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Antihyperlipidemic effect of *Berberis orthobotrys* in hyperlipidemic animal models

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Abstract

The present study was undertaken to evaluate antihyperlipidemic effect of aqueous methanolic extract of *Berberis orthobotrys* in experimentally induced hyperlipidemic animal models. Atorvastatin was used as standard drug. The crude extract (50 and 100 mg/kg) significantly prevented the increase in low density lipoprotein (LDL), very low density lipoprotein (VLDL), total cholesterol (TC), triglyceride (TG), atherogenic index and coronary risk index in high fat diet, cholesterol, fructose and olive oil induced hyperlipidemic rat models whereas in ethanol treated mice, the extract reduced VLDL, TG and TC levels. The high density lipoprotein (HDL) level was significantly increased in high fat diet, olive oil and cholesterol induced hyperlipidemic rats. The reduction in serum level of above parameters with 100 mg/kg dose of extract was comparable with atorvastatin. It is concluded that *B. orthobotrys* has atheroprotective and lipid lowering potential.

Introduction

Atherosclerosis is a complex fibro fatty and inflammatory disease characterized by proliferative chemotaxis of macrophages, T-lymphocytes and platelets, causing damage to coronary smooth muscles (Schwartz et al., 1993; Jain et al., 2007). Syndrome X or hyperlipidemia is an elevation of cholesterol, cholesterol ester, phospholipids and triglyceride (TG) (Mokdad et al., 2003; Gou et al., 2007). Derangement in lipid metabolism and elevation of plasma lipoprotein level are the leading cause of type II diabetes mellitus and coronary heart disease particularly atherosclerosis (Carr and Brunzell, 2004). Cholesterol (0.3–0.5 g/day) is absorbed from diet, remaining 70% is synthesize *de novo* in human (Goodman and Gilman, 2001).

Therapeutically HMG-CoA reductase inhibitors (statins) are consider most effective for treating hyperlipide -mia but associated risk such as myopathies limits its use. Moreover, in homozygous hypercholesterimic patients statins are less useful because of less prevalence of LDL- receptors (Chapman et al., 2004). World health organization (WHO) about 25 years ago recognize herbal medicine and start exploring traditional medicines that are being used in developing countries from thousands of years (Akerele et al.,1991).

It's now become a need of time to biologically evaluate the traditional medicines of Pakistan. Berberidaceae a well-known family of several medicinally active compounds indigenous to subcontinent (Pakistan, India, China and Afghanistan). *Berberis orthobotrys*is a yellow brown shrub was reported for its antihypertensive activity. In Pakistan's northern areas local people use this plant commonly for the treatment of hypertension. During a study on hypertension this plant reveals some hypolipidemic effect. Since no pharmacological knowledge is available for this plant, and to conform its activity a research study is conducted to evaluate its possible antihyperlipidemic activity (Alamgeer et al., 2013).

Methods and Materials

Chemicals and drugs

All chemicals were of analytical grade and obtained locally. Cholesterol, HDL, LDL, TG kits were procured from HUMAN pharmaceuticals, Pakistan.

Animals

Sprague Dawley rats weighing 100-300 g and mice weighing 20–30 g were used for the study. All the animals were housed in controlled environment (23-25° C) and received human care according to the requirements of National Institute of Health (NIH) guidelines for the care and use of laboratory animals. All the study protocols were approved by the institutional animal ethics committee Faculty of Pharmacy, University of Sargodha (Approval No. 20-A12 IEC UOS). Experiments performed complied with the rulings of National Research Council (NRC). The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water *ad libitum*.

Plant material

B. orthobotrys roots extract was used for the study, collected from District Gilgit, Pakistan during the month of June 2011 and was identified and authenticated by Dr. Shair Wali, Karakorum International University Gilgit Baltistan, Pakistan. A voucher (BO-15-12) has been deposited in the herbarium, Faculty of Pharmacy, University of Sargodha for future reference.

