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CHOLESTEROL LOWERING POTENTIAL OF SEABUCKTHORN

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ABSTRACT

The present study was designed to investigate the effect of Seabuckthorn on lipid profile, its antioxidant potency and its effect on haemodynamic changes and baroreceptor mediated blood pressure regulatory mechanism in hypercholesterolemic rats. For induction of hypercholesterolemia rats were fed with fructose in drinking water and in vivo experiment had done to investigate the heamodynamic as well as biochemical profile of seabuckthorn. It is a natural product and investigations carried out so far do not report any apparent toxic effect. Based upon the results of the present study, it is recommended that seabuckthorn pulp oil may be supplemented with normal diet for providing protection against hypercholesterolemia. The fall in blood pressure of animals having a normal lipid profile suggests that it may have a hypotensive effect. Hence, its use as a lipid lowering agent needs to be carefully monitored especially in people with cardiac problems. Conclusive evidence shows that baroreceptor modulation of heart rate is impaired in animals and patients with atherosclerosis. It has been suggested that oxygen free radicals produced in atherosclerosis may contribute to baroreceptor dysfunction. Seabuckthorn prevented development of hypertension and reduced insulin resistance in chronically fructose fed rats and reduced vascular superoxide anion production through lowering the NAD (P) H oxidase activity in hypertensive rats.

KEYWORDS: Hypercholestrolemia, Hypertension, Atherosclerosis, Baroreceptor dysfunction, Free radicals.

INTRODUCTION

The World health report 2002 estimates that cardiovascular diseases will become the major cause of morbidity, especially in high-income countries. Cardiovascular disease (CVD)

encompasses a number of different diseases including coronary heart disease, stroke and peripheral vascular disease. However, the underlying pathology for all of these disorders is atherosclerosis [1]. A number of risk factors for CVD have been described, such as hypercholesterolemia (HC), hypertension, smoking, diabetes and obesity [2], [3], [4]. Till date, there have been many studies on mechanisms by which high levels of LDL-cholesterol affect biology of blood vessels and cause atherosclerotic lesion formation. Arterial baroreceptors are known to regulate absolute blood pressure and ultimately help in maintaining adequate circulation to brain and other organs. Baroreflex contributes importantly to neural circulatory control. Abnormalities in arterial baroreflex function have been linked to adverse cardiovascular outcomes [5]. Functional (neural) mechanisms, in addition to structural vascular changes, contribute importantly to altered baroreflex responses in normal and pathophysiological states. Presently available lipid lowering agents are mainly synthetic drugs, which have many side effects. Natural compounds with fewer side effects are being looked into as an alternative therapy. Seabuckthorn contains a series of chemical compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tannins, etc.; many of them possess biological and therapeutic activity. Flavanoids such as leucocyanidin, catechin, flavonol and flavones lower cholesterol, prevent inflammation and vitamin C degradation and probably control formation of atherosclerotic plaques [6]. In addition, the sterols and stanols present in seabuckthorn reduce the absorption of cholesterol. Together, these effects may be beneficial in hypercholesterolemia and ultimately atherosclerosis [7], [8]. To the best of our knowledge, no study has reported the effect of Seabuckthorn pulp oil on lipid profile and baroreflexes in hypercholesterolemia. Hence, the present study was planned to investigate the effect of SBT on serum lipid profile and baroreflex sensitivity in experimentally induced hypercholesterolemia in rats.

METHODS

Animals

Healthy male Wistar albino rats, weighing between 250-300 g, were obtained from the animal house of VPCI. They were housed in cages in groups of four rats per cage and were

kept in room temperature maintained at 25±2°C with a 12-h light/dark cycle. Experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The Institutional Animal Ethical Committee, Vallabhbhai Patel Chest Institute (VPCI), University of Delhi, New Delhi, India, approved this study.

Preparation of Seabuckthorn pulp oil

We procured SBT pulp oil from DIPAS (Defense Institute of Physiological and Allied Sciences). Fruits of seabuckthorn were collected from hilly regions of western Himalayas, India and dried under shade. The extraction was carried out using fruit by deseeding and crushing the berries. The pulp was centrifuged to obtain SBT pulp oil.

