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Bioavailability of antioxidants from seabuckthorn based food products and its effect on antioxidant system in rats

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Abstract

The wistar strain male albino rats were fed with various seabuckthorn products for a period of 30 days, to determine the bioavailability of antioxidants in seabuckthorn based products (juice powder, jam, squash and fruit yoghurt) and concurrent effect on antioxidant system in rats. The excretion of antioxidants in urine and feces; availability of antioxidants in blood and tissues of liver, heart and kidney; Melondialdehyde (MDA), Glutathione in reduced form (GSH) and antioxidant enzymes such as catalase and superoxide dismutase (SOD) were determined in triplicate. The level of antioxidants in seabuckthorn juice powder were significantly ($p<0.05$) greater than jam, squash and fruit yoghurt. Results also proved that the level of antioxidants in seabuckthorn based products was directly proportional to bioavailability which significantly improved antioxidant system in rats by reducing MDA; increasing the GSH, catalase and SOD levels. Thus the seabuckthorn based products were considered to be an antioxidant rich food source to prevent degenerative diseases.

Introduction

Antioxidants are vitamins, minerals and other compounds found in food that can help slow down or prevent the oxidation process. Antioxidants constitute an important defense system against a variety of diseases and environmental stress. The key role played by antioxidants in the body is their ability to scavenge free radicals via donation of an electron or a hydrogen atom or by deactivation of metal ions and reactive oxygen species. The major health benefits of antioxidants includes: scavenging of the formation of free radicals; retarding the lipid per-oxidation; prevent oxidation of LDL cholesterol; repair damage done to the body cells by free radicals; retarding the aging process; increase immune function and possibly decrease the risk of many diseases.

Seabuckthorn *(Hippophae rhamnoides)*, is a deciduous shrub with yellow or orange fruits (Li and Schroeder, 1996) - a unique plant currently being domesticated in several countries like China, Russia, Germany, Finland, Romania, France, Nepal, Pakistan and India. The plant is reported to have considerable medicinal value (Li and Wang, 1998), being useful for the treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers, cardiovascular diseases and perhaps growth of some tumors. Seabuckthorn berries are among the most nutritious and vitamin-rich fruit found in the plant kingdom because of presence of several natural antioxidants viz., vitamin C, E, carotenoids, anthocyanins and phenols (Chauhan *et al*., 2001).

Metabolism and bioavailability of functional ingredients in foods such as antioxidants has recently attracted attention of many researchers. Food ingredients usually have to undergo the process of cooking, ingestion, absorption and metabolism before being transported to each organ and tissue in the body. They are being exposed to seasonings and also heat during cooking, and then to acidic gastric juice, alkaline intestinal juice etc., in the digestive system. They have to be stable consequently under these conditions for their effective uptake into the body. Most of the minor functional ingredients of plant origin can be "strangers" to the human body and may not absorbed in an intact form due to conjugation and enzymatic degradation when they pass through the highly selective digestive and metabolic system. It is therefore, essential to evaluate their biological functions to know whether the compounds actually reach the tissues where they could desirably exert their activities after clearing the obstacles. Antioxidant enzymes provide an important defense against free radicals. They play a prominent role in maintaining the cellular integrity. Among the various antioxidant enzymes, SOD (superoxide dismutase) and catalase are one of the most important one. Another line of defense in reducing hepatic cellular injury is the GSH redox system (Junqueira *et al*., 1986). These antioxidant enzymes will neutralize many types of free radicals. Supplement of these enzymes from source of antioxidant rich foods

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will reduce the energy of the free radicals, stop the free radical from forming in the first place and also interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. Therefore the bioavailability of antioxidants in Seabuckthorn fruit was assessed to study the effect of these products on status of antioxidant enzymes in body system which can be judged over by continuous feeding of rats.

Materials and Methods

Materials

Seabuckthorn berries *(Hippophae rhamnoides)* were brought from Field Research Laboratory (Leh, Himachal Pradesh, India) by airlifting and kept frozen at (-20˚C) until further analysis studies. All other chemical reagents were procured from E. Merck and Loba Chemicals Limited, Mumbai, India.

Development of seabuckthorn products

The seabuckthorn products viz. jam and squash were developed as per the procedure of Girdharilal *et al.* (1960), fruit yoghurt was developed as per the standard protocol of Sukumar De (1960) and fruit juice powder as per the procedure of Abadio *et al.* (2004).