Preparation of extract

B. orthobotrys aqueous methanolic extract (70:30) was prepared using cold maceration process. Plant material (2 kg) was grounded and soaked in 5 L of methanol-aqueous mixture (70:30) for 72 hours at room temperature. After three days of intermittent shaking, filtrate was obtained and evaporated under reduced pressure using rotary evaporator. Crude extract was then air dried to obtain solid mass giving a yield of 15% (Alamgeer et al., 2013).

Determination of antihyperlipidemic effect of *B. orthobotrys* in hyperlipidemic models

Effect of B. orthobotrys on high fat diet fed hyperlipidemic rats

In order to induce hyperlipidemia, method reported by (Santosh Kumar et al., 2012) was followed. HFD is prepared by homogenously mixing banaspati ghee (dalda) and coconut oil in the ration of 3:2 w/w. The rats were divided into five groups of 6 rats each and received the following diets with or without treatment for 56 days orally.

Group 1 served as negative control (received distill water). Group 2 served as positive control and received high fat diet. Moreover, Group 3 was considered as standard group (atorvastatin 80 mg/kg/day + high fat diet) and treated groups were Group 4 (HFD + extract 50 mg/kg p.o body weight) and Group 5 (HFD + Crude extract 100 mg/kg p.o body weight). At the end of the experiment the rats were fasted overnight, blood was drawn by cardiac puncture under mild anesthesia. Serum was separated and stored in refrigerator until assay.

Effect of B. orthobotrys on olive oil fed hyperlipidemic rats

Hypertriglyceridemia and hypercholesterolemia were induced in five groups of 6 rats each by administering olive oil 30 min after giving *B. orthobotrys* extract. Group 1 received distilled water (negative control), Group 2 was given olive oil only (positive control), Group 3 served as standard control (atorvastatin 50 mg/kg/day), Group 4 and 5 were extract treated groups (Crude extract 50 mg/kg/day p.o + olive oil 5 mL /kg p.o) and (Crude extract 100 mg/kg/day p.o + olive oil 5 mL /kg p.o). Olive oil was administered 30 min after extract treatment. Blood samples were withdrawn by cardiac puncture after 4 hours of olive oil treatment. Serum was separated and stored in refrigerator until assay.

Effect of B. orthobotrys on fructose fed hyperlipidemic rats

Acute administration of fructose leads to sharp increase in serum TG and cholesterol level in rats as describe by (Srikanth et al., 2009). Rats were divided into five groups of 6 rats each as describe above. Animals in Group 1 considered negative control (normal saline). Animals of Group 2 were positive control (25% d-fructose in drinking water daily for 21 days). Group 3 animals were standard control (atorvastatin 80 mg/kg/day + 25% fructose) treated animals were Group 4 (Crude extract 50 mg/kg/day + 25% d-fructose) and Group 5 (crude extract 100 mg/kg/day + 25% d-fructose). Fructose solution was administered in drinking water for 21 days. At 21st day, 2 hours after the final treatment, animals were anesthetized and blood from each animal was withdrawn from cardiac puncture. Serum obtained by immediate centrifugation, refrigerated and used for biochemical analysis.

Effect of B. orthobotrys on cholesterol fed hyperlipidemic rats

Rats were randomly divided into five groups of 6 rats each. Group 1 was maintained as negative control (normal saline), Group 2 positive control (cholesterol 500 mg/kg), Group 3 was standard control (atorvastatin 30 mg/kg + cholesterol 500 mg/kg), Group 4 and Group 5 treated groups (crude extract 50 mg/kg and 100 mg/kg + cholesterol 500 mg/kg) respectively. Cholesterol 500 mg/kg/day was administered in olive oil for 30 days. After 30 days of treatment the rats were fasted overnight and sacrificed, blood sample was collected and

centrifuged for collection of serum, later to be used for biochemical analysis.

