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ORIGINAL ARTICLE

Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats

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Abstract Hypolipidemic and antioxidant potency of a dehydrated onion product was evaluated in experimental rats maintained for 6 weeks at 5 and 10% dietary levels. Serum cholesterol especially low-density lipoprotein was significantly reduced by dietary dehydrated onion in hypercholesterolemic rats. This was associated with an increase in high density lipoprotein cholesterol. Blood triglyceride concentration in hypercholesterolemic rats was lower in onion supplemented diet group. Glutathione, ascorbic acid and α-tocopherol in the blood of hypercholesterolemic rats were higher in onion treatment, while lipid peroxides were lower. Hepatic α-tocopherol concentration was higher in rats maintained on onion diets, while lipid peroxides were reduced. Thus, this study has proved significant cholesterol lowering and antioxidant effect of dehydrated onion product.

Keywords Dehydrated onion · Lipid profile · Hypolipidemic effect · Antioxidants

Introduction

The role of serum cholesterol levels and oxidation of low-density lipoprotein in the etiology of atherosclerosis and coronary heart disease are well known. In view of this, there has been a continuous search for hypocholesterolemic and antioxidant food adjuncts. Some of the commonly used spices have been evaluated as possible hypocholesterolemic adjuncts in a variety of experimental situations in animals and humans. The spices – fenugreek, garlic, ginger, onion, red pepper and turmeric are effective as hypocholesterolemic adjuncts under conditions of experimentally induced hypercholesterolemia and hyperlipidemia. Studies in which spices have been shown to influence cholesterol or triglyceride levels in blood and liver, fecal excretion of sterols and bile acids and biliary secretion of cholesterol and bile acids have been recently reviewed (Srinivasan et al. 2004).

Onion (*Allium cepa*) which is extensively used as spice and as vegetable has a strong flavour due to sulphur compounds, one of which has also been identified as lachrymatory factor (Fenwick and Hanley 1985). Hypocholesterolemic activity of onion in experimental rats and rabbits as well as in humans has been reported (Srinivasan et al. 2004). Fresh onion, onion juice, essential oil and aqueous extract of onion have been examined in these studies. Decreased blood cholesterol concentration has been generally observed in a majority of these studies under conditions of normal diet high-fat diet, and high cholesterol diet. Among the other health beneficial effects of onion, decreased blood fibrinolytic activity observed in rabbits fed a high cholesterol diet was effectively countered by dietary onion juice in addition to hypocholesterolemic effect (lowering of blood fibrinogen concentration) (Sharma et al. 1975a, b). The pronounced crenation and aggregation of erythrocytes in rabbits fed a high-cholesterol diet was reversed by dietary onion extract (Vatsala and Singh 1981, 1982). Enrichment of erythrocyte membranes with cholesterol was suppressed by dietary onion in hypercholesterolemic rats which was accompanied by changes in the erythrocyte membrane enzymes – alkaline and acid phosphatase, 5'-nucleotidase, total and Mg^{2+}-ATPase (Singh and Kanakaraj 1984). Significant reduction in experimentally induced hypertriglyceride levels in blood, aorta and liver has been documented in rabbits as a result of administration of onion extract (Sebastian et al. 1979). The effects of onion have been ascribed to its sulphur compounds, which oxidize thiol compounds either present as free or in combination with protein. Hypolipidemic effect of dietary onion has been evidenced in streptozotocin diabetic rats (Babu and Srinivasan 1997).

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Amelioration of diabetic renal lesions by dietary onion owing to this beneficial hypocholesterolemic influence has also been documented (Babu and Srinivasan 1999). Flavonoid compounds present in plant foods have been increasingly understood to bestow antioxidant properties. Quercetin is the major flavonoid present in onions.

A dehydrated onion product was developed at this Institute which involved homogenization of the onion pulp along with corn starch and gum acacia followed by drum drying. This product retained the major flavonoids present in onion–quercetin and kaempferol to the maximum extent (30 mg/ 100 g dehydrated onion). The objective of this animal study was to evaluate the hypolipidemic and antioxidant potency of this dehydrated onion product in experimental rats.

Materials and methods

Dehydrated onion powder was developed at Department of Lipid Science and Traditional Foods of CFTRI, Mysore from locally available onion (*Allium cepa*; Bellary red variety) and the fresh onion powder produced was used in this study. All fine chemicals used were from M/s. Sigma-Aldrich Co., St.Louis, USA. All other chemicals and solvents used were of analytical grade obtained from M/s Qualigen Chemicals, Mumbai, India. The solvents were distilled before use.

