LIPID LOWERING ACTIVITY OF THE FRUIT JUICE OF *HIPPOPHAE RHAMNOIDES L.* (SEABUCKTHORN) IN HYPERLIPIDEMIC MODELS OF WISTAR ALBINO RATS

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Summary

Lipid lowering effect of fruit juice of *Hippohae rhamnoides* L. was evaluated in triton and diet induced hyperlipidaemic models of wistar albino rats. The juice at 0.3 and 0.5ml/kg body weight inhibited the elevation in serum cholesterol and triglyceride levels on Triton WR 1339 administration rats. The fruit juice at the same dose level significantly attenuated the elevated serum total cholesterol and triglycerides with an increase in high-density lipoprotein cholesterol in high-fat diet-induced hyperlipidaemic rats. The standard dose atrovastatin in the former and gemfibrozil in the later studies showed slightly better effects.

Keywords: *Hippohae rhamnoides* L., Antihyperlipidaemic, High-fat diet, Triton WR 1339, HDL-C, LDL-C, TC, CHD.

Introduction

Hyperlipidemia (elevated levels of triglycerides or cholesterol) and reduced high- density lipoproteins (HDL-C) occur as a consequence of several interrelated factors that may be lifestyle, genetic, metabolic or other conditions that influence plasma lipoprotein metabolism[1]. Elevated serum concentrations of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) appear to increase the risk of individual in developing coronary heart disease (CHD) [2].

Lipid lowering therapy is indicated in primary and secondary prevention of cardiovascular diseases in addition to the management of all other risk factors including smoking, diabetes and obesity[3]. The current antihyperlipidemic therapy includes principally statins and fibrates. The former corrects the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and later acts by enhancing the clearance of triglyceride rich lipoproteins[1]. The investigation of lipid lowering activity on nutraceutical will be useful strategy in the discovery of new lead molecules eliciting improved activity by regulating through different mechanism of action. The plant juice maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology.

Hippophae rhamnoides L., a member of the Elaeagnaceae family, is a perennial plant native to Europe and Asia[4] and also widely distributed in the fields of north and east Anatolia[5]. Its fruits are orange colored, sour to taste, single seeded and 3-7 mm in diameter[6] and contain carotenes (a, b, d) vitamins C, E, riboflavin, folic acid, tannins, sugar, glycerides of palmitic, stearic and oleic acids, polyphenols and some essential amino acids[7-9]. Fruits of *H. rhamnoides* L. have been used extensively in traditional medicine in Turkey as well as China and former Soviet Republics to treat constipation, gastric ulcer, skin wounds and influenza infections[5]. Beneficial effects of HRe-1 have been shown in experimental gastric ulcer models[10, 11]. Antioxidant activity of *H. rhamnoides* L. has been shown *in vitro*, cell culture and animal studies. Different fractions of seabuckthorn (*H. rhamnoides* L.) fruits inhibit 2, 2-azobis (2, 4-dimethylvaleronitrile) and ascorbate-iron induced lipid peroxidations *in vitro*[12]. *Hippophae rhamnoides*, as well as vitamin E, decreases the malondialdehyde(MDA) content in hyperlipidemic rabbit serum cultured smooth muscle cells[13]. Seed oil of *Hippophae rhamnoides* L. inhibits MDA formation of liver induced by CCl4, acetaminophen and ethyl alcohol and also prevents the acetaminophen-induced glutathione depletion in liver[14]. The prevention of glutathione depletion by HRe-1 is also reported in gastric tissue in ethanol administered rats[10].

Bioactive oil has also been obtained from the young branches and leaves and has been incorporated into an ointment for treating a wide variety of skin damage, including burns, bedsores, eczema and radiation injury. The oil is also taken internally for diseases of the stomach and intestine. The berries are relatively high in essential fatty acids, which are important for the maintenance of a healthy skin. Seabuckthorn oil absorbs ultraviolet light and since the oil is also known to be useful for promoting skin health, it is particularly suitable for sun-care cosmetics[15]. The plant reported to posses anti ulcer activity and antioxidant activity[16, 17].

Materials and Methods

Plant material: The juice of *Hippophae rhamnoides* (seabuckthorn) was gifted by Redhill Pharmaceutical, Hyderabad, India.

Chemicals and reagent: Triton WR 1339 (Sigma USA) and Folin-Ciocalteaue reagent (Sd fine) were from commercial sources. Serum Cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were determined using kits of Qualigens fine chemicals.