Effect of B. orthobotrys on ethanol-induced hyperlipidemic mice

Hypertriglyceridemia was introduced in four groups of 6 mice each by administering 10% and 26% ethanol (Silwa et al., 2001). Group 1 and 2 are control groups (normal saline) and (ethanol 26%). Group 3 and 4 treated with ethanol 26% + Crude extract (50 mg/kg/day, 100 mg/kg/day) given 30 min after ethanol 26% for 4 days. 10% ethanol was added to the drinking water of ethanol and *B. orthobotrys* extract treated groups, whereas the Group 1 only received water. Blood samples were collected from cardiac puncture of animals and total cholesterol (TC) and TG level is determined using commercially available kits (Ferreira et al., 2013). Measurement of serum lipid profile- total cholesterol, TG, high density lipoprotein (HDL) were estimated by using standard kits of HUMAN Pharmaceuticals. The low density lipoprotein (LDL), very low density lipoprotein (VLDL), atherogenic index (AI) and coronary risk index (CRI) were calculated by using the following formulas as given below (Friedwald et al., 1972; Abbot et al., 1988; Alladi et al., 1989).

VLDL=TG/5…… (1)

LDL=TC-(HDL+VLDL)…….. (II)

Al was calculated by the formula given below:

AI=TC-HDL/HDL…… (III)

CRI was calculated by formula (Arun et al., 2012).

CRI= TC/HDL……..... (IV)

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM) and statistical analysis was carried out by using two-way ANOVA using GraphPad Prism 5.0. Differences were considered significant at $p<0.05$.

Results

In control group the values of LDL, HDL, VLDL, TC, TG, AI and CRI were increased. Extract at the dose of

Table I

Effect of crude extract of *B. orthobotrys* (50, 100 mg/kg) on serum lipids in high fat diet-induce hyperlipidemia

No.	Groups	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	AI	CRI
I	Normal control	86.7 ± 1.7	65.2 ± 0.4	25.4 ± 0.5	45.5 ± 1.8	13.0 ± 0.1	2.3 ± 0.1	3.3 ± 0.1
II	High fat diet	266.9 ± .3[a]	246.2 ± 8.1[a]	14.4 ± 0.6[a]	212.2 ± 1.9[a]	49.2 ± 1.6[a]	17.5 ± 0.8[a]	18.5 ± 0.8[a]
III	Atorvastatin + High fat diet	76.8 ± 0.3[a]	56 ± 0.7[a]	37 ± 0.8[a]	28.4 ± 0.5[a]	11.2 ± 0.0[a]	1 ± 0.0[a]	2.0 ± 0.0[a]
IV	Extract (50 mg/kg) + High fat diet	144.2 ± 1.1[a]	14.7± 0.4[a]	23 ± 0.9[ns]	118.0 ± 0.9[a]	2.9 ± 0.0[a]	5.2 ± 0.2[a]	6.2 ± 0.2[a]
V	Extract (100 mg/kg) + High fat diet	155.7± 1.2[a]	26.2 ±1.1[a]	18.7± 0.8[**]	131.0 ± 2.0[a]	5.2 ± 0.2[a]	7.3 ± 0.4[a]	8.3 ± 0.40[a]

Results are expressed as means ± SEM; n = 6; Key: where, [ns]= Non-significant and [a]p<0.001, highly significant vs. normal control