Animal treatment: Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with standard guidelines and with due approval from this Institute's Animal Ethics Committee. Male 'Wistar' rats (8 per group) weighing 110-115 g each and housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 6 weeks. The basal diet consisted of (%): casein 21; cane sugar 10, corn starch 54, NRC vitamin mixture 1, Bernhardt-Tommarelli modified national research council (NRC) salt mixture 4 and refined peanut oil 10. The hypercholesterolemic diet consisted of 0.5% cholesterol and 0.125% bile salts at the expense of an equivalent amount of corn starch in the basal diet wherein the peanut oil was also replaced with hydrogenated vegetable fat. The test material was incorporated into the basal diet / high-cholesterol diet at 5 and 10 g/100 g replacing an equivalent amount of corn starch. At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. Blood was collected by heart puncture and serum was separated by centrifugation. Liver was quickly excised, weighed and stored frozen till lipid extraction.

Lipid profile: Total lipids were extracted according to Folch et al. (1957) and estimated gravimetrically. Cholesterol (Searcy and Bergquist 1960), triglycerides (Fletcher 1968) and phospholipids (Stewart 1980) were determined in the lipid extracts of serum and liver. Serum cholesterol and triglyceride associated with high-density lipoprotein (HDL) fraction were determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of Warnick and Albers (1978) low-density lipoprotein – very low-density lipoprotein (LDL-VLDL) precipitate was extracted with chloroform: methanol (2:1 v/v) and used for cholesterol and triglyceride determination.

Lipid peroxides: Plasma lipid peroxides were estimated by the fluorimetric measurement of thiobarbituric acid complex (Yagi 1984). The fluorimetric measurement was carried out at an excitation wavelength of 515 nm and emission wavelength of 553 nm and compared with the standards prepared by reacting 0.5 nmole 1,1,3,3-tetraethoxy-propane with thiobarbituric acid reagent. Lipid peroxide in liver tissue was determined by the method described by Ohkawa et al. (1979) involving photometric measurement of thiobarbituric acid complex extracted into butanol. Absorbance of butanol extract was measured at 532 nm and compared with that of standard tetraethoxypropane treated similarly.

Antioxidant molecules: Total thiols in blood plasma and liver were measured spectrophotometrically by using Ellman's reagent according to the method described by Sedlak and Lindsay (1968). Glutathione in blood plasma/ liver was estimated by using Ellman's reagent according to Beutler et al. (1963). Ascorbic acid was estimated spectrophotometrically by measuring the 2,4-dinitrophenyl-hydrazone derivative of dehydroascorbic acid according to Omaye et al. (1973). α-Tocopherol in liver and blood plasma was determined by the HPLC method described by Zaspel and Csallany (1983) using ODS C18 column and an UV-visible detector (295 nm) and a solvent system consisting of acetonitrile-methanol (1:1).

Statistical analysis: Results are expressed as mean ± SEM and comparisons between groups were made by means of an unpaired Student's t-test Snedecor and Cochran (1976). Differences were considered significant at $p < 0.05$.

Results and discussion

Blood lipid profile: There was a 162% increase in blood total cholesterol concentration as a result of high-cholesterol feeding to rats (Table 1). This increase in circulatory cholesterol in high-cholesterol feeding was predominantly seen in the LDL-fraction. Total cholesterol in serum was significantly reduced in hypercholesterolemic rats maintained on 10 and 5% onion diets. The extent of reduction was 20.7 and 23.6% in 10 and 5% onion diet groups. The reduction in blood cholesterol was seen essentially in the LDL-fraction of serum cholesterol (33 and 37% decrease in the 5 and 10% onion groups, respectively). Another favourable effect of dietary onion in hypercholesterolemic animals was an increase in HDL-cholesterol in blood. This increase in HDL-cholesterol of hypercholesterolemic rats was ~11% (p0.05). Moderate decreases in total cholesterol (12%) and LDL-cholesterol (23%) as a result of dietary onion at 10% level in the serum of normal rats was also observed.

