile

Aqueous methanol extract of *S. bucharica* showed dose dependent antihyperlipedimc effect. Lipofundin administered to all group except normal control caused significant rise in lipid parameters. LIPO group at 8th day produced significant increase in TC (86.6 ± 4.40 mg/dL) and TG (118 ± 7.26 mg/dL) as compared to normal control (65 ± 2.8, 72.66 ± 4.33 mg/dL) with level of significance $P<0.001$. LIPO group at 23rd day also showed significant rise in serum total cholesterol (84.33 ± 4.25 mg/dL) and serum triglyceride (112 ± 6.24 mg/dL) in comparison of normal control with level of significance $p<0.001$ and $p<0.01$ respectively. Atorvastatin (10 mg/kg) and *S. bucharica* at dose of 500 mg/kg caused remarkable ($p<0.001$) reduction in TC and TG level and showed comparable results. Atorvastatin and *S. bucharica* reduced TC to 61 ± 4.94 mg/dL

and 62.66 ± 3.71 mg/dL respectively than LIPO 23rd day. Atorvastatin and *S. bucharica* reduced serum TG to 72.66 ± 4.33 mg/dL and 82.66 ± 5.81 mg/dL respectively than LIPO 23rd day. Both of these groups also showed significant reduction of LDL i.e 11.33 ± 2.02 mg/dL and 10.46 ± 1.84 mg/dL respectively than the LIPO 23rd day (32 ± 2 mg/dL) with level of significance $p<0.01$. SB also caused reduction in HDL and VLDL levels but the effect of extract on these parameters was not significant (Fig.1).

Effect on the atherogenic index (AI) and coronary risk index (CRI)

Aqueous methanol extract of *S. bucharica* showed dose dependent effect on AI and CRI. Atorvastatin (10 mg/kg) and aqueous methanol extract of *S. bucharica* at dose of 500 mg/kg showed significant reduction in AI to 0.73 ± 0.06 and 0.75 ± 0.05 respectively in comparison of LIPO 23rd day (1.63 ± 0.15) with level of significance $p< 0.01$. In case of CRI, Atorvastatin (10 mg/kg) and aqueous methanol extract of *S. bucharica at* dose of 500 mg/kg showed significant reduction in CRI to (1.73 ± 0.06 and 1.75 ± 0.05) than the LIPO 23rd day (2.30 ±0.46) with level of significance $p< 0.05$ (Fig.2).

Effect of Salvia bucharica as antihyperlipidemic agent against Fructose treated rats

Effect on lipid profile

Aqueous methanol extract of *Salvia bucharica* showed significant dose dependent antihyperlipedimc effect in fructose induce hyperlipidemia. Fructose group produced significant increase in total cholesterol (106.66 ± 6.76 mg/dL) and triglyceride (196.66 ± 7.26 mg/dL) as compared to normal group (71 ± 2.30, 91.66 ± 6.00 mg/dL) with level of significance $P<0.001$. The Fructose + SB 250 and Fructose + SB 500 showed significant reduction in total cholesterol to 91 ± 4.61 mg/dL and 82 ± 3.75 mg/dL respectively than Fructose group with level of significance $p<0.05$ and $P<0.001$ respectively. The Fructose + SB 250 and Fructose + SB 500 also showed significant reduction in triglycerides to 131.66 ± 5.36 mg/dL and 81 ± 6.24 mg/dL respectively than Fructose group with level of significance $p<0.05$ and $P<0.001$ respectively. Fructose + ATOR and Fructose + SB500 also caused significant reduction of VLDL level to 14.53 ± 0.86 mg/dL and 17 ± 0.57 mg/dL respectively in comparison of Fructose group (39.33 ± 1.45) with level of significance $p< 0.001$ and $p< 0.01$ respectively (Fig.3).