Atorvastatin and Gemfibrozil were obtained from DR. Reddy's.Lab, Hyderabad and Sun pharmaceuticals, India respectively.

Animals: Male wistar strain albino rats (150-250g) were obtained from Central Animal House of Institute of Pharmacy and Technology Salipur, Cuttack, Orissa. The animals were housed under standardized environmental conditions (at normal room temp, with a 12 Hour light and dark cycle) and fed with standard pellet chow feed and water *adlibitum*. The animal protocol was approved by Institutional Animal Ethical Committee of I. P. T., Salipur, Cuttack, Orissa, India with registration number 1053/ac/07/CPCSEA. All the experiments were performed as per the CPCSEA guidelines.

Triton induced hyperlipidemic study[18]

The use of Triton WR 1339 induced hyperlipidemia through accelerated hepatic cholesterol synthesis was suggested as an important approach to screen the action of hypolipidemic drugs[19]. Male wistar rats weighing 200-250g were divided into 4 groups of 6 animals each. Group-1(Vehicle control) received 0.3% w/v carboxy methyl cellulose (CMC) orally for one week. Group 2 and 3 were treated with the juice of seabuckthorn at the dose 0.3 and 0.5ml/kg body weight respectively. The Group 4 received atrovastatin 1mg/kg body weight once daily for one week. On seventh day, 200mg/kg Triton WR 1339 (isoocctyl polyoxyethylene phenol) was injected (ip), to all the four groups of rats immediately after drug administration. Serum total cholesterol and triglycerides were estimated for individual animals in autoanalyser (Microlab 100) on seventh day previous to drug treatment and after 24 hr of Triton administration. Blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes and serum was separated in cooling centrifuge (Remi, C24) by centrifuging at 2500 rpm for 10 min. The observations made were recorded in Table-2.

High-fat diet-induced hyperlipidaemic study[20]

Hyperlipidemia was induced in male wistar rats weighing 150-180g by feeding them with a high fat diet, (Table 1) for 4 weeks. High-fat diet increased the serum cholesterol and triglycerides to about 75-80% of the normal levels and reduced the HDL-C levels significantly (Table-3). The rats with significantly higher values of serum cholesterol and triglyceride values compared to that of normal animals were considered to be hyperlipidaemic and six hyperlipidaemic animals were grouped for one treatment. Group 1received 0.3% w/v CMC and served as vehicle control while group 2 and group 3 hyperlipidemic rats were orally treated with the juice of seabuckthorn at the dose of 0.3 and 0.5ml/kg body weight respectively, once a day for one week. Animals of fourth group of hyperlipidaemic animals were administered the standard drug gemfibrozil 50mg/kg body weight for one week. All the four groups were kept on the same high fat diet throughout drug treatment. Serum total cholesterol, triglyceride and HDL-C of the non-fasted animals were estimated on seventh day after 1hr of dosing. Atherogenic index was calculated using the formula:

$$\text{Atherogenic Index} = \frac{Total\ Cholesterol}{HDL-C}$$

Table 1- Constituents of high fat diet

Ingredients	Quantity(g/100g)
Cornflour	25
Milk power	15
Sucrose	15
Casein	5
Egg yolk	3
Lard	35
Salt mixture	1
Cholesterol	1

Statistical analysis

Data are represented as mean ±SEM (Standard error of mean). The group means were compared for significant difference ($p<0.01$) by Student's t test in triton model and paired t test in diet model.

Results and discussion

The systemic administration of the surfactant Triton to rats resulted in an enormous elevation of serum cholesterol and triglycerides at 24 hr (Table 2). The fruit juice of *H. rhamnoides* inhibited highly significant elevation in cholesterol by 21.729 and 43.311% at 0.3 and 0.5ml/kg dose levels, respectively as compared to that of untreated vehicle control group. Triglyceride level was lowered in *H. rhamnoides* juice treated rats by 19.719 and 51.110 % at 0.3 and 0.5ml/kg doses, respectively in comparison to that vehicle control rats (Table 2). Atrovastatin, the lipid controlling mechanism of which is inhibition of synthesis of cholesterol in the liver, was employed as the standard drug in Triton induced model. The treatment with atrovastatin resulted in a slightly better effect than *H. rhamnoides*. These results indicate that *H. rhamnoides* fruit juice may interfere with cholesterol biosynthesis as Triton accelerates the hepatic synthesis of cholesterol[21].

