Australian Journal of Basic and Applied Sciences, 5(8): 1344-1353, 2011
ISSN 1991-8178

Effects of Dietary Consumption of Garlic on Delaying Cataract Induced by Sodium Selenite in Rats

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Abstract: Commercial garlic is widely used for certain therapeutic purposes, including cardiovascular disorders, anti-microbial, anti-cancer, and treatment of hyperglycemia. Garlic posses a strong antioxidant protective effect by its ability to scavenge free radicals. Several studies reported the correction of serum lipid profile in response to consumption of garlic powder, thus assumed to have a protective effect against atherosclerosis. The present study aims to investigate the role of dietary consumption of garlic on delaying or protecting from cataract formation. Materials and Methods: Twenty seven Wister rat pups were divided into four groups. Group one (n=6) received basal diet and served as control. The rats in group 2 (n=8) were injected subcutaneously with sodium selenite (30µmol/kg body weight) to induce cataract and fed on basal diet. Group 3 (n=6) were given 5% garlic powder added to their basal diet. Group 4 selenite-induced cataract (n=7) also administered 5% garlic powder in their basal diet. Development of cataract was assessed one week later, and its density was graded by slit lamp biomicroscopy. Total phenolic compounds of garlic were determined**.** After the end of experiment (two months), all rats were fasted overnight. Blood samples were collected from the eye vein and then the crystalline lenses were excised. All planned samples were prepared as will be described. The levels of serum lipid profiles were determined. The antioxidants and oxidative stress parameters such as superoxide dismutase, catalase, reduced glutathione, total antioxidant capacity, malondialdehyde and nitric oxide were assessed. Fas ligand (FAS-L) as apoptotic marker was also assessed in the blood and lens. The crystalline lens protein patterns on sodium dodocyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were identified and analyzed by computerized program. Results: All control rat lenses were clear. Development of cataract was reduced by 85.7% in the group supplemented with 5% garlic. Selenite induced cataract group developed bilateral complete opacification. The consumption of 5% garlic in cataractous group led to significant decrease in serum total lipids (15.5**%),** total cholesterol (TC) 10.3%, triacylglycerol (TAG) 26.7**%**, and low density lipoprotein cholesterol (LDL-C) 19.5**%**. There was a significant increase in the level of serum high density lipoprotein cholesterol (HDL-C) 18.0**%** when compared to selenite-induced cataract group. Also significant decrease in the activities of catalase, superoxide dismutase, reduced glutathione and total antioxidants. A significant increase in the levels of malondialdehyde, nitric oxide and Fas-L were noticed in cataractous group when compared to control group. The levels of all previous parameters were improved after treatment with 5% garlic. Conclusion: Our findings indicate that garlic inhibits selenite-induced cataract formation by inhibiting lipid peroxidation, oxidative stress and act as anti-apoptotic agent that can delay progress of cataract formation.

Key words: garlic-selenite-induced cataract- phenolic compounds- oxidative stress-lipid profile-Fas-L-nitric oxide- delay cataract.

INTRODUCTION

Garlic was found in Egyptian pyramids and ancient Greek temple. Medical applications of garlic were mentioned in India, Egypt and Rome (Rivlin *et al*., 2006). Interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Sasaki *et al*., 2002). Garlic has strong

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antioxidant properties and it has been suggested that garlic can prevent different diseases (Jalal *et al*., 2007 and Jung *et al*., 2008).

With regard to cataract, the selenite model was selected because of the rapid, effective and reproducible cataract formation. Although the rate of opacification in the selenite model is much more rapid than in human cataract, it has many general similarities to human cataract (Shearer *et al*., 1983 and 1997). Selenite induces bilateral nuclear cataract within 4 to 6 days when administrated to suckling rat pups before completion of the critical maturation period of the lens.

