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Effect of seabuckthorn seed oil in reducing cardiovascular risk factors: A longitudinal controlled trial on hypertensive subjects

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ABSTRACT

Background and aims: The present study aimed at investigating whether dietary supplementation of seabuckthorn seed oil which is rich in omega fatty acids at an oral dose of 0.75 ml could affect cardiovascular risk factors and reduce hypertension and systolic blood pressure.

Methods: Toxicological evaluation and efficacy of seabuckthorn seed oil in reducing high fat diet induced dyslipidemia was initially conducted on adult male Sprague Dawley rats. 32 normal and 74 hypertensive and hypercholestrolemic human subjects participated in the randomized, controlled, double blind longitudinal study. Seabuckthorn seed oil or sunflower oil placebo was orally supplemented at a daily dose of 0.75 ml for 30 days.

Results: Supplementation of seabuckthorn seed oil at a daily dose of 0.75 ml for 30 days resulted in normalization of blood pressure in hypertensive subjects. Dietary supplementation of seabuckthorn seed oil markedly reduces cholesterol, oxy-LDL and triglycerides in hypercholesterolemic subjects though it's effect on subjects with normal blood pressure and cholesterol is less pronounced. Seabuckthorn seed oil supplementation also improves circulatory antioxidant status in both normal and hypertensive subjects.

Conclusion: The present study demonstrates the efficacy of seabuckthorn seed oil in reducing dyslipidemia, cardiovascular risk factors and hypertension in human population which may be due to presence of omega 3, 6 and 9 fatty acids in the oil. The improvement in antioxidant status can be attributed to presence of beta carotene and vitamin E in seabuckthorn seed oil.

The trial was registered with Clinical Trial Registry of India (Clinical trial registration number - CTRI/2015/11/006368).

1. INTRODUCTION:

Hypertension or elevated blood pressure is often associated with hyperlipidemia which is a major component of metabolic syndrome [1]. Together, they lead to the onset and progression of cardiovascular disease (CVD) and increase the risk of heart attack, stroke and cardiac failure. Other complications associated with increased blood pressure include peripheral vascular disease, renal damage, retinal hemorrhage and visual impairment [2]. Based on the estimates of World Health Organization (WHO), nearly one billion human population had clinical hypertension by the year 2008 [3]. Recent projections forecast the prevalence of hypertension in human population to be as high as 7.2% by year 2030 [4].

Poor lifestyle, diet and occupational stress have been largely attributed to the rise in prevalence of CVD in human population. A study by Norboo et al has shown increased prevalence of hypertension in migrated human population at high altitude which could be attributed to environmental factors [5]. Our previous studies on human subjects staying for prolonged duration at high altitude showed increase in circulatory homocysteine which is also an important cardiac risk marker. Worldwide, approximately 140 million people live at altitude >2500m above sea level owing to their occupational needs, while several others visit high altitude regions for mountaineering expeditions or recreational purposes [6]. The prevalence of CVD between populations living at low and high altitudes varies displaying controversial results. While several studies conducted at moderately low altitudes suggest association of hypertension with high altitude dwelling, others report that the incidence of hypertension was low in high altitude regions when compared with sea level residents [7]. Nevertheless, considering the burgeoning evidence on association of cardiac risk factors with CVD and stroke, increase of these factors could be highly detrimental in high altitude conditions. In this lieu, nutritional

interventions targeting multiple cardiac risk factors such as hypercholesterolemia, hypertriglyceridemia and hypertension could provide better and effective therapeutic usage as compared to compounds targeting individual risk factors [8].

Several studies have investigated the efficacy of dietary nutraceuticals in controlling dyslipidemia and hypertension [9]. Nutritional supplementation of Omega fatty acids in particular, have been shown to have antihypertensive properties [10]. Amongst the trans-himalayan herbs, seed oil of *Hippophae rhamnoides* (commonly known as seabuckthorn) has been proven to be an excellent source of omega-3 (linolenic), omega-6 (linoleic), omega-7 (palmitoleic acid) and omega-9 (oleic acid). It also contains beta carotene and vitamin E which are chain breaking antioxidants. Despite these unique nutritional properties, the information on the anti-hypertensive efficacy of seabuckthorn seed oil is sparse in existing literature. This pilot study aimed at evaluating the effect of nutritional supplementation of seabuckthorn seed oil on dyslipidemia and cardiac risk factors that contribute to hypertension and CVD in animal models and human subjects at high altitude.