Effect on atherogenic index (AI) and coronary risk index (CRI)

Aqueous methanol extract of *Salvia bucharica* showed dose dependent effect on atherogenic index and coronary risk index in fructose induced hyperlipidemic rats. Atorvastatin (10 mg/kg) and aqueous methanol extract of *Salvia bucharica* at dose of 500 mg/kg showed significant reduction in AI to 0.93 ± 0.14 and 0.62 ± 0.08 respectively than Fructose treated group (1.43 ± 0.12) with level of significance $p< 0.01$ and $p<0.001$ respectively. In case of CRI, Atorvastatin (10 mg/kg) and aqueous methanol extract of *Salvia bucharica* at dose of 500 mg/kg showed significant reduction in CRI to 1.93 ± 0.14 and 1.62 ± 0.08 in comparison to Fructose group (2.43 ± 0.12) with level of significance $p<0.01$ and $p<0.001$ respectively (Fig.4).

Beneficial potential of Salvia bucharica against fructose induced vascular dysfunction

Salvia bucharica aqueous methanol extract showed effective improvement in endothelial dysfunction induced by fructose in dose dependent manner. In fructose treated rats, fructose caused vascular endothelium damage as shown by reduction in endothelium dependent vasorelaxation of aorta rings by acetylcholine after phenylephrine induced contraction. While Atorvastatin group and *Salvia bucharica* 500 mg/kg group protected the vascular endothelium damage and significant vasorelaxation was observed by acetylcholine as compared to fructose control group in dose dependent manner with level of significance $p<0.001$. *Salvia bucharica* 250 mg/kg group do not show remarkable relaxation at lower doses but at dose of 10^{-5} mol/L of acetylcholine, it showed significance relaxation with p value of 0.01 (Fig.5).

Effect of Salvia bucharica on liver histopathology

The histopathology of liver section of normal rats showed normal cell structure while the liver section of fructose fed rats showed the presence of destructive alteration and extensive fatty changes characterized by focal necrosis and damaged sinusoidal spaces and narrowing of central vein. The liver section of rats treated with *Salvia bucharica* at dose of 250 mg/kg showed less effect on necrosis, central vein damage and fatty changes. While *Salvia bucharica* at dose of 500 mg/kg and atorvastatin at dose of 10 mg/kg showed hepatoprotective activity in rats and histopathological changes significantly attenuated by these groups (Fig.6)

Toxicity profile

Acute toxicity study and determination of LD_{50}

At graded doses, from 250 mg/kg up to 5000 mg/kg body weight of aqueous Methanol extract of *Salvia bucharica* did not show any sign of adverse reactions and no changes in animals up to 24 h after the administration of the extract to mice. LD_{50} value of aqueous Methanol extract of *Salvia bucharica* was found to be greater than 5000 mg/kg.

Sub-acute Toxicity Test

No significant difference in body weight, and weight of liver, heart and kidney was observed between the control and extract-administered groups after 14 days in sub-acute study. In addition, no mortality was recorded throughout the period of observation. There was the decrease in level of serum ALT (U/L) and AST (U/L) but the creatinine (mg/dL) and urea (mg/dL) levels were comparable to normal control (Table 1).

Parameters	Normal	Treated
Body weight (g)	33.75 ± 3.25	32 ± 4
Heart weight (g)	0.16 ± 0.02	0.21 ± 0.02
Liver weight (g)	1.78 ± 0.15	1.64 ± 0.03
Kidney weight(g)	0.47 ± 0.07	0.54 ± 0.03
Urea (mg/dL)	22 ± 02	14.5 ± 0.5
Creatinine(mg/dL)	0.7 ± 0.1	0.6 ± 0.1
ALT (U/L)	175 ± 05	79** ± 10
AST (U/L)	157.5 ± 2.5	116.5 ± 9.5

Table 1

Subacute toxicity study of *Salvia bucharica*

Values are expressed as mean ± SEM. Statistical analysis was performed by using 2 way ANOVA. ** = $p<0.01$ (compared with normal group)