In cataractous state, an enormous production of reactive oxygen species takes place leading to characteristic membrane permeability and changes including leakage of structural proteins which is implicated in the opacity of the lens. The opacification of the crystalline lens occurs by aggregation of cytoplasmic lens proteins due to modifications in the intermolecular interactions that include oxidative stress (Shearer *et al*., 1997). The role of garlic in preventing age-related diseases has been investigated extensively over the last 10–15 years. Alireza *et al*. (2009) evaluated the inhibitory impacts of the aqueous extract of garlic on the formation of cataract induced by sodium selenite and suggested that, such herbal remedy may be considered for treatment of cataract.

This study aims to investigate the way and the role of garlic dietary consumption that can decrease or delay selenite-induced cataract formation rate in pup rats.

MATERIALS AND METHODS

Diet Preparations:

The experiment was done in the animal house of Research Institute of Ophthalmology. A commercial diet was used as basal diet. This diet consists mainly of 21% protein, 6 % fat, 3% fiber, and 6% of vitamins and minerals mixture, methionine and choline chloride. The other type of diet was prepared by addition of 5% Garlic powder to the diet as mentioned by Kweon *et al*. (2003). Dried powdered garlic was purchased from Spicy Trade Company, Helwan, Cairo.

Experimental Design:

Twenty Seven Wister rat pups with an average body weight of 25±4 g were obtained from the animal house of Research Institute of Ophthalmology. Four rat mothers having rat pups aging 10±1 days were included in this study. Each rat mother and their pups were housed in one cage and served as groups from one to four.

The Experiment Included Four Groups:

Group (**a**) control fed on basal diet (6 pups). Group (**b**) selenite-induced cataract fed on basal diet (8 pups). Group (**c**) Positive control fed on basal diet containing 5% garlic (6 pups). Group (**d**) selenite-induced cataract fed on basal diet containing 5 % garlic (7 pups).

The rat pups in the experimental groups (**b&d**) received a single subcutaneous injection of sodium selenite (30μmol/kg) according to the method described by Orhan *et al*. (1999), while control group was injected with normal saline (0.3 ml). Water and diets were available *ad libitum* for all rat pups. The progression of cataract was under observation till the end of the experiment.

Slit Lamp Biomicroscopic Examination and Lenticular Opacification:

At the final examination, the pupils were dilated with tropicamide (0.5%) and phenylephrine hydrochloride (2.5%). To assess the onset and maturation states of cataract, slit lamp biomicroscopic examination was carried out at regular intervals and the stages were designated as described by Suryanarayana *et al*. (2005). Briefly, lenses were examined on alternate days and opacities observed were graded into four stages: clear, stage 0; no vacuoles present or clear lens, stage 1: vacuoles of less than one third of the lens radius, stage 2: vacuoles located at the periphery of the lens occupying an area between one third and two thirds of the radius from the periphery, stage 3: vacuoles extending up to two thirds of the radius from the periphery (nuclear opacity may be seen), stage 4: vacuoles cover the entire lens, which appears white to the naked eye. The incidence of cataract appearance was expressed as the percentage of total lenses in each group.

Blood Samples:

At the end of experiment (two months), the rats were fasted overnight, anaesthetized and blood samples were withdrawn from the eye vein and divided into two portions:

The first portion was collected into sterile dry tube to separate serum by centrifugation at 3500 rpm and kept in deep freezer under –30ºC to be used to determine lipid profile, total antioxidant capacity, malondialdehyde, nitric oxide, FAS-L and electrophoreses of lens protein.

The second portion (1ml) was taken in vacutest sterile tube with EDTA interior for the determination of the superoxide dismutase activity and reduced glutathione. Plasma was used for determination of catalase activity.

The eyes were enucleated, the lenses were excised, carefully decapsulated and washed in 0.15 M isotonic sodium chloride solution. Lens homogenate was prepared according to each method for different determinations.

Biochemical Analyses:

Serum total lipids (TL) was determined according to the method described by Zollner and Kirsch (1962), triacylglycerols (TAG) was analyzed according to the method described by Fossati and Prencipe (1982), serum total cholesterol (TC) was measured according to the method of Allain *et al*. (1974), high density lipoprotein-cholesterol (HDL-C) was measured by using method of Lopes-Virella *et al*. (1977), and low density lipoprotein-cholesterol (LDL-C) was estimated by Tietz (1999).