2. Materials and Methods

2.1 Seabuckthorn seed oil extraction and characterization:

Seabuckthorn (SBT) (*Hippophae rhamnoides*) seeds were harvested from Ladakh region of trans-Himalays and oil was extracted by supercritical CO_2 extraction method for 3 hours with 350 bar pressure and temperature at 50^0C. The CO_2 flow rate was set at 150 L/kg of seabuckthorn seed oil per hour [11]. The seed oil was characterized for total fatty acids and bioactive compounds and was encapsulated in gelatin (0.75 ml oil per capsule) by Ambe

Phytochemicals (Ambe Phytochemicals Pvt. Ltd., Delhi). The SBT soft gel capsules thus obtained were rich in omega fatty acids as described in Table 1.

2.2 *Animal studies*

The study was approved by the institutional ethics committee of Defence Institute of High Altitude Research (DIHAR), Leh, India. Male adult Sprague Dawley rats weighing 220±10 gm were housed in clean polypropylene cages under conventional conditions with controlled temperature (21-22^{0}C) and humidity (55-60%) and 12h light/dark cycle. Food and water was made available to the animals *ad libitum* and utmost care was taken to minimize the sufferings of animals. Sub-acute, acute, sub-chronic and chronic toxicity studies were performed according to methods laid down by OECD test guidelines 1995.

Rats were randomly divided into three groups viz; group 1 (n=6) fed with control diet; group 2 (n=6) fed with high fat diet and group 3 (n=6) fed with high fat diet + SBT seed oil at a dose of 150µl/kg b.w. for the period of 30 days after dose optimization. Blood was collected by retro orbital puncture at day 0 and day 30 after overnight fasting. Serum estimations for total cholesterol, HDL-Cholesterol, LDL-Cholesterol, Triglycerides and total serum anti-oxidant were performed.

2.3 *Site of human study and ethics*

The present study is a unicentric, randomized, placebo controlled, interventional study, investigating the efficacy of seabuckthorn seed oil (0.75ml/day; encapsulated) in volunteers having hypertension associated with hypercholesterolemia. Systolic blood pressure (SBP) ≥140 mm Hg was defined as 'hypertension' and serum cholesterol level ≥ 200 mg/dl was defined as 'hypercholesterolemia'. The study was conducted in Ladakh region of India (4200-4600 m

above MSL). Human volunteers with hypertension and hypercholesterolemia who were unwilling to take standard prescribed drugs for hypertension were recruited for the experimental group of the study. The volunteers were briefed about the procedures, purpose and expected outcome of the study and informed written consent was obtained from each participant prior to enrollment in the study. The sample size for the study was estimated taking type I error rate of 0.05 and type II error rate of 0.20 (power 80 %). The study was approved by the Institutional Ethical Committee on Biomedical Research on Human Subjects and was enrolled with Clinical Trial Registry of India (Clinical trial registration number - CTRI/2015/11/006368).

2.4 *Study population and study drug*

150 male volunteers who had continuously stayed in Ladakh region for more than 12 months were initially recruited for the study. The study population had similar dietary pattern based on a pre-defined high altitude ration scale and had similar pattern of physical activity. Preliminary screening was performed based on eligibility criteria L1 viz. age, gender, education, monthly income and medical examination. Physical examination was performed in the presence of a clinician and a medical questionnaire regarding medical history and general health status was administered to the participants (Table 2a). The volunteers qualifying L 1 screening, were subjected to screening with eligibility criteria L2 comprising of core behavioral measures (CBM) like core alcohol consumption (section A), core smoking behavior (section C), and core physical activity (section P) in compliance to WHO guidelines [12] (Table 2b).