Table II

Effect of crude extract of *B. orthobotrys*

No.	Groups	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	AI	CRI
I	Normal control	68.0 ± 0.4	58.2 ± 0.4	41.7 ± 0.2	42.5 ± 0.2	11.6 ± 0.0	0.6 ± 0.0	1.5 ± 0.0
II	Olive oil	99.7 ± 0.4[a]	100.0 ± 0.0[a]	44.2 ± 0.9[ns]	66.5 ± 1.1[a]	20.1 ± 0.9[a]	1.2 ± 0.0[ns]	2.2 ± 0.1[ns]
III	Atorvastatin + Olive oil	52.0 ± 0.4[a]	59.6 ± 0.4[a]	60.0 ± 1.5[a]	43.7 ± 0.7[a]	11.9 ± 0.1[a]	0.0 ± 0.0[ns]	0.82 ± 0.0[ns]
IV	Extract (50 mg/kg) + Olive oil	54.0 ± 2.7[a]	42.5 ± 0.6[a]	55.5 ± 0.9[a]	50.7 ± 0.8[a]	8.6 ± 0.1[a]	0.1 ± 0.9[ns]	0.9 ± 0.0[ns]
V	Extract (100 mg/kg) + Olive oil	66.2 ± 0.6[a]	34.2 ± 1.2[a]	50.5 ± 0.9[a]	57.5 ± 0.9[a]	6.8 ± 0.2[a]	0.2 ± 0.0[ns]	1.2 ± 0.8[ns]

Results are expressed as means ± SEM; n = 6; Key: where, [ns]= Non-significant and [a]p<0.001, highly significant vs. normal control

Table III

Effect of crude extract of *B. orthobotrys* (50, 100 mg/kg) on serum lipids in fructose-induce hyperlipidemia

No.	Groups	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	AI	CRI
1	Normal control	83.4 ± 1.0	75.0 ± 0.91	32.0 ± 1.5	36.3 ± 2.7	15.0 ± 0.1	1.6 ± 0.1	2.5 ± 0.0
II	Fructose	111.5 ± 4.5^{c}	113.5 ± 5.7^{c}	19.5 ± 0.6^{b}	41.3 ± 3.5^{ns}	22.7 ± 1.1^{ns}	10.7 ± 7.6^{a}	4.3 ± 0.3^{ns}
III	Atorvastatin + Fructose	67.7 ± 0.2^{c}	77.2 ± 0.8^{c}	60.7 ± 0.8^{b}	38.5 ± 1.0^{ns}	15.4 ± 0.1^{ns}	0.1 ± 0.0^{b}	1.06 ± 0.0^{ns}
IV	Extract (50 mg/kg) + Fructose	56.5 ± 2.7^{c}	69.2 ± 2.0^{c}	23.7 ± 0.6^{ns}	18.9 ± 2.1^{ns}	13.8 ± 0.4^{ns}	1.3 ± 0.0^{a}	2.3 ± 0.0^{ns}
V	Extract (100 mg/kg) + Fructose	73.7 ± 1.1^{c}	85.0 ± 1.5^{c}	20.8 ± 0.4^{ns}	35.8 ± 1.3^{c}	17.0 ± 0.3^{a}	2.4 ± 0.1^{a}	3.5 ± 0.1^{ns}

Results are expressed as means ± SEM; n = 6; Key: where, ^{ns}= Non-significant; ^{a}p<0.05 (significant), ^{b}p<0.01 and ^{c}p<0.001 (highly significant) vs. normal control

Table IV

Effect of crude extract of *B. orthobotrys* (50, 100 mg/kg) on serum lipids in cholesterol-induce hyperlipidemia

No.	Groups	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	AI	CRI
1	Normal control	40.2 ± 0.8	48.7 ± 1.3	18.0 ± 0.4	17.5 ± 0.2	12.6 ± 1.6	1.2 ± 1.0	2.2 ± 0.0
II	Cholesterol	188.7 ± 0.4^{c}	111.7 ± 0.6^{c}	34.0 ± 0.4^{c}	152.0 ± 0.4^{c}	22.3 ± 0.1^{c}	4.5 ± 0.5^{b}	5.5 ± 0.3^{b}
III	Atorvastatin + Cholesterol	34.0 ± 1.0^{c}	32.7 ± 0.4^{c}	12.0 ± 0.7^{c}	14.7 ± 0.4^{c}	6.5 ± 0.9^{c}	1.8 ± 0.2^{a}	2.5 ± 0.1^{a}
IV	Extract (50 mg/kg) + Cholesterol	110.2 ± 1.0^{c}	88.7 ± 1.9^{c}	32.0 ± 0.4^{c}	55.7 ± 0.2^{c}	17.7 ± 0.3^{ns}	2.4 ± 2.0^{ns}	3.4 ± 0.3^{ns}
V	Extract (100 mg/kg) + Cholesterol	115.7 ± 0.4^{c}	102.7 ± 0.4^{c}	29 ± 0.7ns	53.0 ± 0.8^{c}	20.5 ± 0.0^{c}	2.9 ± 0.1^{ns}	4.2 ± 0.2^{ns}