Triton induced hypercholesterolaemia, though simple and rapid for evaluating hyperlipidemic compounds, is rather artificial. Hence the lipid controlling potential of *H. rhamnoides* fruit juice was further validated in diet-induced hyprlipidemic rat model. When male wister albino rats were kept on high-fat diet supplemented with 1% cholesterol for 4 weeks, there was elevated serum cholesterol levels and triglyceride levels were almost doubled whereas, HDL-C levels were reduced significantly as indicated by low value of atherogenic index (Table 3). Elevated circulating lipid levels may be the outcome of inhibitory effect of high dietary fat intake on lipogenesis[22].

The treatment of hyperlipidemic rats with *H. rhamnoides* fruit juice for one week brought down the elevated serum total cholesterol and triglycerides improving the HDL-C levels as shown by reduced atherogenic index (Table 3). Similar to Gemfirozil (50mg/kg) the standard fibrate drug used, the extract may have enhanced the breakdown of lipids, thus modifying the altered lipid metabolism induced by high fat-diet. Increase in HDL levels and reduction in LDL shows the intensive conversion of LDL to HDL and clearance of circulating lipids. Total cholesterol/HDL-C ratio of > 4.5 is associated with increased coronary heart disease (CHD) risk and the ideal ratio is ≤3.5[23]. A significant reduction in the atherogenic index on *H. rhamnoides* fruit juice treatment demonstrates the protective efficacy of the extract against atherogenesis. Consequently the lipid regulating efficacy of fruit juice of *H. rhamnoides* would be beneficial in the prevention of plaque formation leading to atherosclerosis and CHD accelerated by high fat diets.

Table 2: Effects of the fruit juice of *H. rhamnoides* L. (seabuckthorn) on lipid profile in triton induced study.

Group	Total cholesterol(mg/dl)		Triglycerides(mg/dl)	
	0 hr	24 hr	0hr	24 hr
1	47.0±3.2	182.3±4.2	73.32 ± 5.57	356.2 ± 24.75
2	51.5±2.7	157.4±3.4*	69.30±2.4	296.4 ± 28.66*
3	46.3±2.9	123.0±2.6*	71.2±3.1	209.5 ± 26.34*
4	49.7±1.3	119±4.3*	67.5±7.9	197.3±21.42*

Values are mean±SE of 6 rats in each group
*-P<0.001 compared with vehicle (untreated) control

Table 3: Effects of the fruit juice of *H. rhamnoides* L. (seabuckthorn) on lipid profile of hyperlipidemic wistar rats in diet-induced hyperlipidemia.

Group	Total cholesterol(mg/dl)			Triglycerides(mg/dl)			Atherogenic index (total cholesterol/HDL-C)		
	Normal value	On induction of hyperlipidemia		Normal value	On induction of hyperlipidemia		Normal value	On induction of hyperlipidemia	
		0^{th} day	7^{th} day		0^{th} day	7^{th} day		0^{th} day	7^{th} day
1	51.6±2.6	72.1±3.1	78.6±2.9*	69.8±2.9	132.6±2.1	173.6±4.3*	3.65±0.23	3.87±0.12	5.28±0.27*
2	49.8±2.3	75.6±1.8	64.8±4.3*	71.7±2.3	134.8±2.6	111.3±3.1*	3.37±0.20	4.13±o.o8	3.02±0.07*
3	49.3±2.1	76.3±1.5	55.2±1.2*	68.8±3.7	136.2±2.3	95.32±2.1*	3.17±0.32	4.12±0.17	2.46±0.08*
4	48.3±2.7	75.2±2.6	48.6±1.8*	66.3±3.1	135.4±2.7	76.8±2.7*	3.23±0.26	4.36±0.17	2.75±0.28*

Values are mean ±SE of 6 rats in each group
*- Represent values significantly different in paired t test as compared to 0 day values (P<0.01)

The lipid lowering activity of *H. rhamnoides* fruit juice may be attributed to the phytoconstituents present, such as carotenes (a, b, d), vitamins C, E, tannins & polyphenols. Tannins have also been reported to increase faecal bile acid excretion there by leading to reduction of serum cholesterol levels[23, 24]. Phenolic active principle present in *Anenthum graveolens* were observed to be responsible for lowering TC and LDL-C and elevating HDL-C in hypercholesterolemic rats[25].

The finding of the study reveal that the fruit juice of *H. rhamnoides* can effectively control the blood levels in dyslipidemic conditions by interfering with biosynthesis of cholesterol and utilization of lipids.

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