Of the 150 volunteers enrolled for the study, 106 volunteers who qualified both L1 and L2 were recruited for the study (Figure 1). The study population was divided into two groups viz; Group 1 which served as control group (n= 32), consisting of healthy participants (SBP <

140 mm Hg, Total serum cholesterol < 200) treated with SBT soft gel capsules (hence forth referred to as Cohort 1) and Group 2 (n=74) consisted of volunteers with hypertension and hypercholesterolemia treated with either sunflower oil placebo capsules or SBT soft gel capsules (Ambe Pharmaceuticals Pvt. Ltd., India). Group 2 was further randomized into two cohorts viz; Cohort 2 comprising of volunteers (n=37) supplemented with SBT soft gel capsules at a daily dose of 0.75 ml f and Cohort 3 consisting of volunteers (n=37) supplemented with placebo (0.75 ml sunflower oil encapsulated in gelatin). All the volunteers were supplemented either with SBT or placebo soft gel capsules for a period of 30 days. Compliance with treatment was ensured through daily supervised intake of capsules and counting of capsules. All the physiological measures and blood samples were collected at baseline and one month follow up for all the groups.

2.5 End Points

The primary end points were change in total serum cholesterol and systolic blood pressure from baseline to the end of treatment regime.

2.6 Randomization

After acquiring baseline data, participants with hypertension and hypercholesterolemia (Group 2) were randomly assigned for receiving SBT soft gel capsules (Cohort 2) or matching placebo (gelatin encapsulated sunflower oil) capsules (Cohort 3). Both SBT and placebo capsules were identical in weight, size, color, shape and consistency and were packed in identical bottles. The batch consistency of ingredients in each type of capsule was independently verified through random selection and characterization. The random number table method for allocation

of SBT and placebo capsules was complied and the participants were blinded to the treatment paradigm during the length of the study.

2.5 *Physiological Measurements*

Height was measured using portable anthropometer and weight was taken using digital weighing machine. Hip and waist circumference were measured using standard technique. The body mass index was calculated and represented in kg/m^2. Volunteers were made to relax for 10 minutes prior to their blood pressure measurement in sitting position. Blood pressure was measured using automated machine based on cuff oscillometric principle (OMRON-HEM 7120). Blood pressure was measured thrice and the mean value was taken into consideration.

2.6 *Biochemical Assessment*

After 12h overnight fasting, blood samples were drawn from the median cubital vein of all the volunteers under aseptic conditions in vacutainer tubes. Serum was isolated by centrifugation at 7000 rpm for 10 minutes for the biochemical estimations. Serum total cholesterol, HDL-cholesterol and triglyceride were measured using dry chemistry method, Reflotron system (Roche Diagnostic GmbH, Mannheim, Germany) [13]. Measurement was done with 30 ul of serum sample on test strips. Precinorm U and Reflotron check (Roche Diagnostic GmbH, Mannheim, Germany) strips were used for functional test of the system. LDL and VLDL concentrations were calculated using friedewald formula, LDL – C = TC – (HDL- C + TG/5) [14]. Oxidized LDL was estimated using Human Oxidized LDL ELISA kit (Cell biolabs, Inc.) as per manufacturer's instruction. Homocysteine was determined using enzyme immune assay method (Abnova homocysteine ELISA kit KA 1242). Total serum antioxidant was also estimated using ABTS method taking Trolox as reference standard [15]. All the samples were

run in triplicates and mean value was considered for further statistical analysis. The inter and intra assay CV's for lipid profile measurements were less than 5.2% while the inter and intra assay for CV's for homocysteine were 3.8 and 4.6 respectively.

2.7 *Statistical Analysis*

Data was analyzed using SPSS 22.0 statistical software (SPSS, Chicago, IL, USA). One way analysis of variance (ANOVA) with Duncan's Post Hoc test was performed for comparisons between groups. Paired t-test was performed to evaluate significant changes in physiological and biochemical values in two point of the study i.e. baseline and follow up after 30 days. Bivariate Pearson correlation (r) was analyzed for evaluating the association between change in systolic blood pressure and lipid profile at baseline and day 30.Statistical results were considered significant at P-values < 0.05. The data was archived in the laboratory record centre.

Results

3.1 Animal studies

No mortality or change in food and water intake, body weight and behavior was observed during the toxicity studies. Histo-pathological examination of vital organs did not show morphological alterations or any signs of histotoxicity on sub-acute, sub-chronic and chronic oral administration of SBT seed oil (Figure 2). While high fat diet resulted in dyslipidemia in rats as indicated by increase in cholesterol and triglycerides; supplementation of SBT seed oil at an oral dose of 150µl/kg b.w. to rats on high fat diet reduced hypercholesterolemia to significant levels as shown in Table 3.