S.NO	RT	m/z	Height	Proposed compound	Formula	Molecular mass	Volume
1	0.624	317.0605	9419	Pyriftalid	C15 H14 N2 O4 S	318.0685	41722
2	0.631	215.0353	178161	Isobergaptene	C12 H8 O4	216.0415	601787
3	0.635	195.0528	41041	1,9-Dimethyluric acid	C7 H8 N4 O3	196.0601	140304
4	0.636	259.0154	6965	(E)-C-HDMAPP	C6 H14 O7 P2	260.0213	27630
5	0.637	165.0418	24523	1-Methylxanthine	C6 H6 N4 O2	166.049	80893
6	0.637	539.1489	13865	N,N'-Bis(g-glutamyl)-3,3'-(1,2-propylenedithio)dialanine	C19 H32 N4 O10 S2	540.1564	76070
7	0.642	353.0956	8684	ST638	C19 H18 N2 O3 S	354.1019	68786
8	0.644	135.0306	19328	Hypoxanthine	C5 H4 N4 O	136.038	66414
9	0.651	179.061	64093	1,10-Phenanthroline	C12 H8 N2	180.0683	310019
10	0.664	162.0784	25426	6-Dimethylaminopurine	C7 H9 N5	163.0851	135796
11	0.664	191.055	7493	BEC	C5 H12 B N O4 S	192.0627	31962
12	0.665	387.1209	5761	Abu-TyrMe-OH	C19 H20 N2 O7	388.1285	40713
13	0.669	397.1204	5093	Cys Asn Tyr	C16 H22 N4 O6 S	398.126	21530
14	0.672	209.0681	15863	1,3,7-Trimethyluric acid	C8 H10 N4 O3	210.0747	70857
15	0.673	105.0194	12779	Glyceric acid	C3 H6 O4	106.0267	55835
16	0.681	339.101	49686	Arteglasin A	C17 H20 O5	304.1328	223744
17	0.683	125.0011	11752	Dimethyl phosphate	C2 H7 O4 P	126.0085	55970
18	0.69	281.0925	15635	Saphenic acid methyl	C16	282.0988	60085

				ester	H14 N2 O3		
19	0.726	133.0153	99638	3,3-Dimethyl-1,2-dithiolane	C5 H10 S2	134.0227	537518
20	0.773	253.0955	5190	Dyphylline	C10 H14 N4 O4	254.1024	29745
21	0.918	128.0356	76089	N-Acryloylglycine	C5 H7 N O3	129.043	304158
22	0.918	253.0939	34414	Dyphylline	C10 H14 N4 O4	254.1012	183670
23	0.947	306.0607	7035	Narciclasine	C14 H13 N O7	307.0681	34367
24	1.088	117.0193	25487	Erythrono-1,4-lactone	C4 H6 O4	118.0266	123693
25	1.174	161.0461	6892	Levoglucosan	C6 H10 O5	162.0537	39568
26	8.088	361.0983	15790	Asp Asp Asn	C12 H18 N4 O9	362.1058	92942
27	8.257	225.1139	8737	Pyridine-2-azo-p-dimethylaniline	C13 H14 N4	226.1213	69143
28	8.341	186.1137	13375	KAPA	C9 H17 N O3	187.121	172528
29	9.007	265.0599	13514	7-Acetoxy-7,8-dihydroiodovulone I	C23 H33 I O6	532.133	161454
30	9.041	393.0876	17206	Met-His-OH	C16 H18 N4 O6 S	394.0952	110163
31	9.528	187.098	21206	Nonic Acid	C9 H16 O4	188.1053	157743
32	9.577	269.1042	18700	Idebenone Metabolite (Benzenebutanoic acid, 2,5-dihydroxy-3,4-dimethoxy-6-methyl-)	C13 H18 O6	270.1115	213324
33	10.209	375.1227	5742	5-O-Methylchamanetin	C23 H20 O5	376.1301	34356
34	10.437	221.1193	5279	(6S)-dehydrovomifoliol	C13 H18 O3	222.1265	37628
35	10.964	809.4353	7486	Lucyoside J	C42 H66 O15	810.443	63612