Results are expressed as means ± SEM; n = 6; Key: where, ^{ns}= Non-significant; ^{a}p<0.05 (significant), ^{b}p<0.01 and ^{c}p<0.001 (highly significant) vs. normal control

100 mg/kg significantly (p<0.001) prevented the rise in the values of LDL, VLDL, TG, TC, compared to the control while, it significantly (p<0.01) increased HDL cholesterol level. Similarly at the dose of 50 mg/kg extract also significantly (p<0.001) prohibited the rise in the levels of TC, TG, LDL and VLDL respectively. The values of AI and CRI were less in treated groups compare to control but were significant. Most pronounced effects in these parameters were seen at 100 mg/kg in rats on high fat diet (Table I).

The *B. orthobotrys* extract, at a dose of 100 mg/kg, produced a significant (p<0.001; p<0.05) antihyperlipidemic effect after 4 hours treatment. The extract demonstrate a remarkably decrease in LDL, VLDL, TG, TC compared to control while, it significantly (p<0.05) increased HDL cholesterol level. *B. orthobotrys* extract at 50 mg/kg dose also significantly (p<0.001; p<0.05) prevented the rise in the levels of TC, TG, LDL and VLDL levels respectively. The values of AI and CRI decreased but were non-significant (p<0.05), resulting in increased degree of protection (Table II).

In fructose-induced hyperlipidemic rats, *B. orthobotrys* extract at the dose of 100 mg/kg significantly (p<0.001; p<0.05) prevented the rise in the values of LDL, VLDL, TG, TC and AI compared to the control, while, a non-significant (p<0.05) increased is observed in HDL cholesterol level. Similarly at 50 mg/kg dose, *B. orthobotrys* extract also significantly (p<0.001; p<0.05) prevented the rise in the levels of TC, TG, and VLDL respectively. The values of AI, CRI were less in treated groups as compared to control but were non-significant (p<0.05) (Table III).

The *B. orthobotrys* extract at the dose of 100 mg/kg significantly (p<0.001), (p<0.05) prevented the rise in the values of LDL, VLDL, TG, TC compared to the control while, increase in HDL cholesterol level is non-significant (p<0.05), Similarly at the dose of 50 mg/kg significantly (p<0.001) prevented the rise in the levels of TC, TG, and LDL respectively. The values of VLDL, AI and CRI were less in treated groups compare to control but were non-significant (p<0.05) (Table IV).

Albino mice treated with *B. orthobotrys* extract (50 and 100 mg/kg) p.o daily significantly (p<0.001) prevent rise in VLDL, TG, TC levels, and most pronounced