Animals and Diet

A total of thirty Wistar strain male albino rats weighing between 100-120 g were randomly divided into 5 groups of six each. The first group was fed with control casein diet .The second group was fed with seabuckthorn jam, third group with seabuckthorn squash, fourth group with seabuckthorn fruit yoghurt and fifth group with seabuckthorn fruit juice powder. The seabuckthorn products were fed at a level of 10 g along with conventional balanced casein diet (10 g) for respective group of rats and for control group, which was fed at 10 g casein diet for a period of 30 days. The animals had a free access to water and food was adlibitum. The food intake of each rat was recorded daily. The animals were kept in accordance with the laboratory animal guidelines on the care and use of animals.

Collection of faeces, urine, blood and organ samples

On 28th day, animals were shifted from stainless steel cages to metabolic cages for collection of faeces and urine samples. The faeces were collected in petri dishes for 2 days, blended, dried at 60°C, weighed and powdered in a mixer grinder for further analysis. The urine samples were collected in conical flasks for 2 days, filtered and analyzed.

The rats were fasted for 12 hrs before sacrifice. Rats were anesthetized with anesthetic ether and sacrificed. Blood was removed directly from heart and transferred into tubes. Heart, liver and kidney were excised and washed with ice cold physiological saline (0.92%) to remove the adhering blood, pressed dry with a filter paper, weighed and transferred into paper foil polyethylene pouches and were kept frozen (-24°C) until further analysis. The determination of antioxidants in seabuckthorn based products viz. total phenol was estimated as per the procedure of Jayaprakasha *et al*. (2003); vitamin E and vitamin C contents as per the method of Desai (1984) and Ranganna (1986), respectively.

Biochemical analysis

The total phenol content in tissues, urine and faeces were determined as per the procedure of Jayaprakasha *et al*. (2003). Vitamin E content of tissues, urine and faeces were determined according to procedure described by Desai (1984). The vitamin C in tissues, urine and faeces were determined by the method of Roe and Keuther (1943). The probability of absorption of each antioxidant in body was calculated based on difference from quantity of antioxidants fed and their losses in urine and faeces. Liver total reducing thiols glutathione (GSH) were determined according to the method of Ellman (1958). The concentration of malondialdehyde (MDA) was estimated as per the procedure of Girotti and Deziell (1983). Catalase was assayed in tissue according to the method described by Cohen *et al.* (1970). Superoxide dismutase (SOD) was estimated as per the procedure of Flohe and Otting (1984).

Statistical analysis

All determinations were in triplicate and data were subjected to analysis of variance (ANOVA) using Microsoft Excel 2000 (Microsoft Corporation, Washington, USA). LSD was used as post hoc comparison to specify the difference in mean within group and the difference in mean was indicated with different alphabets.

Results and Discussion

Antioxidants content of various seabuckthorn products and its daily intake

The Table 1 show the antioxidants i.e. total phenols, vitamin C and vitamin E intake / day by rat from the seabuckthorn products, their probability of absorption, loss through urine and faeces. The intake of total phenols, vitamin C and E per day was higher for fruit juice powder incorporated diet followed by jam, squash and fruit yoghurt diet from 10 g each of

Table 1. Total phenol, vitamin C and vitamin E content of various seabuckthorn products and its daily intake

Product	Total phenols (mg/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)	Quantity of products fed (g)	Phenols (mg) intake in 10 g diet*	Loss in urine mg/dl/	Loss in faeces mg/dl/	Probability of absorption 10 gram diet*	Vitamin C (mg) intake in mg/dl/ day	Loss in urine mg/dl/ day	Loss in faeces mg/dl/ day	Probability of absorption in body (mg)	Vitamin E (mg) intake in 10 gram diet*	Loss in urine mg/dl/ day	Loss in faeces mg/dl/ day	Probability of absorption in body (mg) diet*
Jam	226.8 ± 2.0	175.1 ± 0.6	76.2 ± 2.0	10.0	22.7[a]	2.2±0.6[b]	2.5 ±0.4[b]	18.0[a]	17.5 [a]	1.7 ± 0.3 [b]	1.9 ± 0.4 [b]	13.9 [a]	7.6 [a]	0.7 ± 0.3 [b]	0.8 ± 0.4 [b]	6.1 [a]
Squash	136.1 ± 2.0	105.1 ± 0.5	45.7 ± 1.9	10.0	13.6 [a]	1.5 ± 0.5 [b]	1.9±0.7 [b]	10.2 [a]	10.5 [a]	1.1 ± 0.5 [b]	1.5 ± 0.6 [b]	7.9 [a]	4.6 [a]	0.5 ± 0.2 [b]	0.6 ± 0.3 [b]	3.5 [a]
Fruit yoghurt	24.5 ± 1.8	20.9 ± 0.6	12.0 ±1.8	10.0	2.4 [a]	0.2 ± 0.1 [b]	0.5 ± 0.2 [b]	1.7 [a]	2.1 [a]	0.2 ± 0.1 [b]	0.5 ± 0.2 [b]	1.4 [a]	1.2 [a]	0.1 ± 0.1 [b]	0.3 ± 0.1 [b]	0.8 [a]
Juice powder	453.6 ± 1.1	384.2 ± 0.6	152.4 ± 1.8	10.0	45.4 [a]	4.5± 0.9 [b]	5.0± 0.8 [b]	35.9 [c]	38.4 [a]	3.8 ± 0.6 b	4.2 ± 0.8 [b]	30.4 [c]	15.2 [a]	1.5 ± 0.4 [b]	1.7 ± 0.6 [b]	12.0 [a]
Control	ND	ND	ND	Nil	Nil	ND	ND	Nil	Nil	ND	ND	Nil	Nil	ND	ND	Nil