36	11.037	327.2194	7061	11-hydroperoxy-12,13-epoxy-9-octadecenoic acid	C18 H32 O5	328.2266	60013
37	11.422	1087.5373	8231	Araloside C	C53 H84 O23	1088.5447	78353
38	11.424	566.2674	12848	Calendasaponin C	C54 H86 O25	1134.5501	123199
39	11.431	329.2336	13903	5,8,12-trihydroxy-9-octadecenoic acid	C18 H34 O5	330.241	108743
40	12.325	267.126	5617	DMABA-d6 NHS ester	C13 H8 D6 N2 O4	268.1334	36836
41	12.877	647.3939	5339	3alpha-O-trans-Feruloyl-2alpha-hydroxy-12-ursen-28-oic acid	C40 H56 O7	648.4008	47391
42	13.145	293.1786	48842	Tetradecyl sulfate	C14 H30 O4 S	294.186	395783
43	13.22	207.1398	15514	(5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol	C13 H20 O2	208.1471	190675
44	13.43	293.1778	6925	Tetradecyl sulfate	C14 H30 O4 S	294.185	51767
45	13.459	309.1706	35949	methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate	C17 H26 O5	310.178	350994
46	14.026	293.1803	13269	Tetradecyl sulfate	C14 H30 O4 S	294.1876	108467
47	14.183	194.0827	5814	O-Benzyl-L-Serine	C10 H13 N O3	195.09	41932
48	16.628	555.2942	40000	PI(P-16:0/0:0)	C25 H49 O11 P	556.301	631571
49	19.329	381.2357	5805	STS-135	C24 H31 F N2 O	382.2428	69167

Table 2

List of compounds identified by LC-MS analysis of DB-Cr

Assessment of anti-oxidant activity

Free radical scavenging activity of Salvia bucharica against DPPH (1,1-Diphenyl-2-picryl hydrazyl)

Aqueous methanol extract of *Salvia bucharica* showed remarkable concentration dependent DPPH scavenging activity. The results were more profound than standard (Ascorbic acid) at almost all the concentration except 100 ug/mL. At 100 ug/mL the plant showed comparable result with ascorbic acid having values of 98.62 ± 0.01 and 98.61 ± 0.01 respectively.

Phytochemical analysis of aqueous methanol extract of Salvia bucharica using LC-MS

LC-MS analysis of aqueous methanol extract of *Salvia bucharica* revealed the presence of several compounds of which 49 were identified. The major compounds found in chemical analysis of *Salvia bucharica* were belonging to flavonoid, phenol, alkaloids and tannins class. As shown in figure Methyl xanthine, glyceric acid, nonic acid, octadecenoic acid, araloside and tri methyluric acid were commonly identified in aqueous methanol extract of *Salvia bucharica.*

Discussion

Lipofundin is a fat emulsion which is used as an energy source in parentral nutrition, but experimental studies evidenced the development of atherosclerotic lesion, lipid peroxidation and oxidative stress in rabbits [29, 30]. In the present investigation, it was observed that lipofundin caused significant increase in total cholesterol and triglyceride level (86.6 ± 4.40 and 118.33 ± 7.26 mg/dL respectively) in rabbits after intravenous administration for 8 days. In order to assess the possible effect of *S. buchrica* on lipid profile, aqueous methanol extract in doses of 250 and 500 mg/kg was administered in lipofundin treated hyperlipidemic rabbits. 500 mg/kg dose of extract produced significant ($p<0.001$) reduction in level of triglycerides, total cholesterol and LDL as compared to lipofundin treated negative control group (Table 3.1, Fig.3.1). It is well known that elevated total cholesterol level plays major role in development of coronary artery diseases [31] and triglycerides play key role in regulation of lipoprotein interaction thus maintaining metabolism of lipids [32]; Hence, attenuation of these factors by extract may contribute to the possible antihyperlipidemic potential of *Salvia bucharica* which is in agreement with the previous findings [33]. Formerly, it has been established on the basis of clinical and animal studies that chronic administration of fructose contributes to the development of various abnormalities such as dyslipidemia, hypertension, insulin resistance, oxidative stress and decreased glucose utilization [34]. Present study demonstrated that administration of fructose (25% w/v) in drinking water for 28 days provoked marked increase ($p<0.001$) in the level of TC, TG and VLDL, which is an indication of development of hyperlipidemia. Further, the results showed that fructose treatment also caused endothelium dysfunction in aorta of treated rats [35].

Subsequently administration of aqueous methanol extract of SB remarkably decreased the levels of lipid profile in serum of treated animals indicating antihyperlipidemic potential, however, the HDL-C level remains unaffected. This valuable effect might be related to enhanced activity of endothelium bound lipoprotein lipase which is involved in hydrolysis of triglycerides to fatty acids [36] or by inhibition of pancreatic lipase as previously, it has been reported that high fructose diet increases serum pancreatic lipase activity, which is involved in cleavage of dietary triglycerides and thus leads to digestion of lipids [16]. Therefore, inhibition of pancreatic lipase can cause reduction in fat absorption hence, cholesterol level in blood could be decreased.