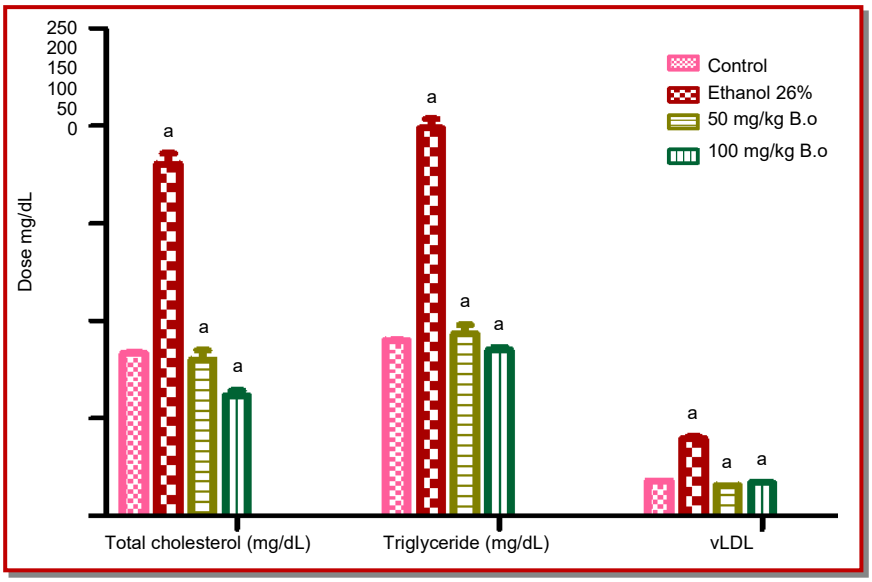

Figure 1: Effect of administration of ethanol, 50, and 100 mg/kg crude extract on serum lipids ethanol induce hyperlipidemic mice. Values are expressed as mean ± SEM for 6 animals. p value <0.001 for level of highly significance

reductions in these parameters were seen at 100 mg/kg in mice fed with 26% ethanol treatment for 4 days (Figure 1).

Discussion

It is noteworthy that the *B. orthobotrys* extract produced a significant reduction in TC, TG, LDL, VLDL, levels, increase in the HDL level and a non-significant decrease in AI and CRI, indicating its antihyperlipidemic effect. Maximum decrease in serum lipids and lipoprotein's level was observed at 100 mg/kg. Reduction in the level of serum lipids of *crude extract* treated animal models was much higher than in positive control. This is in agreement with the previous findings that hyperlipidemic rats appear to have a stronger response to antihypertensive agents (Santosh Kumar et al., 2013).

Moreover, in rats the HFD and cholesterol induce hyper-lipidemia has been reported to be significant due to lipid peroxidation evoked by HFD and high cholesterol diet (Byron et al., 2002), while fructose fed hyperlipidemia has been associated with increased availability of precursor molecule glycerol-3-phosphate and excess free fatty acids while the diminished activity of LPL result in increased content and impaired clearance from circulation as describe in previous studies. Fructose also cause increase in cholesterol synthesis that is in line with a previous findings that addition of fructose to cultured rat hepatocytes increase HMG-CoA reductase activity 3 folds (Spence et al., 1993; Panchamoorthy et al., 2005). Moreover, ethanol interaction with lipid metabolism is complex. Presence of ethanol becomes a perfect fuel for liver and displaces fat as an energy source this blocks fat oxidation and favors fat accumula-tion (Leiber et al., 1961).

Various species of Berberis contain certain constituents like alkaloids and phenolic compounds that show anti-oxidant properties by reducing oxidative stress. Lipid peroxidation is a free radical mediated process which has been reported to be implicated in variety of disease states (Byron et al., 2002; Alamgeer et al., 2012).

Furthermore, extract may alter activity of cholesterol ester transfer protein (CETP), a key enzyme in HDL-c metabolism that reverse cholesterol transport which increase in HFD and HCD, or may be due to its activity on the LCAT, enzyme involved in the trans-esterification of cholesterol and its flux from cell membrane into HDL, or may be due to its effect on HMG-CoA reductase, whose level signifies an increase in cholesterol synthesis, decrease fatty acid oxidation in citric acid cycle and decrease lipoprotein secretion (Leiber et al., 1961; Panchamoorthy et al., 2005; Spence et al., 1993; Emily et al., 2005; Zulet et al., 1999). HDL-c level increase with *B. orthobotrys* extract may be attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol o-acyltransferase (LCAT) (Khanna et al., 2008).

Thus the antihyperlipidemic effect of *B. orthobotrys* extract in HFD, HCD, olive oil or ethanol induced hyperlipidemia might be due to certain hypolipidemic, antioxidant, and imbalance in lipid metabolism or enzyme inhibitor constituents of plant extract.

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