Catalase activity (CAT) was determined according to the method described by Aebi, (1984); superoxide dismutase activity (SOD) was assessed by using the method described by Marklund and Marklund (1974); total antioxidants capacity (TAO) was measured according to the method of Koracevic *et al*. (2001); reduced glutathione (GSH) was assessed according to the method of Beutler *et al*. (1963); malondialdehyde (MDA) was determined according to the method described by Draper and Hadley (1990); and nitric oxide (NO) was assessed according to the method of Moshage *et al*. (1995). APO-1 Fas was assessed in serum and lens by a competitive enzyme linked immuno-sorbant assay (ELISA) Kit, using human (APO-1 Fas) as standard to assess apoptosis according to the manufacturer's instructions (Biosource International, INC., Camarillo, California, U.S.A.) (Tanaka *et al*., 1996).

Analysis of Eye Crystalline Lens Proteins:

The protein patterns of the soluble fraction of the lens homogenate were analyzed by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). A 12% (w/v) polyacrylamide separating gel and 4% (w/v) polyacrylamide staking gel were used. Coomassie Brilliant Blue was used to detect the lens protein bands. The gels were scanned, photographed and analyzed using Bioimage software. Total protein was estimated according to the method described by Lowry *et al*. (1951).

Identification of Phenolic Compounds by HPLC:

The phenolic compounds present in garlic sample were identified according to the method described by Duke *et al*. (2003). A known weight of air dried plant sample was used. Identification of individual phenolic compounds of the garlic was performed on JASCO HPLC, using hypersil C_{18} reversed-phase column (250 x 4.6 mm) with 5 particle size.

Statistical Analysis:

All statistical calculations were carried out with the statistical package for social sciences (SPSS) software program (version 10.0 for Windows). The values are expressed as the mean ± SE. The data were statistically analyzed using analysis of variance (ANOVA) and significant difference of the means was determined using Duncan's multiple range tests at the level of $P< 0.05$.

Results:

Slit Lamp Examination and Degree of Opacification:

Table 1: Grading of Cataract on the Basis of Slit-Lamp Examination.

Groups	Number of rats	Stages of cataract					% of cataract
		Stage0	Stage1	Stage2	Stage3	Stage4	
Normal	6	6	-	-	-	-	0%
Cataract	8	-	-	-	-	8	100%
Normal fed on garlic	6	6	-	-	-	-	0%
Cataract fed on garlic	7	6	1	-	-	-	14.3%

Table (1) shows cataract grade distribution in experimental groups. All control rat lenses were clear - stage zero (Figures 1-1 and 1-2). In selenite cataract group eight of eight rats 100% developed bilateral stage 4 cataract (Figure 1-3) whereas one rat only developed a bilateral 14.3% of lenses- stage 1 in selenite cataract group fed on 5% garlic (Figure 1-4).

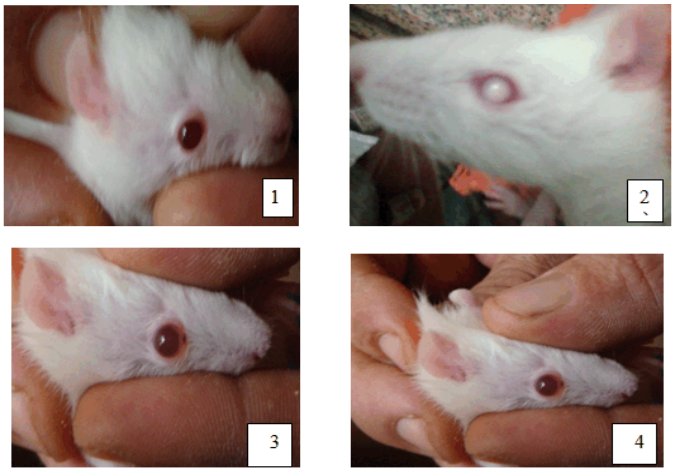

Fig. 1: Cataract formation observed in one sample from each group.
1: control group; 2: selenite cataract group; 3: control fed on 5% garlic; 4: selenite cataract fed on 5% garlic

Phenolic Compounds in Garlic:

Table 2: Phenolic Compounds in Garlic.