*Only 10g diet of seabuckthorn products was mixed with 10g diet of control without total phenol, vitamin C and vitamin E
Mean values in the same row bearing the common superscript do not differ significantly (p>0.05)
ND: Not detected

Table 2. Effect of feeding of seabuckthorn products on total phenol content, vitamin C and vitamin E content ** in tissues after 30 days

	Total phenol				Vitamin C				Vitamin E			
Group*	Blood** (µg/dl)	Liver** (µg/g)	Heart** (µg/g)	Kidney** (µg/g)	Blood** (µg/dl)	Liver** (µg/g)	Heart** (µg/g)	Kidney** (µg/g)	Blood** (µg/dl)	Liver** (µg/g)	Heart** (µg/g)	Kidney** (µg/g)
Jam	110.2 ± 1.2[a]	32.0 ± 1.7[b]	7.1 ± 1.0[c]	10.3 ± 1.5[c]	79.2 ± 1.9[a]	29.8 ± 1.2[b]	6.4 ± 1.0[c]	7.2 ± 1.4[c]	35.1 ± 1.5[a]	13.4 ± 1.4[b]	2.8 ± 0.7[c]	3.0 ± 0.9[c]
Squash	48.2 ± 1.3[a]	28.2 ± 1.2[b]	5.4 ± 1.1[c]	8.2 ± 0.9[c]	43.2 ± 1.8[a]	18.2 ± 1.4[b]	3.4 ± 0.7[c]	4.2 ± 1.1[c]	19.2 ± 1.0[a]	8.9 ± 1.3[b]	1.2 ± 0.5[c]	2.2 ± 0.7[c]
Fruit yoghurt	10.4 ± 1.0[a]	3.0 ± 0.8[b]	ND	1.1 ± 0.6[b]	9.0 ± 1.0[a]	2.0 ± 0.8[b]	ND	1.0 ± 0.6[b]	5.0 ± 1.2[a]	1.0 ± 0.5[b]	ND	ND
Juice powder	221.6 ± 1.8[a]	64.2 ± 1.6[b]	14.3 ± 1.7[c]	19.2 ± 1.4[c]	174.8 ± 1.6[a]	63.1 ± 1.3[b]	12.2 ± 1.2[c]	16.3 ± 1.7[c]	70.0 ± 1.8[a]	25.3 ± 0.8[b]	5.1 ± 1.0[c]	6.0 ± 1.1[c]
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

*No of rats=6
** Mean ± SD of triplicate analysis
Mean values in the same row bearing the common superscript do not differ significantly (p>0.05)
ND: Not detected

Table 3. Effect of feeding of seabuckthorn products on antioxidant system** in rat liver

Groups*	MDA n mol/g	GSH m moles / g	SOD x10^2 ***	Catalase x 10^4 n mol/g
Jam	0.87 ± 0.23 ^{a}	15.70 ± 0.86 ^{a}	1.62 ± 0.10 ^{a}	0.42 ± 0.001 ^{a}
Squash	0.92 ± 0.17 ^{a}	15.25 ± 0.94 ^{a}	1.58 ± 0.18 ^{a}	0.30 ± 0.002 ^{a}
Fruit yoghurt	0.97 ± 0.19 ^{a}	15.01 ± 0.57 ^{a}	1.51 ± 0.12 ^{a}	0.25 ± 0.005 ^{a}
Juice powder	0.70 ± 0.26 ^{a}	16.25 ± 0.45 ^{a}	1.90 ± 0.11 ^{a}	0.62 ± 0.006 ^{a}
Control	1.27 ± 0.20 ^{b}	14.82 ± 0.39 ^{b}	1.40 ± 0.14 ^{b}	0.18 ± 0.004 ^{b}

*No of rats=6
** Mean ± SD of triplicate analysis
Mean values in the same column bearing the common superscript do not differ significantly ($p>0.05$)
*** Units / min / mg protein

seabuckthorn products mixed in the diet.