Blood triglyceride concentration of hypercholesterolemic rats was significantly lower in both 10 and 5% onion treated groups compared to corresponding control (Table 1). The decreases in serum total triglyceride concentration were ~31% in onion powder treated rats. This reduction was seen predominantly in the LDL-fraction (26 and 22% in 2 onion groups, respectively). Blood triglyceride level remained unchanged in normal rats as a result of onion treatment. Blood phospholipid was higher in normal rats under onion treatment as compared to respective controls. On the other hand, blood phospholipid concentration was significantly ($p \leq 0.05$) lower in hypercholesterolemic rats under onion treatment as compared to the respective control.

Liver lipid profile: Hypercholesterolemic rats featured higher concentration of cholesterol, triglyceride and total lipid in the liver compared to their normal counterparts (Table 2). Hepatic cholesterol was slightly higher in hypercholesterolemic rats as a result of onion treatment at 5% level. This increase over the control was also reflected in the total lipid content of liver tissue as a result of onion treatment at 5% level. Such an effect of onion on hepatic cholesterol or total lipid was, however, not evidenced when fed at 10% dietary concentration. Hepatic triglyceride and phospholipid concentrations were unaffected by onion treatment in both normal and hypercholesterolemic rats.

Decreased cholesterol concentration both in blood as well as in liver has been reported in rats maintained on normal diet by treating with onion (Bakhsh and Chugtai 1985) and aqueous extract of onion (Augusti and Mathew 1973). Similarly, decreased cholesterol concentration both in blood as well as in liver has been reported in rats maintained on high-sucrose diet and treated with essential oil of onion (Adamu et al. 1982) and in rats maintained on high-fat diet and treated with allylpropyl disulfide (Wilcox et al. 1984). Decreased blood cholesterol has been evidenced in rabbits maintained on high-cholesterol diet and treated with onion (Sharma et al. 1975a, Sainani et al. 1979a), onion juice (Jain 1976) or essential oil of onion (Bordia et al. 1975). Decreased blood and liver triglyceride level has been reported as a result of onion treatment to rabbits maintained on high-sucrose diet (Sebastian et al. 1979). Lowered blood cholesterol levels have been evidenced as a result of onion treatment in clinical trials involving normal subjects (Bhushan et al. 1977, Sharma and Sharma 1979, Sainani et al. 1979b) and in hyperlipemic subjects (Jain and Andleigh 1969, Jain 1971, Bordia et al. 1974, Sharma et al. 1975c).

Sharma et al. (1975a, b) have studied the effect of onion juice on rabbits fed a high-cholesterol diet. Rabbits fed high-cholesterol diet for 24 weeks showed elevated levels of serum cholesterol and of plasma fibrinogen and decreased fibrinolytic activity. Addition of onion juice to the diet reversed these changes. The elevated levels of serum cholesterol and plasma fibrinogen were reduced, and blood fibrinolytic activity increased. Onion juice at a dose equivalent to 25 g of onion/kg body weight/day, when incorporated in the high-cholesterol diet prevented rise in serum cholesterol.

Effects of an aqueous extract of onion on the sucrose fed rabbits have been investigated (Sebastian et al. 1979). Long term administration of sucrose significantly increased

Table 1 Influence of dietary dehydrated onion on blood lipid profile

Animal group	Cholesterol			Triglycerides			Phospholipids
	Total	LDL-VLDL	HDL	Total	LDL-VLDL	HDL	
Basal - Control	55.3 ± 0.40	35.8 ± 2.90	20.6 ± 0.19	113.9 ± 4.43	66.6 ± 2.99	47.3 ± 1.64	103.2 ± 2.12
Basal - Onion (10%)	48.5 ± 0.57**	27.4 ± 2.45**	20.2 ± 0.59	121.6 ± 5.89	76.9 ± 4.77	44.7 ± 1.46	112.0 ± 2.80*
Basal - Onion (5%)	52.9 ± 1.35	31.8 ± 3.09	21.2 ± 1.14	110.2 ± 3.70	78.1 ± 3.37	44.2 ± 1.23	122.1 ± 5.36*
HCD - Control	145.2 ± 4.62	117.8 ± 2.71	27.4 ± 0.57	148.6 ± 4.11	99.8 ± 3.71	48.8 ± 3.90	108.8 ± 3.74
HCD - Onion (10%)	115.2 ± 4.25**	84.8 ± 6.64**	30.4 ± 0.84*	117.7 ± 3.61**	73.5 ± 2.06**	44.2 ± 1.22	92.5 ± 2.06**
HCD - Onion (5%)	110.9 ± 4.15**	80.6 ± 3.98**	30.3 ± 1.15*	116.8 ± 1.81**	77.9 ± 2.78**	38.9 ± 4.30	87.0 ± 4.23**

Values expressed as mg/dl are mean ± SEM of 8 animals in each group
Significant *increase and **decrease compared to corresponding control.