Phenolic Compounds	mg/100g garlic	Phenolic Compounds	mg/100g garlic
Pyrogallic acid	780	Pinostrobin	510
Salicylic acid	2030	Daidzin	90
Protocatechuic acid	1440	Genistein	40
P-cumuric acid	140	Catechines	760
Eugenol	1660	Genistin	40
Quercetin	150	Myricetin	120
Pinocembrin	60	Rutin	380
3,5 dihydroxy isoflavone	390	Luteolin	200

Table (2) shows that, garlic contains high amounts of salicylic acid, eugenol, protocatechuic acid, pyrogallic acid, catechines, pinostrobin, 3,5 dihydroxy isoflavone, rutin, luteolin, and P-cumuric acid. Garlic also contains appreciable amount of the flavonids myricetin and quercetin. Also Daidzin, Genistein and Genistin were found in garlic in a low concentrations.

Lipid Profile:

Table (3) shows the effect of supplementation with 5% garlic on serum total lipids, triacylglycerols, total cholesterol, high and low density lipoprotein-cholesterol in the control and experimental groups. There were significantly high levels of all lipid parameters except HDL-C in cataractous group as compared to the control group ($P<0.001$-0.0005). It was interesting to note that the supplementation with 5% garlic to normal rats showed a significant decrease in the levels of all previous lipid parameters. The percentage decreases were 19.9%, 9.1% and 24.4% concerning total lipids, total cholesterol and triacylglycerols, respectively. Also the cataractous rats fed on 5% garlic (group d) showed significant decrease in the levels of all lipid parameters except the level of HDL-C compared to cataractous group. The level of HDL-C in cataractous group showed improvement by 18.0% after feeding 5% garlic (table 3).

Antioxidant Parameters:

The data in table (4) reported that, the mean values and ±SE of catalase was (283.8±8.45U/L), superoxide dismutase activity (111.0±2.16 U/ml), reduced glutathione (65.2±0.82mg/dl) and total antioxidants capacity (1.13±0.07mmol/L) in control group. A significant decrease in the levels of catalase (156.2±9.15, $P<0.0007$),

superoxide dismutase (73.2±3.90, P<0.0002) and total antioxidants (0.49±0.03, P<0.0001), and reduced glutathione (44.9±0.56, P<0.0001) was noticed in cataractous group. Meanwhile, the percent of change were -44.9%, -34.0%, -56.6% and -31.1% respectively when compared to control group.

Table 3: Means±S.E., P Values and % of Change for Serum Total Lipids (TL), Total Cholesterol (TC), Triacylglycerols (TAG), High Density Lipoprotein-Cholesterol (HDL-C), and Low Density Lipoprotein-Cholesterol (LDL-C) in Control and Experimental Groups.

Groups Parameters		Normal (a)	Cataract (b)	Normal fed on garlic (c)	Cataract fed on garlic (d)	P-values (significant differences between groups)
TL mg/dl	mean±S.E	372.1±21.04	478.3±15.99	298.1±16.96	404.4±12.27	a,b (<0.0005); a,c(<0.006);
	%Change		28.5%*	-19.9%*	8.7%*	
					-15.5%#	a,d (N.S); d,b(<0.002).
TC mg/dl	mean±S.E	90.1±2.03	116.1±3.59	81.9±1.96	104.1±3.22	a,b (<0.0002); a,c(<0.049);
	%Change		28.8*	-9.1*	15.5%*	
					-10.3%#	a,d (<0.003); d,b (<0.006).
TAG mg/dl	mean±S.E	82.4±2.09	121.8±3.13	62.3±1.38	89.2±2.80	a,b (<0.0001); a,c(<0.0003);
	%Change		47.8%*	-24.4%*	8.3%*	
					-26.7%#	a,d (N.S). d,b (<0.0007).
HDL-C mg/dl	mean±S.E	43.3±1.34	39.9±1.49	51.4±1.42	47.1±2.14	a,b (N.S); a,c(<0.004);
	%Change		-7.8%*	18.7%*	8.7%*	
					18.0%#	a,d (N.S); d,b(<0.004).
LDL-C mg/dl	mean±S.E	31.3±2.12	49.8±2.62	19.5±1.73	40.1±2.21	a,b(<0.0001); a,c(<0.0003);
	%Change		59.1%*	-37.7%*	28.1%*	a,d (0.004);
					-19.5%#	d,b(<0.001).