3.2 Human studies

193 *Study Population*

194 Total number of recruited volunteers fulfilling the inclusion criteria were divided into
195 cohort 1 (n=32), cohort 2 (n=37) and cohort 3 (n=37). The cohort 2 and cohort 3 participants
196 comprised of 'hypertensive and hypercholestrolemic subjects' with no other remarkable
197 difference in their baseline characteristics (Table 4). While cohort 1 (control group) and cohort 2
198 received SBT seed oil supplementation, cohort 3 received placebo (sunflower oil) capsules orally
199 at daily dose of 0.75 ml for a period of 30 days. Of all participants initially recruited 29
200 participants of cohort 1, 34 of cohort 2 and 31participants of cohort3 showed 100% compliance
201 with the drug treatment paradigm and completed the study.

202 *3.2.1 Physiological Data*

203 Physiological tests (Table 5) were done at baseline with follow up after 30 days. A
204 significant change was found in systolic blood pressure (SBP) and diastolic blood pressure
205 (DBP) in SBT oil supplemented subjects between baseline and follow up. The mean systolic
206 blood pressure in cohort 2 decreased by 9.57 mmHg (95% CI -7.26; -11.89) ($p < 0.001$) and mean
207 diastolic blood pressure was reduced by 4.96 mmHg (95% CI -2.72; -7.20) ($p < 0.001$)
208 respectively on supplementation of SBT seed oil for 30 days. The reduction in systolic and
209 diastolic blood pressure was significantly lower in cohort 2 (-4.31 mmHg, 95% CI -2.63; -6.11)
210 ($p < 0.05$) after 30 days supplementation when compared to cohort 3 (placebo group) (1.48
211 mmHg, 95% CI -0.14; -2.46) ($p = 0.15$). However, individuals in cohort 1 (control group)
212 receiving SBT supplementation showed moderate reduction in systolic blood pressure in
213 comparison to baseline (-6.49 mmHg, 95% CI -3.11; -10.83) ($p < 0.001$). No change in BMI and
214 WHR was observed in any of the cohort during the study.

3.2.2 Lipid Profile

The mean reductions in serum total cholesterol, triglyceride, LDL-cholesterol, oxy-LDL and oxy-LDL to HDL-cholesterol ratio were significantly greater in SBT supplemented cohort 2 when compared to baseline values and cohort 3 (placebo group) (Table 5). Serum total cholesterol level was significantly lower in Cohort 2 after 30 days supplementation of SBT seed oil (-44.85 mg/dL, 95% CI -31.61; - 58.08) when compared to baseline values ($p < 0.001$). The mean reduction in serum triglyceride levels in cohort 2 when compared to baseline was also significant (-14.99, CI -11.22; -18.76) ($p < 0.001$). There was significant reduction in serum total cholesterol (-27.12 mg/dL, 95% CI -16.44; - 42.10) ($p < 0.001$) and serum triglyceride (-2.01 mg/dL, 95% CI -0.98; - 4.06) ($p = 0.23$) in cohort 2 when compared to cohort 3. Serum LDL-Cholesterol, was also significantly lower on supplementation of SBT oil in cohort 2 when compared to baseline with a mean reduction of -42.13 mg/dL (95% CI -13.04; -61.22) ($p < 0.001$). Serum LDL-cholesterol in Cohort 2 was significantly reduced after 30 days of SBT oil supplementation when compared to cohort 3 (-36.01 mg/dL, 95% CI -21.91; -59.22) ($p < 0.001$). Oxy-LDL levels also decreased significantly at follow up in cohort 2 when compared to baseline (-8.18, 95% CI – 21.3; -4.16) ($p < 0.001$). Oxy-LDL concentration was significantly lower in cohort 2 after 30 days of supplementation when compared to cohort 3 (-4.48 (95% CI – 1.90; -9.33) ($p < 0.05$) .Oxy-LDL to HDL ratio was also significantly lower in cohort 2 subjects when compared with baseline with mean difference of -0.31 (95% CI; $p < 0.05$). The mean reduction in oxy-LDL to HDL ratio in cohort 2 when compared with cohort 3 after 30 days supplementation of SBT seed oil was -0.41 (95% CI; $p < 0.05$). In the subjects in cohort 1 (control group) the mean reduction in total serum cholesterol, triglyceride, LDL-cholesterol, oxy-LDL and oxy-LDL to HDL cholesterol after supplementation of SBT seed oil for 30 days when compared to