PLAN OF STUDY

Hypercholesterolemia was induced by feeding rats with 10% fructose for 3 weeks in drinking water. SBT pulp oil was given in a dose of 2ml/kg body weight orally. Study was conducted in 20 rats, which were randomly divided into following groups. Group I: Control rats fed with normal pellet diet: Rats of this group were maintained on normal balanced diet and water for 3 weeks. Group II: Rats fed with 10% fructose: For induction of hypercholesterolemia, rats were maintained on normal pellet and 10% fructose in drinking water for 3 weeks. Group III: Rats given 10% fructose and seabuckthorn pulp oil: rats of this group were maintained on normal pellet and 10% fructose in drinking water and seabuckthorn pulp oil for 3 weeks. Seabuckthorn pulp oil (2ml/kg/day) is administered through oral route with the help of oral feeding needle. Group IV: Rats fed with normal pellet diet along with seabuckthorn: In order to study the effect of seabuckthorn on normal rats, seabuckthorn was administered in the dose of 2ml/kg/day, orally with the help of oral feeding needle for 3 weeks.

Biochemical Determinations

After completion of experiment, under deep anesthesia blood samples were collected by direct heart puncture after opening the chest of the animal. Blood was allowed to clot and then centrifuged for 10 min at 5000 rpm, to obtain the serum. Serum sample was transferred into dry and clean vials and stored at -20°C for biochemical analysis at a later date and determined by commercially available spectrophotometric assay kits (Monozymes, India), as per the manufacturer procedure given.

Arterial blood pressure and heart rate

Rat was anesthetized with urethane dissolved in distilled water and injected intraperitoneally (i.p.) at a dose of 1gm/kg body weight. Disappearance of pedal reflexes indicated adequate anesthesia. Rat was placed on a small table and secured by tying the limbs. A middle incision was given in the neck region; retracting the pretracheal muscle exposed trachea and a transverse incision was given in between two rings. A cannula was introduced into the opening to allow free breathing without obstruction. Body temperature of the rat was maintained at 37-38^{o}C. Femoral artery of one side was exposed and a polyethylene catheter filled with heparin solution (500 IU/ml, v/v) was inserted in the artery through a small incision for recording arterial blood pressure (ABP). The catheter was attached to 23-gauge needle connected via three-way stopcock to a pressure transducer (Statham-P23D). Femoral vein of the other limb was cannulated for injecting drugs (Ashraf et al., 2005). Prior to recording ABP, catheter was flushed with heparinized saline solution (500 IU/ml, v/v) to prevent formation of any blood clot, which might interfere with normal recording of ABP. The pressure recording system was calibrated with the help of a mercury manometer before each experiment. Arterial blood pressure was measured after 20 min of stabilization period. Systolic, diastolic, mean arterial pressures and heart rate were displayed and recorded on Power Lab data-acquisition system (4SP, AD Instruments, Australia) with a computerized analysis programme.

Measurement of baroreflex sensitivity (BRS)

BRS was measured by administering 20µg/ml/kg phenylephrine (vasoconstrictor) and sodium nitroprusside (vasodilator) through venous catheter. The resultant changes in heart rate at corresponding rise or fall in SBP were measured at different time intervals (every 2 sec). The relationship between increase in SBP evoked by phenylephrine and associated bradycardia or decrease in SBP evoked by sodium nitroprusside and associated tachycardia was assessed by regression analysis for individual animal. The regression coefficient (slope of regression line), expressed as beats per minute per mm of mercury (beats/min/mmHg) was taken as an index of baroreflex sensitivity.

Statistical analysis

The data are presented as mean ± SEM. Statistical analysis was done by analysis of variance (ANOVA) followed by tukeys multiple comparison method. P value for statistical significance was set at 5% i.e., $p< 0.05$.

RESULTS

No significant difference in the diet intake and body weight was observed among the various groups.