The asterisk (*) denotes that data and the percentage change compared to control group. The asterisk (#) denotes the percentage change is compared to selenite-induced cataract group. The p values significantly different at P<0.05.

The results of the control fed on garlic showed a significant increase in the levels of catalase, superoxide dismutase, reduced glutathione and total antioxidants capacity, the mean and ± S.E. values were 325.0±18.3 U/L; 127.4±4.22 U/ml; 76.2±0.61mg/dl and 1.42±0.02mmol/L, respectively.

After selenite injection of rats fed on garlic, the levels of catalase, superoxide dismutase, reduced glutathione and total antioxidants capacity showed significant improvement with a percentage value of 44.8%, 19.9%, 24.9% and 102.9%, respectively as compared to cataract group (table 4).

Oxidative Stress and Apoptotic Marker:

The data in table (5) shows that, in cataract group, the levels of malondialdehyde, nitric oxide, and Fas-L was significantly high. The mean ±SE of malondialdehyde (4.38±0.13); nitric oxide (4.16±0.07); and Fas-L (467.5±5.91) were noticed in cataractous group. The percentage changes were 76.6%, 34.2% and 34.2%, respectively as compared to control group. The percentage reductions observed in rats fed on garlic were -18.9%, -27.4% and -21.0%, respectively compared to control group (table 5). Cataract group fed on garlic showed a significant reduction in the levels of malondialdehyde, nitric oxide, and Fas-L. The mean±SE of malondialdehyde (3.05±0.06, P<0.0001), nitric oxide (3.24±0.06,p<0.0004); and Fas-L (375.7±5.71, p<0.0002) were reported. The percentages reductions were -30.4%, -22.1% and -19.6%, respectively.

Biochemical Assessment of Lens Crystalline:

Results in table (6) show that, lenses of cataract group demonstrated a marked reduction in levels of lens total protein (represented as % of change), catalase activity, superoxide dismutase activity, total antioxidants and reduced glutathione as compared to lenses of control group. Cataractous lenses (represented as % of change) also showed the marked increase in levels of malondialdehyde, nitric oxide and Fas-L (represented as % of change) compared to lenses of control group.

Table 4: Means ±S.E., P Values and % of Change for Catalase (CAT), Superoxide Dismutase Activities (SOD), Total Antioxidants (TAO) and Reduced Glutathione (GSH) in Control and Experimental Groups.

Groups Parameters		Normal (a)	Cataract (b)	Normal fed on garlic (c)	Cataract fed on garlic (d)	P-values (significant differences between groups)
CAT	mean±S.E.	283.8±8.45	156.2±9.15	325.0±18.3	226.2±9.38	a,b(<0.0007; a,c(<0.011);
U/L						a,d (0.0003);
	%Change		-44.9%*	14.5%*	-20.3%* 44.8%#	d,b(<0.0006)
SOD	mean±S.E.	111.0±2.16	73.2±3.90	127.4±4.22	87.8±2.45	a,b(<0.0002; a,c(<0.0003;
U/ml						a,d(<0.0005;
	%Change		-34.0%*	14.7%*	-20.9%* 19.9%#	d,b (<0.003).
GSH	mean±S.E	65.2±0.82	44.9±0.56	76.2±0.61	56.1±0.62	a,b(<0.0009; a,c(<0.0001;
mg/dl						a,d(<0.0002)
	%Change		-31.1%*	16.3%*	-14.0%* 24.9%#	d,b(<0.0001)
TAO	mean±S.E.	1.13±0.07	0.49±0.03	1.42±0.02	0.99±0.01	a,b(0.0001);
mmol/L						a,c(<0.0008;
	%Change		- 56.6%*	25.7%*	-12.4%*	a,d(<0.018); d,b(<0.0005)
					102%#	

Legends as shown in table (3).