baseline values were -18.04 mg/dl (95% CI; p <0.001),-41.73 mg/dl (95% CI; p <0.001), - 21.01 mg/dl (95% CI; p <0.001), -05.89 (95% CI; p <0.05) and -0.12 (95% CI; p <0.05) respectively. Systolic blood pressure in SBT supplemented cohort was observed to be positively correlated with reduction in cholesterol, triglyceride an LDL-cholesterol with pearson's correlation of 0.87(p <0.001),0.56(p <0.05) and 0.67 (p <0.05) respectively (Table 6).

3.2.3 Homocysteine

The serum homocysteine level was found to be elevated in all the study cohorts at high altitude with baseline values higher than normal reference range of 3.0 µmol/L - 12.0 µmol/L. Baseline values of cohort 1, cohort 2 and cohort 3 were 32.09±14.22 µmol/L, 38.20±16.57 and 36.25±13.67 µmol/L respectively. The mean difference in the homocysteine values at follow up after 30 days of SBT oil supplementation was found to be non-significant when compared to baseline as well as between the cohorts (Table 4).

3.2.4 Total Antioxidant Status

Significant improvement in serum total Antioxidant status was observed in subjects supplemented with SBT oil capsules. The mean difference between 30 day follow up and baseline in cohort 2 was higher by +36.27 µmol Trolox Equivalent/L (95% CI +50.83; +13.45) and significant (p < 0.001). The difference in antioxidant status, between cohort 2 and cohort 3 was 23.11 µmol Trolox Equivalent/L (95% CI 09.34; 54.35) (p < 0.001) on 30 days of supplementation of SBT seed oil (Table 4) .The mean difference in antioxidant status in Cohort 1 after 30 days supplementation of SBT seed oil was +46.13 µmol Trolox Equivalent/L (95% CI +60.13; +10.02) (p < 0.001) when compared to baseline.

4. Discussion

The present study demonstrates the nutraceutical efficacy of seabuckthorn (SBT) seed oil in ameliorating dyslipidemia in both animal models and human volunteers. The study also demonstrates efficacy of SBT supplementation in lowering blood pressure in young male hypertensive human volunteers at high altitude. SBT seed oil is highly rich in poly unsaturated fatty acids (PUFA), particularly omega-3, 6 and 9 as well as carotenoids and flavonoids which show significant antioxidant and cardioprotective activity [16]. Seabuckthorn seed oil is reported to contain more omega fatty acids in an optimal ratio of nearly 1:1.6:1.5 proportion of omega 3, 6 and 9 per equal serving than any other oil [17-18]. Conversely, cod liver oil which is widely used as a natural dietary supplement for reducing cardiac risk factors, is rich only in omega 3 fatty acids and has been reported to have adverse effects that include Vitamin A toxicity and hypertensive disorders[19]. Omega-3 fatty acid from fish oil has been reported to have hypotriglycerdemic effect in experimental animals as well as humans [20]. The derivatives of linoleic acid have been reported to have cholesterol lowering effect in animals [21]. Studies also reveal that omega -3 fatty acid improves endothelial function by modulating e-NOS and increases endothelium – derived relaxing factor (EDRF) thereby resulting in relaxation of arteries and vessels [22]. On the basis of several studies and case reports, American Heart Association has reported reduction of risk factors contributing towards coronary heart diseases on supplementation of Omega 6 PUFA and has advocated for at least 5% to 10% of energy intake through omega – 6 PUFA [23]. In the present study, supplementation of SBT seed oil at a daily dose of 0.75ml to human volunteers with hypertension and hypercholesterolemia for a period of 01 month during the present study resulted in normalization of systolic and diastolic blood pressure and reduction in circulatory cholesterol. SBT seed oil supplementation also reduced triglycerides and LDL which have been previously reported to be detrimental cardiac

risk factors that lead to CVD [11]. The unique composition of omega fatty acids in seabuckthorn seed oil viz., linolenic acid (omega-3, C 18:3) 17.47 %, linoleic acid (omega-6, C 18:2) 28.02 %, palmitoleic acid (omega-7, C 16:1) 4.89 %, oleic acid (omega-9, C 18:1) 26.24 % probably contributes towards the hypolipidemic effect that was observed during the present study. This is further supported by previous findings on ability of omega fatty acids to reduce synthesis of triglycerides [24].