LC-MS analysis of *S. bucharica* evident the presence of arasolide, derivative of oleanolic acid, Somova and its colleagues [37] had established the antihyperlipidemic effect of olenolic acid therefore, it could be assumed that lipid lowering effect offered by *S. bucharica* may at least be partially linked to the presence of araloside. Further, Lipoic acid derivative, 3,3-Dimethyl-1,2-dithiolane, has been identified as an active constituent of *S. bucharica* and former reports [38] evinced the noticeable cholesterol lowering effect exerted by lipoic acid in rabbits, in addition dithiolane also improves the insulin sensitivity and reduced serum triglyceride level [39]. Therefore, it could be deduced that *S. bucharica* mediated antihyperlipidemic effect observed in present study may be due to the presence of these active principles. Cholestrol lowering effect exerted by *S. bucharica* could be attributed to the presence of saponins and methylxanthines as previous studies have demonstrated the hypocholestrolemic and lipid lowering properties of saponins [40] and methylxanthines [41].

Literature has shown that terpenes have potential to decrease the activity of HMG-COA reductase thus limiting the synthesis of cholesterol [42], as Salvadiol; a terpenoid is active constituent of *S. bucharica* [7]. Hence, the aforementioned findings suggest that antihyperlipidemic effect of extract might be linked with the presence of terpenes.

To provide the further basis for antihyperlipidemic effect of SB, vascular reactivity to various concentrations of acetylcholine (10^{-9}—10^{-4} mol/L) was studied in rat thoracic aorta. Aqueous methanol extract obtained from aerial parts of *Salvia bucharica* potentially prevented the loss of endothelium functional integrity that was significantly compromised in fructose treated rat thoracic aorta. As endothelial dysfunction endorses dyslipidemia, atherosclerosis and hypertension, so, in the light of present findings, it can therefore be deduced, that *Salvia bucharica* produced antihperlipidemic effect which might be attributed to multiple possible pathways including anticipation of endothelial dysfunction.

Additionally, it is popularly known that chronic administration of fructose culminates in fat deposition in hepatocytes and increased liver weight [43]. Hence, to evaluate the possible protective effect of *Salvia bucharica*, histopathological studies of liver tissues were performed. The result presented less alteration in hepatic vein and sinosidal spaces were examined in extract treated groups, when compared to fructose treated group reflecting protective role of *Salvia bucharica* on hepatocytes. Moreover, observation of hepatocytes clearly demonstrated that size of hepatocytes and central vein in *Salvia bucharica* treated rats was equivalent to normal rats and was also comparable to standard treated group.

It is evident from recorded results that atherogenic index (AI) and coronary risk index (CRI) was significantly ameliorated in both models (lipofundin and fructose induced hyperlipidemia) by treatment of aqueous methanol extract of *Salvia bucharica*. Atherogenic index being an important indicator of coronary risk is of great consideration [44].

Reactive oxygen species are involved in oxidation of low density lipoprotein, major contributor in endothelial dysfunction [5].The significant finding of present work is the antioxidant effect of *S. bucharica.* The results of DPPH radical scavenging assay predicted that *S. bucharica* showed more pronounced effect as compared to ascorbic acid (Fig 7). Hence, it could be proposed from aforementioned findings that antihyperlipidemic effect of *Salvia bucharica* might be attributed to the presence of terpenes and free radical scavenging potential of plant.

To assess safety profile aqueous methanol extract of *S. bucharica* was administered in mice, findings of acute toxicity study evinced that LD_{50} of extract is above 5000 mg/kg, which makes the extract a candidate of class 5 that is lowest toxic. Further results of subacute toxicity study showed no toxic effects thus referring the extract to be quite probably safe. Aqueous methanol extract obtained from aerial parts of *Salvia bucharica* M. Pop possesses antihyperlipidemic activity in the experimental animals at the dose of 500 mg/kg. It significantly reduced lipid profile ($p<0.05$) in the lipofundin and fructose induced hyperlipidemia. Furthermore, in fructose fed rats, studies on the isolated aorta showed the vascular protective activity and histopathological examination displayed that this extract has hepatoprotective potential. Toxicity studies demonstrated that this plant extract is safer upto 5000 mg/kg and no sign of morbidity shown. However the results are too much encouraging but further research work is required for scientific validation and to isolate the compound for further studies.