Table 5: Means ±S.E., P Values and % of Change for Malondialdehyde (MDA), Nitric Oxide (NO) and Fas-L in Control and Experimental Groups.

Groups Parameters		Normal (a)	Cataract (b)	Normal fed on garlic (c)	Cataract fed on garlic (d)	P-values (significant differences between groups)
	mean±S.E.	2.48±0.14	4.38±0.13	2.01±0.11	3.05±0.06	a,b(<0.0001;
						a,c(<0.015);
MDA						a,d (0.002);
nmol/ml	%Change		76.6% *	-18.9% *	20.9%*	d,b(<0.0001).
					-30.4%#	a,b(<0.00005);
	mean±S.E.	3.10±0.05	4.16±0.07	2.25±0.08	3.24±0.06	a,c(<0.0003);
NO						
µmol/L	%Change		34.2%*	-27.4%*	4.5%%*	a,d(<0.026);
					-22.1%#	d,b (<0.0004).
	mean±S.E.	348.3±6.01	467.5±5.91	275.0±5.63	375.7±5.71	a,b(<0.00002);
						a,c(<0.0001);
Fas-L						
Pg/ml	%Change		34.2%*	-21.0%*	7.9%*	a,d (0.002);
					-19.6%#	d,b(<0.0002).

Legends as shown in table (3).

Feeding 5% garlic to control rat pups caused a small increase in the levels of total lens proteins, catalase, superoxide dismutase activities, total antioxidants capacity and reduced glutathione as compared to lenses of control group; while it produced reduction in levels of malondialdehyde, nitric oxide and Fas-L as compared to lenses of normal control group.

In case of cataract group fed on garlic the data showed a low reduction in the levels total lens protein, catalase activity, superoxide dismutase activity, total antioxidants and reduced glutathione as compared to control group; while it produced increase in levels of malondialdehyde, nitric oxide and Fas-L when compared to lenses of control group.

Effect of Garlic on Lens Protein Profile:

In cataractous lenses, aggregations of proteins are related to lens membrane leakage. The selenite cataract group rats showed a significant decrease in total protein in comparison to the controls (Table 6). There was a remarkable increase of total protein contents in garlic-administered rats (control and selenite induced cataract fed on garlic). The SDS-electrophoresis of the soluble protein fraction showed an aggregated band at 21.2 kDa in relation to the controls and with a clear disappearance of this band in control group and selenite groups fed on garlic (Fig. 2).

Table 6: Mean and Percentage of Change for Total Proteins (TP), Catalase Activity (CAT), Superoxide Dismutase Activity (SOD), Total Antioxidants (TAO), Reduced Glutathione (GSH), Malondialdehyde (MDA), Nitric Oxide (NO), and Fas-L in the Soluble Fraction of the Eye Lens Homogenate of Control and Experimental Groups.

Parameters Groups		TP mg/g lens	CAT U/ mg Lens protein	SOD U/ mg Lens protein	TAO mmol/ mg lens protein	GSH µg/ mg Lens protein	MDA nmol/ mg lens protein	NO µmol/ mg lens protein Fas-L	Fas-L Pg/ mg lens protein
Normal	Mean	0.67	1.2	1.26	9.4	108.7	3.3	0.65	4.07
Cataract	Mean	0.47	0.7	0.66	7.1	65.8	5.8	1.24	6.9
	% Change	-30.5	-41.7	-47.6	-24.5	-39.5	74.2	90.7	69.5
Normal with garlic	Mean	0.71	1.4	1.36	9.6	120.6	2	0.35	3.17
	% Change	5.2	16.7	7.9	2.1	10.9	-39.9	-46.15	-22.1
Cataract with garlic	Mean	0.58	1.05	1.12	8.8	94.9	3.4	0.67	5.17
	% Change	-14.1	-12.5	-11.1	-6.4	-12.7	2.1	3.1	27

*The mean of duplicate sample.

