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Research Article

ATTENUATION EFFECT OF CAFFEIN AND/OR NIFEDIPINE AGAINST SELENITE-INDUCED CATARACTOGENESIS

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Abstract

The current study was performed to investigate the anti-cataractogenic effect of single and combined treatment with caffeine and nifedipine in sodium selenite induced cataract. Seventy five healthy albino Wistar rats were divided into 5 groups; the first group received intraperitoneal injection of saline and served as control, 2nd group received a single subcutaneous injection of 30 nmol/g body weights of sodium selenite to induce cataract. 3rd and 4th groups received 5.15 µmoles of caffeine and 0.1 mg/kg of nifedipine respectively, starting two days prior to the administration of selenite and continuing such treatment till the end of experiment. Last group got the combined treatments of caffeine and nifedipine in the same regimen. The results indicated a very highly significant decrease ($P<0.001$) in total soluble lens protein and Na^+-K^+ATPase activity, a very highly significant increase ($P<0.001$) in calcium lens content in selenite cataract group. Also significant changes in the molecular weight and area under peaks estimated in profile of electrophoretic mobility of lens proteins were found in selenite group. All these changes reduced markedly in caffeine and nifedipine group. But complete protection appeared in animal group that treated with both of caffeine and nifedipine. Nutrients with antioxidant capabilities can potentially delay against these changes such as caffeine but blockade of L-type Ca^{++} channels in the lens are needed as nifedipine to complete protection from cataractogenesis.

Keywords: caffeine, cataract, lens, nifedipine, rats.

1. Introduction

The eye lens tissue is a unique part of both the human and the animal body. It is very different from any other soft tissue. The main feature of the lens that is essential to sight is its transparency. The natural selection guided eye lens features towards a structure and components capable of preserving transparency of the constitutes of the lens for many years, giving evidence of the possible presence of reactive radical species and exposure to visible and ultraviolet radiation [1]. The lens is avascular and non-innervated structure which the main functions to deviate light onto the retina and make minor changes in focus by accommodation [2]. In order to make light refraction effectively, the lens must keep its transparency. Lenses contain a protein called crystalline that accounts for around 90% of the soluble proteins in the lens and play a crucial role to maintain lens transparency [3]. Cataract, loss of the lens transparency, is the main cause of blindness all over the world. The selenite cataract is just a rapidly induced and appropriate model of cataractogenesis. Looking for preventive methods to delay the onset of cataract can serve to narrow the gap between the incidence of cataract blindness and the provision of surgery. Even in modern ophthalmology, the effective medical treatment for cataract is surgery but it has its limitations. Development of a drug which could prevent or postpone the onset of cataract will lessen this burden and decrease the number of blind patients waiting for cataract surgery. It is generally accepted that oxidative stress is a significant factor in the pathogenesis of cataract both in experimental animal model [4] and in cultured lens systems [5]. Depending on oxidative theory of cataract formation, reactive oxygen species can denature lens proteins and lens fiber cell membrane. Therefore, nutrients with antioxidant capabilities can potentially provide protection against these progressions [6].

Caffeine ($C_8H_{10}N_4O_2$) is an effective scavenger of reactive oxygen species, particularly hydroxyl radicals, singlet oxygen and to some extent peroxyl radicals. It was demonstrated that the antioxidant action of caffeine was similar to that of glutathione and significantly higher than the action of ascorbic acid [7]. In the human body, caffeine is demethylated by the hepatic cytochrome P450 1A2 (CYP 1A2) to the main metabolites 1-methylxanthine and 1-methyl uric acid that also have been shown to have significant antioxidant activity [8]. Blockade of L-type Ca++ channels in the lens is one of the possible pharmacological interventions to prevent cataracts. Both systemic and topical administration of Nifedipine ($C_{17}H_{18}N_2O_6$), an L-type Ca^{++}- channel blocker, have been reported to prevent cataract formation in

animals [9,10]. Ca^{++} influx into lens cells via L-type Ca^{++} channels has been suggested to be participated in the mechanism of this process. Since calcium ion overload and free radical toxicity have been recognized as the major two players in the causation of cataract. The current study was performed to investigate the anti-cataractogenic effect of single and combined treatment with nifedipine and caffeine in sodium selenite induced cataract.