In addition to omega fatty acids, an inverse relationship between natural antioxidants and cardiovascular disease (CVD) risk has also been reported in several epidemiological studies [25]. Oxidative stress is a major contributing factor for progression of vascular dysfunction and pathology of atherosclerosis. Populations with higher degree of lipid peroxidation are more likely to develop cardiovascular complications on ageing [26]. Several reports suggest that consumption of fruits; vegetables and red wine which are rich source of natural antioxidants have protective effect against cardiovascular diseases [27]. Vitamin E being the chain breaking antioxidant is most abundant naturally occurring antioxidant in humans and is effective against LDL oxidation [28]. The vitamin E content in SBT seed oil has been reported to be two times higher than wheat oil, nine times higher than corn oil, thirty five times higher than soyabean oil and two times higher than sprouted grams [29-30]. Another lipid soluble, naturally occurring antioxidant is beta carotene that has been reported to be good quencher of oxygen free radicals and its efficacy under low partial pressure of oxygen has been found to be more effective [31]. SBT seed oil used in the present study was rich in both these lipid soluble antioxidants. Antioxidant flavanoids from seabuckthorn have been reported to prevent endothelial cell injury by suppressing the oxy-LDL effect and modulating NO synthesis via endothelial nitric oxide synthase [32]. We also observed a decrease in circulatory oxy-LDL along with improved

antioxidant status in hypertensive subjects administered with SBT seed oil which could be attributed to the presence of natural antioxidants viz., vitamin E and beta carotene in the oil. Hence, combined efficacy of omega fatty acids and natural antioxidants which are present in SBT seed oil has the advantage of synergistic as well as complimentary action of bio-molecules and together contributes towards reduction of cardiac risk factors.

5. Conclusion

The present pilot study demonstrates the efficacy of dietary supplementation of SBT seed oil in reducing dyslipidemia and hypertension in male human population at high altitude with no observed adverse health effect. SBT seed oil also reduces oxy-LDL (an important cardiac risk factor) in this study population. The results warrant further investigation in clinical set ups on a larger population size and at lower altitudes.

5.1 Study limitations

There were few limitations in the present study which could not be met due to logistic reasons at the remote location where the study was conducted. The main limitation is the short-term evaluation of the SBT capsules in reducing hypertension and hypercholestrolemia. The second limitation is exclusion of other risk factors contributing towards the progression of cardiovascular disease that includes apolipoproteins, C-reactive protein and HbA1c.

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6. Authors Contribution

The data from human studies was collected by Vivek Vashistha and Dr Kalpana Barhwal conducted the animal studies and drafted the manuscript. The study was designed and supervised by Dr Sunil Kumar Hota, Dr Om Prakash Chaurasia contributed towards extraction and characterization of SBT seed oil, Ashish Kumar contributed towards recruitment of human volunteers and Dr Bhuvnesh Kumar reviewed the manuscript and contributed towards data interpretation.

7. Acknowledgement

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FIGURE LEGENDS

Table 1

Table showing composition of fatty acid, minerals and bioactive compounds in CO_2 super-critically extracted seabuckthorn seed oil.

Table 2

a) Basic inclusion criteria for volunteers enrolled in the study (Eligibility criteria L1).

b) Eligibility Criteria L2 for core behavioral measures (CBM), Beck Depression Inventory (BDI), Lake Louise Score kidney function test, liver function test and blood glucose. Values depicted in percentage or as Mean±SD of the study population

Table 3

Effect of SBT supplementation on HDL, LDL cholesterol, triglycerides and antioxidant status of rats administered with high fat diet. Values depict Mean±SEM, * denotes $p<0.05$ when compared to control and # denotes $p<0.05$ when compared to control + high fat diet using paired t-test.