Table 2 Influence of dietary dehydrated onion on hepatic lipid profile

Animal group	Total lipids	Cholesterol	Triglycerides	Phospholipids
Basal - Control	45.4 ± 2.63	4.41 ± 0.22	12.3 ± 0.86	14.6 ± 0.83
Basal - Onion (10%)	50.5 ± 2.40	5.04 ± 0.38	14.0 ± 1.43	15.4 ± 0.50
Basal - Onion (5%)	51.2 ± 3.90	4.88 ± 0.11	13.7 ± 1.30	16.4 ± 0.89
HCD - Control	146.4 ± 5.56	55.7 ± 1.20	33.4 ± 1.48	14.5 ± 0.64
HCD - Onion (10%)	152.1 ± 6.21	58.6 ± 1.93	30.5 ± 1.75	14.8 ± 0.75
HCD - Onion (5%)	162.4 ± 4.09*	62.5 ± 1.79*	29.6 ± 2.14	13.1 ± 1.01

Values expressed as mg/g fresh tissue are mean ± SEM of 8 animals in each group
* Significant increase compared to corresponding control

triglyceride levels in normal rabbits while administration of onion extract significantly reduced serum, liver and aorta triglycerides and serum and liver proteins. The effects of onion have been ascribed to its sulphur containing principles, which oxidize thiol compounds either present free or combined in protein. Babu and Srinivasan (1997) have evidenced hypolipidemic effect of dietary onion in streptozotocin diabetic rats. Amelioration of diabetic renal lesions by dietary onion owing to this beneficial hypocholesterolemic influence has also been documented (Babu and Srinivasan 1999).

Antioxidant molecules in blood: Plant foods contain flavonoids and phenolic acids, which have antioxidant activity. Onion is a source of flavonoids, the flavonoids being, quercetin 4'-O-β-glucoside and quercetin 3,4'-O-β-diglucosides. There are only limited reports on the antioxidant potential of onions.

Total thiols in the blood of hypercholesterolemic rats were profoundly higher in onion treatment at 5 and 10% levels as compared to the respective controls (Table 3). This increase in total thiol concentration was 106 and 260% in 10 and 5% onion groups, respectively. Thus, onion at 5% dietary level produced a higher effect on blood total thiol concentration in hypercholesterolemic rats than when fed at 10% level. However, higher blood total thiol concentration (49%) was evidenced only in 10% onion treatment in the case of normal rats. Blood glutathione concentration was significantly increased by 10% onion in hypercholesterolemic rats, the increase being around 18%. α-Tocopherol concentration was significantly higher in hypercholesterolemic rats as a result of onion treatment (1.16 and 1.12 μg/dl, respectively, as compared to 0.953 μg/dl in the corresponding control). α-Tocopherol concentration was also significantly higher in normal rats under 10% onion treatments, the increase being around 23%. Ascorbic acid concentration was also significantly higher in hypercholesterolemic animals fed dehydrated onion (31 and 35% increase over corresponding control). Blood ascorbic acid concentration was similarly higher as a result of onion treatment even in normal rats (by 23 and 22% in 2 respective onion groups). Blood lipid peroxides were lowered by 15-16% in animals fed 10% dehydrated onion in both hypercholesterolemic and normal rats.

Antioxidant molecules in liver: Hepatic α-tocopherol concentration was significantly higher in normal rats maintained on dehydrated onion containing diets, but not in hypercholesterolemic animals (Table 4). This increase in α-tocopherol concentration produced by 10 and 5% onion diet was 53 and 77% in the respective experimental groups. Liver ascorbic acid concentration was higher under onion treatment in both normal (31 and 43% increase) and hypercholesterolemic (25 and 17% increase) animals. On the other hand, hepatic glutathione concentration was lower in experimental rats maintained on dehydrated onion containing diet at 10% level. This decrease being 26 and 20%, respectively in normal and hypercholesterolemic rats. Liver lipid peroxides were significantly lower in rats maintained on onion diets, the decrease being 24–26%.