Haemodynamic Profile

Effect of seabuckthorn on arterial blood pressure and heart rate

Rats fed with fructose showed rise in systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate compared with control rats. Treatment of rats with seabuckthorn along with fructose prevented the rise in systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate compared with hypercholesterolemic rats. Treatment of rats with seabuckthorn on normal diet for 3 weeks prevented the rise in

systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate compared with hypercholesterolemic rats. These are followings data shown in Table-1.

Lipid Profile

Effect of Seabuckthorn on serum lipid profile

Rats given fructose (10%) in drinking water developed hypercholesterolemia marked by significant ($P<0.05$) increase in total cholesterol, triglycerides, LDL-C, and a significant ($P<0.05$) decrease in the level of HDL-C compared with control rats. Treatment with seabuckthorn along with fructose in drinking water showed significant ($P<0.05$) fall in total cholesterol, triglycerides, LDL-C and significant ($P<0.05$) increase in HDL-C level compared with hypercholesterolemic group. Following treatment of rats with seabuckthorn on normal diet for 3 weeks, hypercholesterolemic rats showed significant ($P<0.05$) decrease in total cholesterol, triglycerides, LDC-L, and significantly ($P<0.05$) increased HDL-C level compared with hypercholesterolemic group. These are followings data shown in Table-2

Effect of seabuckthorn on the arterial baroreceptor mediated blood pressure regulatory mechanism

Baroreflex sensitivity was measured as a ratio of bradycardia response to rise in arterial pressure by phenylephrine or as a ratio of tachycardia response to fall in arterial pressure by sodium nitroprusside. Rats given fructose showed significant ($P<0.05$) decrease in baroreflex sensitivity compared to the control rats. Treatment with seabuckthorn along with the fructose showed significant ($P<0.05$) restoration of baroreflex sensitivity compared with hypercholesterolemic group. Rats treated with seabuckthorn and maintained on normal diet did not show any change in baroreflex sensitivity compared with control group. These are followings data shown in Table-3.

DISCUSSION

Hypercholesterolemia, which is characterized by high levels of lipoprotein containing cholesterol in blood, is generally accepted as a major risk factor for the development of atherosclerosis and subsequent myocardial ischemia [9]. Wistar rat as a model of hypercholesterolemia has been chosen for the present study as it shows increase in serum cholesterol due to feeding of fructose rich diet. Rats can develop hypercholesterolemia after feeding the fructose rich diet for a long period of time. But rats have shown functional changes in vascular responsiveness even with a relatively small elevation of plasma cholesterol levels. So, it has been suggested that, rat may be a good model for studying vascular functions during hypercholesterolemia prior to the development of atherosclerotic lesions [10].

Lipid profile

In the present study, fructose rich diet feeding for 3 weeks caused a significant increase in serum TC, LDL-C, TG with a significant decrease in HDL-C in rats. Seabuckthorn treatment along with fructose diet in rats (preventive effect) showed significant reduction in serum TC, LDL-C, TG with a significant increase in HDL-C compared with hypercholesterolemic rats. After treatment with Seabuckthorn along with normal diet for 3 weeks in hypercholesterolemic rats (therapeutic effect) showed further reduction in serum TC, LDL-C, TG with a significant increase in HDL-C compared with hypercholesterolemic rats. Strong evidences suggested that hypercholesterolemia induces oxidant stress by causing reduction in the enzymatic antioxidant defense potential of tissues and generation of oxygen free radical (OFR) like superoxide anions. As a result of these metabolic events peroxidation reactions are accelerated leading to cellular injury [11], [12], [13].

Haemodynamic profile

In this study, hypercholesterolemic rats show significant rise in SBP, DBP, MAP and HR. Another important finding is that Seabuckthorn treatment along with fructose feeding in rats (preventive) completely prevented the rise in SBP, DBP, MAP and HR compared with hypercholesterolemic group. However, hypotensive effect was observed in rats treated with Seabuckthorn and fed with normal diet. Taken together, the present data suggest that Seabuckthorn attenuated the development of hypertension in hypercholesterolemic rats probably through its antioxidative properties.