Conclusion

It is concluded that *Salvia bucharica* has shown dose dependent antihyperlipidemic activity .In addition, Studies performed on the isolated thoracic aorta and histolopathological studies on liver demonstrated that *Salvia bucharica* has endothelial dependent vasoprotective and hepatoprotective activity respectively as compared to fructose fed rats. Furthermore, the plant has been found to be safe in toxicity studies.

Abbreviations

Total cholesterol (TC), Triglyceride (TG), high density lipoprotein cholesterol (HDL-C) VLDL l, Low density lipoprotein (LDL-C), Atherogenic index (AI), Coronary risk index (CRI).

Declaration

Ethics approval

The experimental protocols were approved from Institutional Animal Ethics Committee, College of Pharmacy, University of Sargodha (Approval No. 43A24 IEC UOS).

Informed Consent

Not Applicable

Funding

Not Applicable

Authors’ contributions

Alamgeer and Hafiz Muhammad Irfan designed this study and provide the necessary facilities for experiments. Muhammad Ishfaq Ahmad: Perform the experiments, Hira Asif Collect the results and write the article. Nasser Hadal Alotaibi, Khalid Saad Alharbi, Syed Nasir Abbas Bukhari performed phytochemical studies.

and all other authors wrote and review the manuscript:

Author details

Not Applicable

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

The data supporting findings of this study are available from the corresponding author upon request.

Consent for publication

All of authors consent to publication of this study in Journal of Chinese Medicine.

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Figures

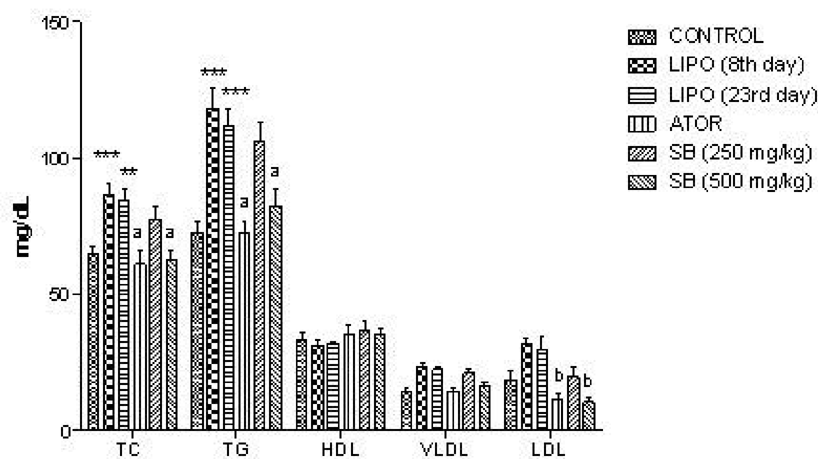

Figure 1

Attenuation in lipid profile by S. bucharica aqueous methanol extract. Values are represented as mean ± SEM. Statistical analysis was performed using 2 way ANOVA. *=p<0.05, **=p<0.01, ***=p<0.001(when compared with CONTROL). a=p<0.001, b=p<0.01 (compared with lipofundin 23rd day control). LIPO= Lipofundin group, ATOR= atorvastatin treated group, SB= Salvia bucharica

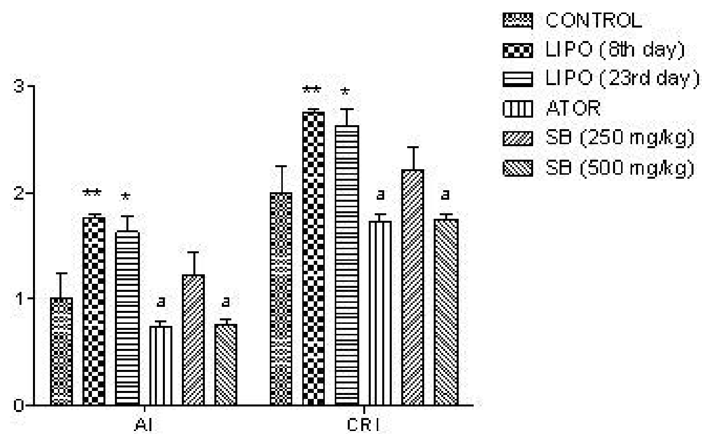

Figure 2

Attenuation in the atherogenic index (AI) and coronary risk index (CRI) by S. bucharica aqueous methanol extract. Values are expressed as mean ± SEM. Where, *=p<0.05, **=p<0.01 (compared with CONTROL). While, a=p<0.001 (compared with lipofundin 23rd day control). LIPO= Lipofundin group, ATOR= atorvastatin treated group, SB= Salvia bucharica