Table 4

435 Baseline demographic values of volunteers in different cohorts. Values depict Mean±SD.

436 **Table 5**

437 Physiological measures and Biochemical measurements at baseline and follow up after 30 days
438 of SBT seed oil supplementation. Values depict Mean±SD, * denotes *P*-value < 0.05 when
439 compared to baseline data and # denotes p-value < 0.05 when compared with cohort 3 (placebo
440 group) using t-test. SBP (Systolic Blood Pressure); DBP (Diastolic Blood Pressure).

441 **Table 6**

442 Correlation of Systolic blood pressure in SBT supplemented cohort 2 with cardio-vascular risk
443 factors. *denotes P-value < 0.05 when compared with baseline. 'r' denotes Bivariate Pearson
444 correlation analysis with no adjustment.

445 **Figure 1**

446 Flow chart depicting study design and recruitment of volunteers at baseline and follow-up after
447 30 days of SBT capsule supplementation.

448 **Figure 2**

449 Histopathological studies for sub-chronic toxicity of seabuckthorn seed oil at a dose of
450 2000µl/kg b.w. for 28 days. Panels show representative histological sections of different organs
451 viz., a) Brain b) Lungs c) Kidney d) Spleen e) Liver and f) Heart. Arrows in the figure denote
452 CA3 neurons (CA3), bronchioles (BCL), Proximal Tubule (PT), Glomerulus (GL), red pulp
453 (RP), arteries (A), hepatocytes (HC), bile duct (BD), cardiac muscles (CM) and connective tissue
454 (CT) as observed at a magnification of 10X.

Seabuckthorn seed oil composition	**Quantity**
Fatty acid composition	
Linolenic acid (omega-3, C 18:3) (%)	17.47
Linoleic acid (omega-6, C 18:2) (%)	28.02
Palmitoleic acid (omega-7, C 16:1) (%)	4.89
Oleic acid (omega-9, C 18:1) (%)	26.24
Saturated Fat (%)	21.06
Grand total of composition, %	97.68
Minerals	
Calcium (mmol/L)	0.60
Phosphorous (mmol/L)	0.17
Zinc (mmol/L)	0.01
Iron (mmol/L)	0.04
Magnesium (mmol/L)	0.12
Selenium (mmol/L)	<0.001
Bioactive compounds	
Vitamin E, mg/100g	48.2
Carotene, IU/100g	97584

VARIABLES	VALUE
Age (Years)	35±10
Birth altitude range (Min-Max, M, MSL)	200-400
Duration of stay at high altitude (Years)	01
Education (Years)	12±2
Income per month (INR)	25000 ±5000
Head injury resulting in loss of consciousness	No
Any form of seizers, delirium tremens or convulsions	No
Allergies to medication, foods, animals, chemicals or other agents	No
Lung diseases such as asthma, emphysema, or chronic bronchitis	No
Surgeries or hospitalizations	No
Diabetes	No
Viral hepatitis	No
Dementia/ Memory Impairment	No
Stroke/ Infarction/Cerebral Hemorrhage	No
Kidney disease	No
GERD symptoms	No
Chest pain	No
Congenital heart disease	No
Neurological Problem/ Epilepsy	No
Cancer	No
Heart attack or any heart problem	No
Familial disorders	No

Core Behavioral Measures (%)	**Values**
Core Alcohol Consumption (Section A)	
No Consumption (%)	100
Mild Consumption (%)	Nil
Moderate Consumption (%)	Nil
Severe Consumption (%)	Nil
Core Tobacco Use (Section C)	
No Smoker	96.42%
Mild Smoker (%)	3.7%
Moderate Smoker (%)	Nil
Core Physical Activity(Section P)	
Mild (%)	80
Moderate (%)	14.5
Severe (%)	5.5
Severe Smoker (%)	Nil
Beck Depression Inventory (BDI)(Score)	6±0.5
Lake Louise Score	0
Biochemical Estimations	
Kidney Function Test	
Blood Urea Nitrogen (6-20 mg/dl)	9.3±3.12
Creatinine (0.9-1.3 mg/dl)	1.0±0.11
Liver Function test	
SGOT (15-37 U/L)	27.8±7.20
SGPT (30-65U/L)	40.1±8.11
Blood Glucose	
Fasting Glucose	83.0±13.91