Baroreflex sensitivity

Results of the present study showed that the bradycardia response to phenylephrine (PE) and the tachycardia response to sodium nitroprusside (SNP) were attenuated in hypercholesterolemic animals. The linear regression analysis of the reflex changes in HR in response to changes in SBP indicated decreased baroreflex sensitivity in hypercholesterolemic rats compared with normal rats. The present work have shown that Seabuckthorn treatment along with fructose feeding in rats improved the baroreflex sensitivity to depressor and pressor responses to SNP and PE, respectively. Further, rats treated with Seabuckthorn and maintained on normal diet did not show any change in baroreflex sensitivity. Seabuckthorn has shown antioxidant activity in our study as well as in other studies [14]. This may explain its beneficial effect on the baroreflex sensitivity in hypercholesterolemic rats as decreased baroreflex sensitivity has been correlated with oxidative stress [15].

Table – I

Parameter	GROUP-I	GROUP-II	GROUP-III	GROUP-IV
SBP	131.6 ± 3.35	156.5 ± 8.41	109.8 ± 5.37	98.71 ± 7.49
DBP	87.16 ± 3.80	108 ± 12.81	64.32 ± 6.12	65.92 ±13.85
MAP	106.6 ± 2.65	129.6 ± 11.07	83.41 ± 4.92	79.83 ± 11.36
HR	365.5 ± 21.65	340 ± 29.76	348.7 ± 20.60	348.7 ± 9.43

Table – II

Parameter	GROUP-I	GROUP-II	GROUP-III	GROUP-IV
TC	93.63 ± 2.58	125 ± 6.88	95.96 ± 5.63	56.76 ± 5.32
TG	68.12 ± 2.35	82.16 ± 4.01	64.25 ± 5.10	44.14 ± 5.99
HDL-C	21.93 ± 2.83	13.28 ± 0.42	16.42 ± 1.05	18.36 ± 1.87
LDL-C	58.07 ± 4.41	95.24 ± 6.53	66.69 ± 5.38	29.57±4.52

Table – III

Parameter	GROUP-I	GROUP-II	GROUP-III	GROUP-IV
PE	4.66 ± 1.25	2.02 ± 1.11	3.74 ± 0.59	3.80 ± 1.41
SNP	5.19 ± 1.08	2.77 ± 1.69	4.24 ± 1.74	4.93 ±1.73

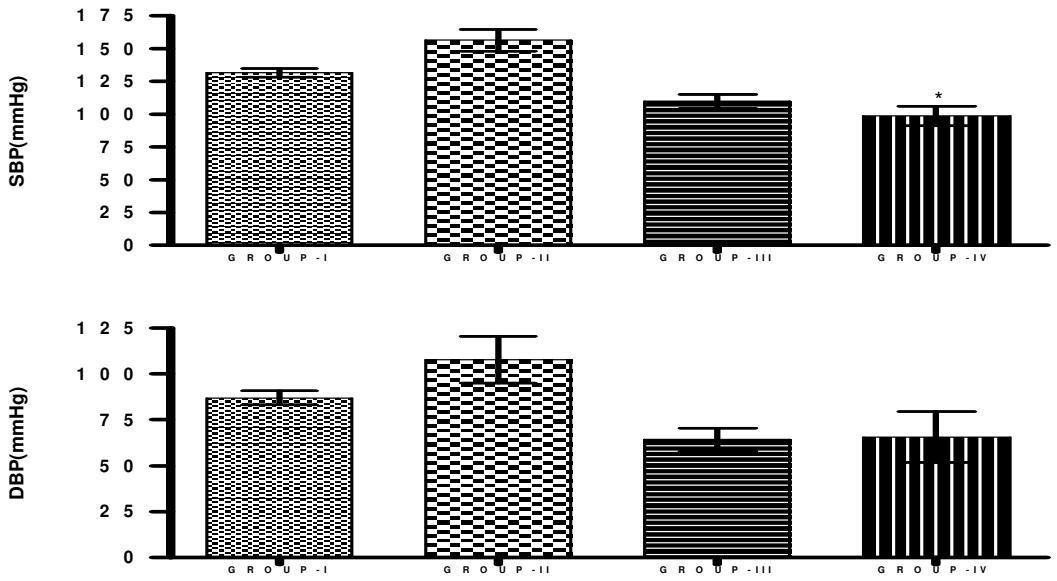

Fig-I-II- Effect of Seabuckthorn on systolic and diastolic pressure in rats, Each value represents mean ± SEM of five animals. SBP-Systolic blood pressure, DBP-diastolic blood pressure.