The excretion of antioxidants was found to be slightly more in faeces when compared to urine in all groups, this might be because of metabolization and excretion of antioxidants through bile (Augustin and Gary, 2000). The level of excretion of total phenols, vitamin C and E was in accordance with the level of intake in the diet by rats.

Effect of feeding of seabuckthorn products on food intake of rats

The jam group recorded more food intake and weight gain/ day followed by squash, juice powder, fruit yoghurt and control groups at a given quantity of the products fed (Figure 1). There was no significant difference ($p>0.05$) in the food intake among the groups, which suggests that the incorporation of seabuckthorn based products did not influence the daily average food intake by rats.

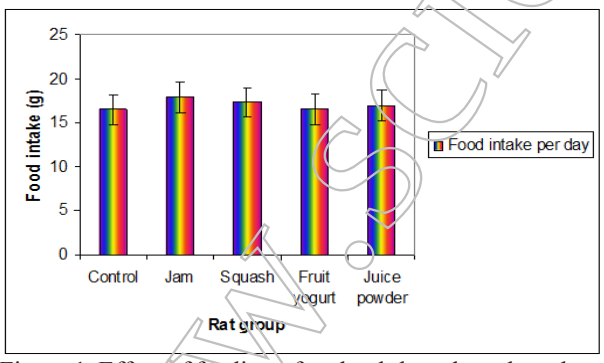

Figure 1. Effect of feeding of seabuckthorn based products on average food intake of rats/day

Bioavailability of antioxidants from seabuckthorn food products

The level of total phenols, vitamin C, and E in tissues of different rat groups after 30 days of feeding period (Table 2) suggests that the retention of these antioxidants in blood were found to be more significantly ($p<0.05$) and also in accordance with the level of intake which may be due to presence of more amounts of metabolites that are formed in the body tissues especially in plasma or by the colonic microflora (Augustin and Gary, 2000). Result also suggests that the metabolites formed in various body tissues as a result of continuous intake may significantly contribute to the antioxidant capacity (Augustin and Gary, 2000). Kim *et al*. (2000) and Henning *et al.* (2006) were also reported similar results of bioavailability of tea polyphenols in rat tissues after continuous feeding; Franke *et al.* (2005) also reported similar bioavailability of vitamin C from orange juice in human tissues; Mitchell *et al.* (1996) were also reported similar bioavailability of vitamin E from fortified breakfast cereals in rat tissues after continuous feeding respectively.

Effect of feeding of seabuckthorn products on antioxidant system in rats

The feeding of seabuckthorn based products reduced the MDA levels with a concomitant increase in the activity of antioxidant enzymes (Table 3). Among the various seabuckthorn products fed, the juice powder group significantly showed more MDA reduction followed by jam, squash and fruit yoghurt when compared to control group. This exhibited the hepatoprotective effect of the seabuckthorn products as reported by Bao *et al.* (2008). The feeding of seabuckthorn products significantly ($p<0.05$) helps in enhancing the liver GSH and antioxidant enzymes such as SOD and catalase when compared to control. Similar effect also predicted by Anurag *et al.* (2007) by feeding curcumin. The activity of antioxidant enzymes increased significantly more for seabuckthorn product fed group i.e. juice powder group followed by jam, squash and fruit yoghurt when compared to control group. Bao *et al.* (2008) and Anurag *et al.* (2007) were also reported similar results of significant decrease in MDA levels and

increase in antioxidant enzymes levels viz., GSH, SOD and Catalase as a result of feeding of bilberry and curcumin.

Conclusion

The continuous feeding of seabuckthorn based products indicated good bioavailability of the antioxidants and the metabolites formed significantly contributed to the antioxidant capacity. Thus it was proved that the consumption of antioxidants from seabuckthorn will help to provide the body with the most complete protection against free radical damage. In a nutshell, these seabuckthorn products could be safely claimed to exert health benefits by enhancing the antioxidant status of the body of consumers.

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