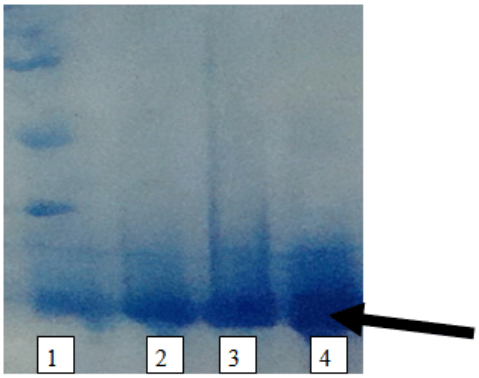

Fig. 2: The effect of 5% garlic powder on protein cross-linking and aggregation of the soluble fraction of the lens. The arrow indicates the cross-linked proteins.1) normal lens, 2) normal fed on garlic, 3) cataract fed on garlic and 4) Cataractous lens.

Discussion:

Cataracts, the opacification of the eye lens, are the most common cause of blindness, accounting for almost half of all cases worldwide (Bethesda, 1998) At present, the treatment for cataracts requires removal of the natural lens that has developed opacification, through surgery, and replacing it with a synthetic lens to restore the vision. Treatment is relatively expensive and there is a significant rate of postsurgical complications (Hirsch and Schwartz, 1983) Therefore, alternative treatments must be used. To date, as a part of better strategic management of cataract, metabolic intervention through natural dietary ingredients is gaining importance in recent times (Vibin *et al*., 2010; Pourkabir *et al*., 2010; Joshua *et al*., 2011).

Garlic is the oldest of all cultivated plants and is widely used because of its high pharmacological significance. Thus, the present study aimed to have an insight on the ameliorative action of garlic against selenite-induced cataract in rats.

Oxidative stress is the result of an imbalance of antioxidants and pro-oxidants. Lens opacity, due to cataract formation, is directly attributed to oxidative processes that occur within the lens. Oxidation, which can be caused by an over abundance of oxidative stress generators, such as molecular oxygen, hydrogen peroxide, and free radicals, produces major insult upon the crystalline lens, which can lead to the loss of glutathione, lipid peroxidation, and decrease in antioxidant enzymes activity (Bhuyan and Bhuyan, 1984; Meister, 1991; and Mitton *et al*., 1993).

We have evaluated the effects of garlic in the inhibition of cataracts formation induced by sodium selenite in rat pups. Results from ophthalmic examination indicate that 5% garlic powder is able to prevent, or at least significantly reduce the opacification of the lens crystalline within an experimental cataract model. This premise is very evident from our study, in that 85.7% of the rats supplemented with 5% garlic did not develop any opacification of the lens, whereas 14.3% of lenses developed cataract (table 1, figure 1 (4). The one hundred percentage of the rat lenses treated with sodium selenite developed lenticular opacification (table 1, figure 1(2).

In the present study the phenolic compounds present in garlic sample were identified by HPLC. Sharma *et al*. (1998) and Rhone and Basu (2008) supported the safety of higher intakes of the phytochemicals catechines and its association with reducing risks of cataracts. Recently Cotlier (2011), reported that salicylic acid lowers blood tryptophan that may delay or prevent the formation of senile cataracts. The observed findings in this study could be well because of the ameliorative action of the bioactive compound salicylic acid, eugenol, protocatechuic acid, pyrogallic acid and catechines as shown in table (2) and suggest that garlic is efficient potential antioxidant against the changes imposed by cataract formation. Our results are in agreement with previous findings (Vinson *et al*., 1998; Jackson *et al*., 2002; and Eidi *et al*., 2006).