Table 3 Influence of dietary dehydrated onion on blood lipid peroxides and antioxidant molecules

Animal group	Lipid peroxides, μmole/dl	Total thiols, mmole/dl	Glutathione, mg/dl	α-Tocopherol, μg/dl	Ascorbic acid, mg/dl
Basal - Control	0.725 ± 0.040	18.6 ± 3.20	0.366 ± 0.030	0.768 ± 0.032	0.78 ± 0.030
Basal - Onion (10%)	0.607 ± 0.038**	27.7 ± 2.70*	0.298 ± 0.034	0.943 ± 0.059*	0.96 ± 0.046*
Basal - Onion (5%)	0.691 ± 0.072	17.2 ± 2.70	0.310 ± 0.027	0.683 ± 0.044	0.95 ± 0.050*
HCD - Control	0.506 ± 0.034	7.6 ± 1.96	0.271 ± 0.013	0.953 ± 0.050	0.89 ± 0.060
HCD - Onion (10%)	0.430 ± 0.019**	15.6 ± 3.00*	0.320 ± 0.023*	1.160 ± 0.065*	1.17 ± 0.030*
HCD - Onion (5%)	0.546 ± 0.014	27.3 ± 0.65*	0.277 ± 0.016	1.120 ± 0.043*	1.20 ± 0.020*

Values are mean ± SEM of 8 animals in each group
Significant *increase and **decrease compared to corresponding control.

Table 4 Influence of dietary dehydrated onion on hepatic lipid peroxides and antioxidant molecules

Animal group	Lipid peroxides, mmole/mg protein	Total thiols, mmole/mg protein	Glutathione, μmole/mg protein	α-Tocopherol, μmole/g liver	Ascorbic acid, μmole/mg protein
Basal - Control	2.35 ± 0.07	0.844 ± 0.042	0.667 ± 0.055	16.7 ± 2.79	0.397 ± 0.025
Basal - Onion (10%)	1.74 ± 0.12**	0.738 ± 0.048	0.496 ± 0.020**	25.5 ± 2.33*	0.521 ± 0.018*
Basal - Onion (5%)	1.79 ± 0.05**	0.928 ± 0.060	0.685 ± 0.034	29.6 ± 1.89*	0.568 ± 0.015*
HCD - Control	1.22 ± 0.06	0.761 ± 0.027	0.628 ± 0.027	44.6 ± 6.00	0.466 ± 0.018
HCD - Onion (10%)	1.32 ± 0.05	0.854 ± 0.038*	0.500 ± 0.017**	43.3 ± 1.83	0.583 ± 0.033*
HCD - Onion (5%)	1.45 ± 0.08	0.913 ± 0.050*	0.595 ± 0.032	42.3 ± 2.06	0.543 ± 0.036*

Values are mean ± SEM of 8 animals in each group
Significant *increase and **decrease compared to corresponding control.

Yamamoto et al. (2005) have recently reported that Welsh onion reduces superoxide generation in rats fed with the high-fat high-sucrose diet when included at 5% level. Shon et al. (2004) have demonstrated that the antimutagenic and antioxidant properties of ethyl acetate extract of red, yellow and white onion against mutagens were related to their phenols and flavonoids which are heat stable. Antioxidant effects of S-methyl cysteine sulphoxide isolated from onion were studied in alloxan-diabetic rats after treating for two months (Kumari and Augusti 2002). It lowered the levels of malondialdehyde, hydroperoxide and conjugated dienes in tissues exhibiting antioxidant effect on lipid peroxidation in experimental diabetes. This is achieved by their stimulating effects on glucose utilization and the antioxidant enzymes, viz. superoxide dismutase and catalase. The ability of flavonoids, especially quercetin to inhibit low-density lipoprotein oxidation *in vitro* has been demonstrated (O'Reilly et al. 2000).

In normal rats, feed intake in onion-10% and onion-5% animal groups were comparable to controls throughout the experimental regimen. On the other hand, feed intake in onion-10% and onion-5% animal groups were lower (13.4 and 13.0 g/day, respectively) than their control counterparts in hypercholesterolemic rats (16.9 g/day) during 5th to 8th week. Body weight gain was moderately lower as a result of 10% onion powder diet in hypercholesterolemic rats. Liver weight was not affected as a result of onion treatment.

In conclusion, the present animal study has evidenced the health beneficial hypolipidemic potency of dehydrated onion product. The beneficial influence of dehydrated onion product on the antioxidant molecules both in circulation as well as in liver is also evidenced here.

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