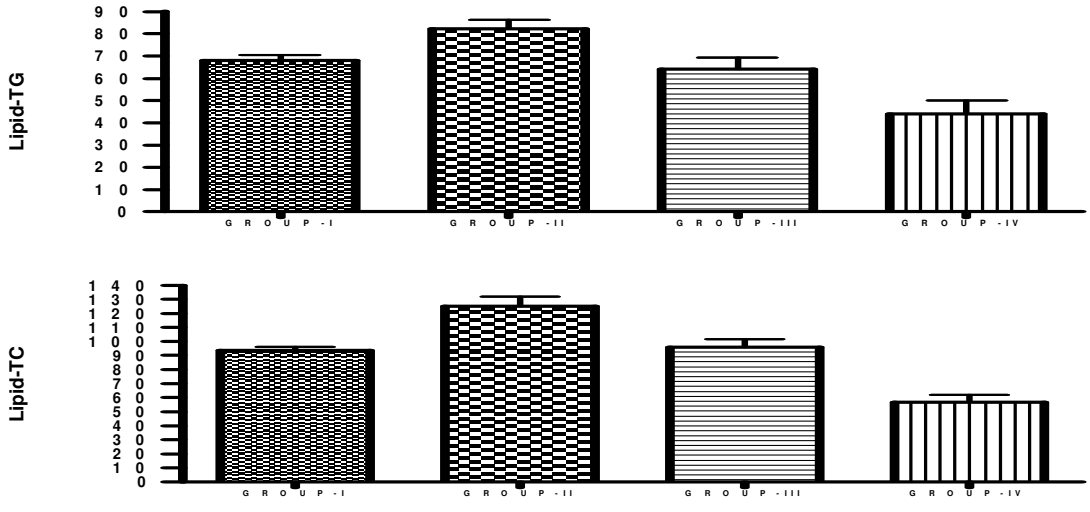

Fig-IV-V- Effect of Seabuckthorn on Total triglycerides and total cholesterol in rats, Each value represents mean ± SEM of five animals. TG- total triglycerides, TC-total cholesterol.

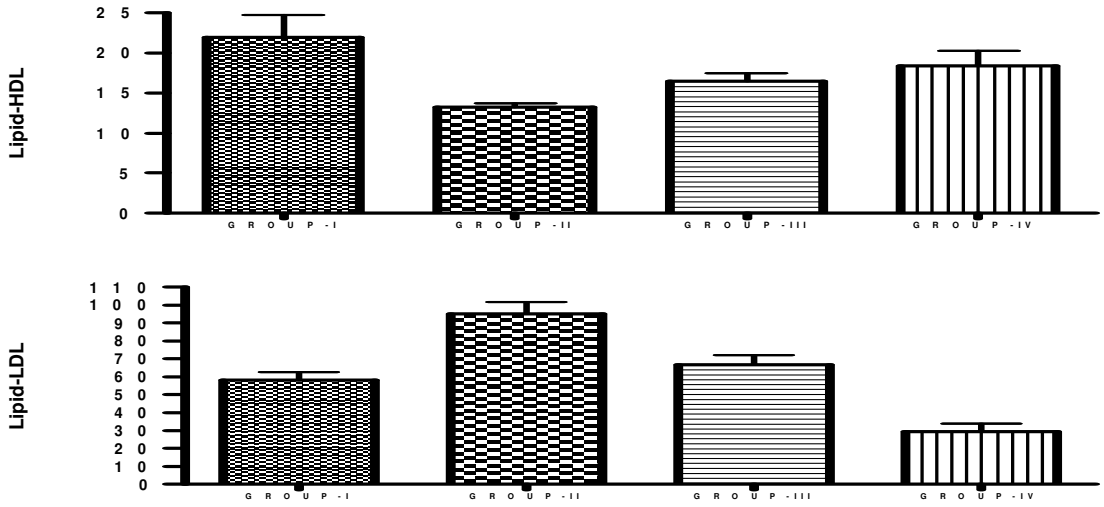

Fig-VI-VII- Effect of Seabuckthorn on Total high-density lipoprotein and low-density lipoprotein in rats pellet diet. Each value represents mean ± SEM of five animals. HDL-High density lipoprotein, LDL-Low density lipoprotein.