A previous report by Bhuyan and Bhuyan (1984) and Babizhayev (1996) found a close relationship among cataract and hyperlipidemia. Also lipid peroxidation has been associated with the cataracts formation. Intake of garlic powder reduced total cholesterol, LDL-C and triacylglycerol and increased HDL-C (Liu and Yeh 2001; and Kojuri *et al*., 2007). Elkayam *et al*. (2003) suggested that garlic prevents hypercholesterolemia. Similarly Pourkabir *et al*. (2010) showed that supplementation with 5% garlic powder for 8 weeks had effect on the lipid profile in the cataractous rats. Our study supported these findings, the results indicate a reduction of about 15.5%, 10.3%, 26.7%, and 19.5% in serum TL, TC, TAG, and LDL-C, respectively and an increase by 18.0% of HDL-C level in response to 5% garlic powder was noticed. These results indicate that routine consumption of garlic in the diet has a beneficial effect in maintaining the serum lipids at low or normal levels and prevent lipid peroxidation and in turn cataract formation.

The glutathione redox system is a major component of overall antioxidant defenses in the cells. It is very efficient free-radical scavenger and protects cells from the toxic effects of reactive oxygen compounds (Lang *et al*., 2000). The selenite induced cataracts in pups have lower concentrations of glutathione in whole blood and lens as reported in this study. The data demonstrated that cataractous group have been linked directly to impaired glutathione status, and garlic powder has been shown to increase glutathione in the blood and lens. Garlic being a strong antioxidant can participate in prevention cataract by its ability to scavenge free radicals, and enhance scavenging systems in the lens, including the activity of each of superoxide dismutase, catalase and reduced glutathione.

In addition, SOD and catalase were found to act as defense enzymes to the oxidative stress in cataract. Significant increase was observed in the activity of previous enzymes (tables 4 and 6) accordingly profound protection against peroxidation damage in the lens upon treatment with 5% garlic powder.

This effect was associated with higher GSH level as well as lower levels of MDA and nitric oxide in response to garlic in normal and cataract groups (tables 4, 5 and 6) as compared to the cataract group. It could be postulated that garlic powder act as a potent source of antioxidant which provide an additional support to the elevation of SOD, catalase activities, and GSH level and decrease in MDA and nitric oxide levels.

Selenite-induced oxidative stress (30µmol/kg body weight) causes nuclear opacity through the calpain proteolysis of lens proteins. It is a strong sulfhydryl oxidant. This study suggested that the role of garlic in delaying cataract formation may be due to the active components which have potent antioxidant properties for maintaining sulfhydryl groups (-SH) of crystalline lens proteins in their reduced form preventing disulfide cross-linkage and prevent aggregation of lens protein. On the other hand, Orhan *et al*. (1999) reported that GSH protects the structural proteins and enzymes from sulfhydryl cross-linking that can disrupt their function. Considering the above facts, results from this study indicate that treatment with garlic decreases the oxidative damage and can prevent the formation of cataracts by maintaining GSH levels.

Apoptosis (Programmed cell death) is involved in a whole array of normal physiologic processes, including immune defense, tissue homeostasis, and development, and any tilt of the balance between life and death within an organism can lead to disease. Thus, the loss of essential cells of postmitotic tissues due to enhanced cell death may play an important role in a number of functional deficiencies and degenerative diseases such as cataract (Gosslau and Chen 2004). Garlic also inhibits the proliferation of human cells and induces apoptosis by increasing intracellular calcium concentrations (Sundaram and Milner 1996). Cho *et al*. (2006) found that Allicin a major component of garlic inhibits apoptosis of macrophages in a depleted nutritional state. Apoptosis as indicated by the level of serum Fas was assessed in the present study and the data shows a decrease in the levels of FAS-L in induced selenite cataract rat after feeding with garlic (5%). Garlic exerts anti-apoptotic action in different ways, due to the variety of compounds present in it such as water and lipid-soluble organosulfur compounds, phenolic compounds, saponins and selenium.

Results in the present study show that the consumption of 5% garlic could protect the animal models against selenite induced cataract. It is proved that garlic contain compounds that are effective antioxidant against oxidative insult. In addition the understanding of the anti-apoptotic, hypolipidemic, and antioxidants action of its active compound could open doors for further investigations in the management of cataract and quality of vision.

In conclusion, the present biochemical and morphological findings prove that: garlic consumption appeared effective to prevent Selenite induced cataract, perhaps through its potent free radical scavenging anti-apoptotic and antioxidant properties.

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