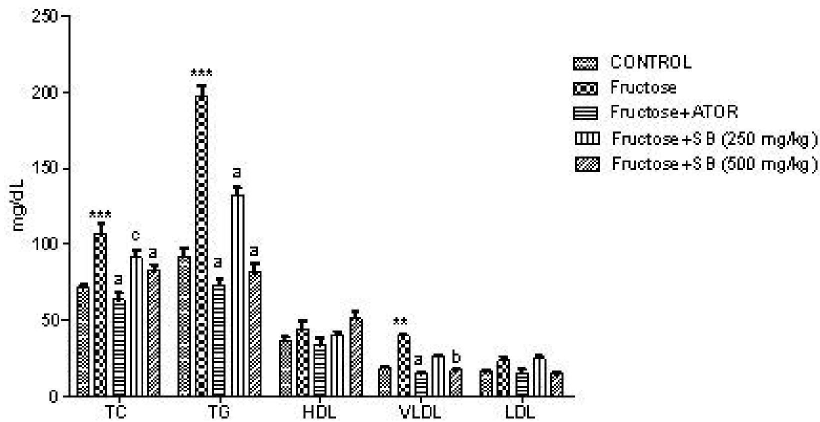

Figure 3

SB mediated changes in serum lipid profile in fructose induced hyperlipidemia. Data is depicted as mean ± SEM. Statistical analysis was performed by using two way ANOVA. **=p<0.01,***=p<0.001 (compared with normal control). a =p<0.001, b=p<0.01, c=p<0.05 (compared with fructose control).

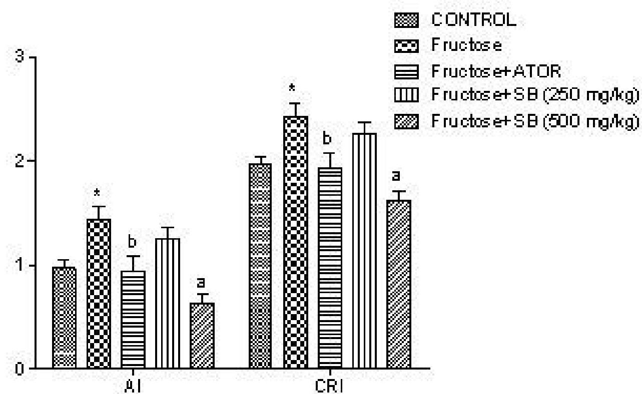

Figure 4

Attenuation in the atherogenic index (AI) and coronary risk index (CRI) by Salvia bucharica. Values are stated as mean ± SEM (n = 5). Where, *=p<0.05 (compared with normal control) and a =p<0.001, b =p<0.01 (compared with fructose control).

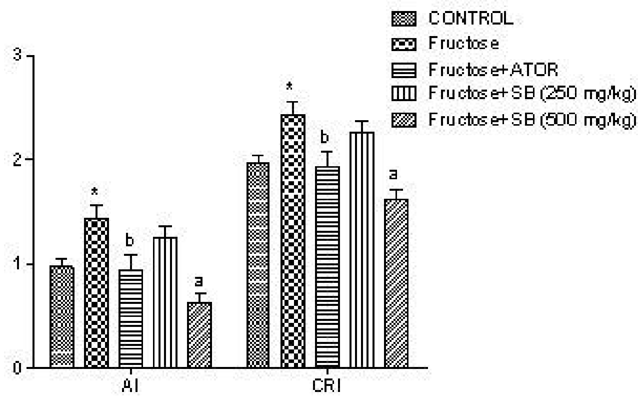

Figure 5

Protective effect of Salvia bucharica in vascular dysfunction induced by fructose. Values are depicted as mean ± SEM (n=5). Where *** =p<0.001 (compared with normal), while a=p<0.001, b=p<0.01, c=p<0.01 (compared with negative control). AI=Atherogenic index CRI=coronary risk index