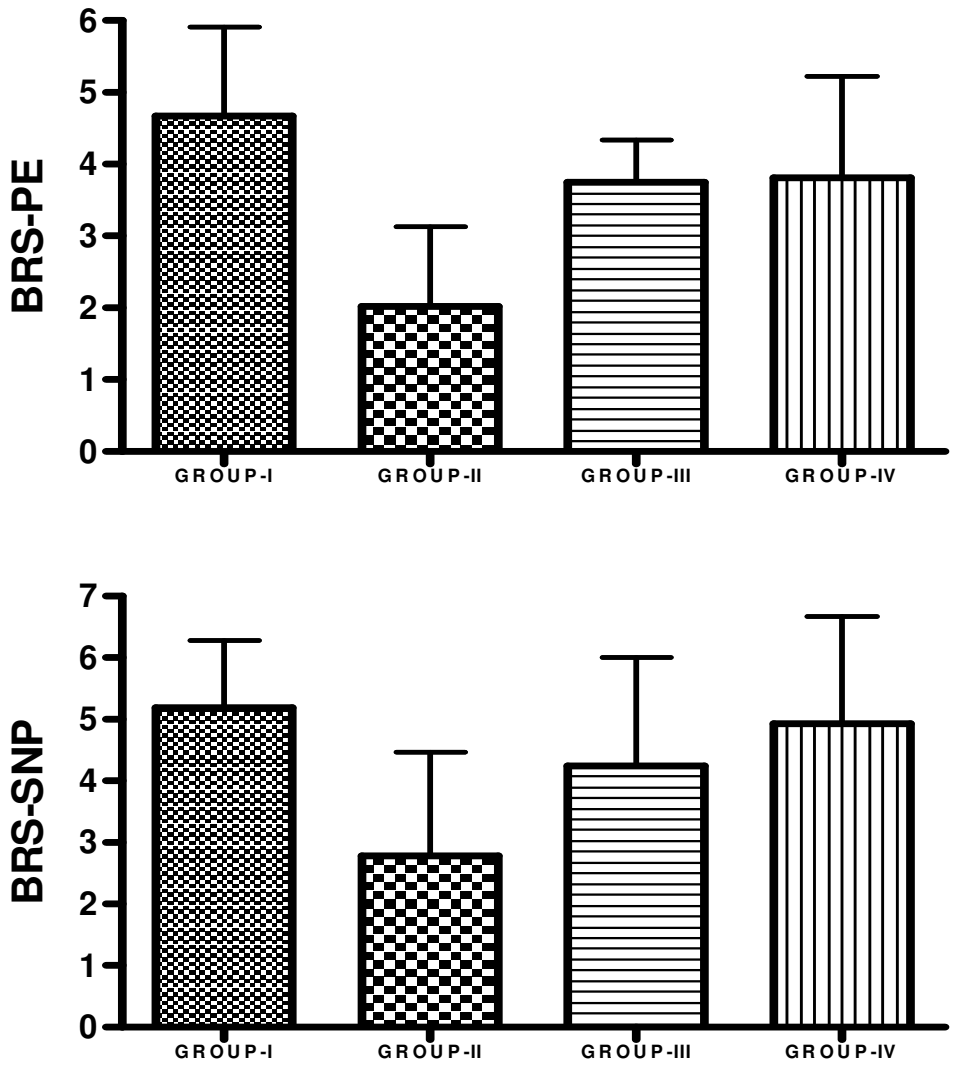

Fig-VIII-IX- Effect of Seabuckthorn on baroreflex sensitivity, BRS -Baroreflex sensitivity PE Phenylephrine, SNP-Sodium nitroprusside. Each value represents mean ± SEM of five animals.

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