	CONTROL RATS	CONTROL RATS+HFD	CONTROL RATS+HFD+SBT OIL
TOTAL CHOLESTEROL (mg/dL)	75.7±2.10	141.7±1.01*	85.4±2.25*#
TRIGLYCERIDE (mg/dL)	72.6±1.90	131.9±2.63*	86.7±1.88*#
HDL-CHOLESTEROL (mg/dL)	35.9±2.03	51.0±1.53*	68.0±1.23*#
LDLCHOLESTEROL(mg/dL)	24.3±1.46	74.6±1.78*	60.2±2.19*#
TOTAL SERUM ANTIOXIDANT(%)	100.8±11.3	92.4±8.70*	165.6±9.50*#

Parameters	Cohort 1		Cohort 2		Cohort 3	
	Baseline (day 0) N=32	**Follow up (day 30) N= 29**	**Baseline (day 0) N= 37**	**Follow up (day 30) N= 34**	**Baseline (day 0) N= 37**	**Follow up (day 30) N= 31**
AGE	33.4±6.1	34.8±5.9	35.0±7.01	35.7±6.03	36.01±5.3	34.6±6.8
PULSE RATE	81±1.19	81±1.07	85±2.35	84±2.01	86±3.12	85±2.91
WHR	0.9±0.04	0.8±0.03	0.9±0.16	0.9±0.10	0.9±0.06	0.9±0.01
BMI	23.1±1.11	23.1±1.32	24.1±1.75	24.1±1.53	23.8±1.20	23.6±1.44

Parameters	Cohort 1		Cohort 2		Cohort 3	
	Baseline (day 0) N=32	**Follow up (day 30) N= 29**	**Baseline (day 0) N= 37**	**Follow up (day 30) N= 34**	**Baseline (day 0) N= 37**	**Follow up (day 30) N= 31**
SBP	130±8.18	123±6.01*	149±6.47	139±4.15*#	147±5.51	142±4.27
DBP	85±3.15	84±4.69	95±5.59	90±4.52*	93±4.67	91±3.76
Total Cholesterol (mg/dL)	167.5±19.40	146.0±11.54*	236.9±22.97	192.1±27.74*#	223.7±23.90	218.5±24.60
HDL-Cholesterol (mg/dL)	38.0±6.92	36.4±7.48	39.9±9.26	36.8±8.12	38.6±9.01	35.0±7.13
LDL- Cholesterol (mg/dL)	109.3±14.70	91.5±15.12*	155.9±23.32	113.7±25.68*#	150.6±21.31	146.0±23.78
VLDL (mg/dL)	24.3±10.45	22.1±9.24	36.0±8.62	28.6±11.29*	35.7±12.01	29.9±10.98*
Triglyceride (mg/dL)	129.2±32.30	104.3±37.41*	155.1±38.53	142.9±26.35*	152.2±24.91	144.8±30.14*
Oxy-LDL	45.0±7.01	39.8±6.88*	51.9±5.82	43.7±6.29*#	50.6±11.82	47.1±7.29
Chol:HDL	4.4±1.03	4.1±0.68	5.8±1.09	4.9±0.97*#	5.6±2.01	5.4±1.25
LDL:HDL	2.8±0.55	2.6±0.39	3.8±1.63	3.0±1.26*#	3.9±1.04	3.7±1.73
Oxy-LDL:HDL	1.1±0.12	1.0±0.09*	1.4±0.03	1.0±0.05*#	1.3±0.15	1.3±0.23
Homocystiene µmol/L	32.0±14.22	30.1±11.83	38.2±16.57	35.8±14.43	36.2±13.67	34.7±15.66
Total Antioxidant µmol Trolox Equivalent/L	515± 19.33	562.2± 24.03*	498±32.88	532±25.01*#	490±30.11	509.5±35.11*

Variables	Correlation coefficient (r)	p- value
Δ cholesterol	**0.87***	**0.001**
Δ triglyceride	**0.56***	**0.04**
Δ HDL chol	-0.34	0.25
Δ LDL chol	**0.67**	**0.02**