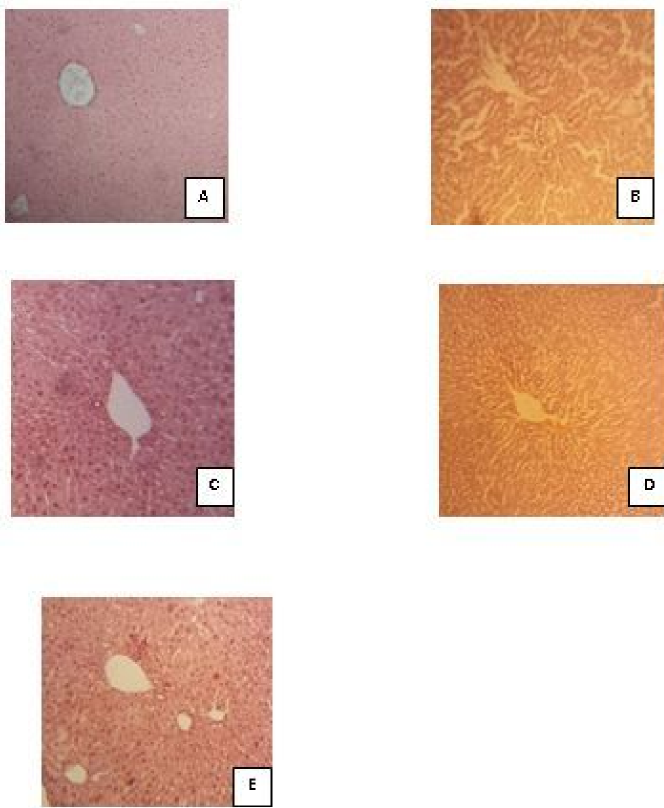

Figure 6

Effect of Salvia bucharica on liver histopathology. A: normal control group; B: fructose control group; C: standard group; D: SB 250 mg; E: SB 500 mg

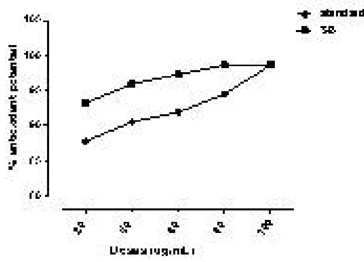

Figure 7

Free radical scavenging activity of Salvia bucharica against DPPH

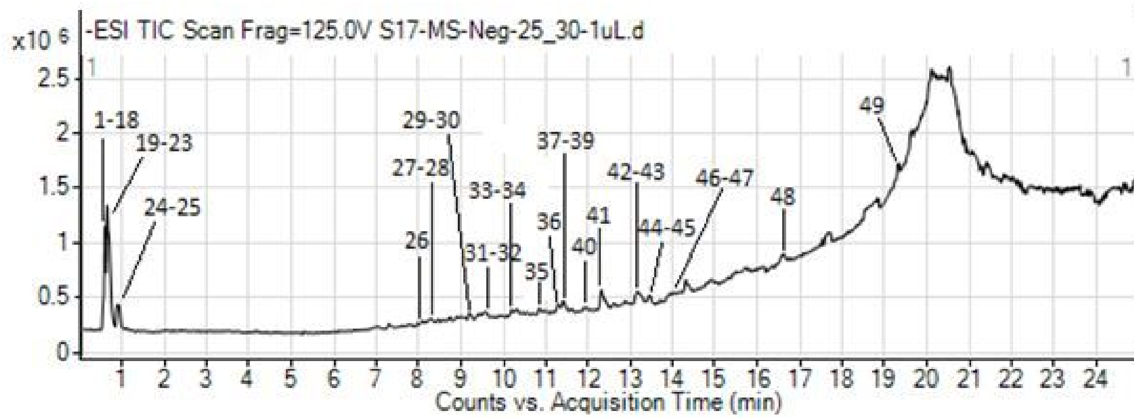

Figure 8

Illustrative figure of LC-MS analysis of Salvia Buchrica

Supplementary Files

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