Materials and Methods

Chemicals supplies: All chemicals used in the experiments were obtained from Sigma Company (St. Louis, MO, USA) with the highest purity grad available.

Animals

Seventy five healthy albino Wistar rats of both sexes weighing 50±10g (20 days old) were used in this study. The rats were randomly selected from the animal house facility at the Research Institute of Ophthalmology RIO, Giza, Egypt. They were housed in special designed cages and maintained under constant air flow and illumination during the experimental periods, the rats were fed with balanced diet (protein 21%, starch 70%, fat 3.5%, fiber 3.5%, minerals and vitamins 2%) and drink water ad libitum.

The animal was handled according to the ARVO (The Association for Research in Vision and Ophthalmology) statements and regulations for the use of animals in research. Rats will be divided into 5 groups; the first group received intraperitoneal injection (i.p.) of saline and served as control (Con group), 2nd group (Sel group) received a single subcutaneous injection of 30 nmol/g body weights of sodium selenite to induce cataract [11]. 3rd and 4th groups, that denoted by Sel+Caf and Sel+Nif, received 5.15 µmoles of caffeine [12] and 0.1 mg/kg of nifedipine [13] respectively, starting two days prior to the administration of selenite and continuing such treatment till the end of experiment. Last group got the combined treatments of nifedipine and caffeine in the same regimen. Animals were decapitated after 5, 15 and 25 days of the experiment and the following measurements were done:

Measurement of Na^+-K^+ATP ase activity on the lens membrane

After animals' decapitation, eyes were enucleate from the eye globe, then the lenses were freed from the eye and their capsules were removed carefully. Each lens capsule was weighed separately then homogenized after putting in extraction medium (0.32M sucrose, 1 µM EDTA and 0.15% deoxycholic acid). Na^+-K^+ ATPase measurement was carried out on the lens membrane according to the method of Bowler and Tirri [14].

Measurement of total soluble lens proteins

The lenses were weighed then homogenized separately in de-ionized water then centrifuged at 16.000 rpm to extract soluble lens proteins. Total proteins in the soluble part of the crystalline lens were determined according to the method of Lowry et al. [15].

Measurement of calcium concentration in lens

Total calcium concentration was determined using calcium kit (Arsenazo III Method). The principle mainly depends on the combination of calcium with Arsenazo III at a neutral pH to form a purple blue colored complex. Intensity of the color formed is directly proportional to the amount of calcium present in the sample [16].

SDS polyacrylamide gel electrophoresis

The separation of soluble lens proteins was depending on their molecular weights by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [17] using 5% stacking gel and 12% separating gel. The data were shown graphically with an automatic scanner (model R-112, manufactured by Beckman) and then subjected to curve narrowing procedure that combines Fourier deconvolution and nonlinear curve fitting.

Statistical analysis

Data was expressed as the mean standard deviations. Comparison between multiple groups was done using analysis of variance (ANOVA). Commercially available statistical software package (SPSS-11, for Windows) was used and the significance level was established at $P<0.05$. All the spectral analysis was performed with OriginPro 9 software (OriginLab Corporation, Northampton, MA, USA).

Results

Figure (1) illustrated a histogram pattern for Na^+-K^+ATPase activity in μmoles of Pi liberated/min/mg protein from lens membrane of rats groups injected with selenite and treated with caffeine and/or nifedipine compared to their control after 5 days,15 days and 25 days, respectively. The value of Na^+-K^+ATPase activity of control ranges (Con) was 13.74±0.45, 13.85±0.56 and 14.11±0.26 μmoles of Pi liberated/min/mg protein. After all periods of injection of sodium selenite (5, 15 and 25 days), there was a very highly significant decrease (V.H.S, $P<0.001$) of the Na^+-K^+ATPase activity. After caffeine treatment (Sel+Caf group) two weeks before selenite injection and continue till decapitation, there was very highly significant decrease (V.H.S, $P<0.001$) after 5 days and highly significant decrease (H.S, $P<0.01$)

appeared after 15 days and 25 days. Nifedipine injection in the same regime leads to statistically highly significant decrease ($p<0.01$) after 5 days and 15 days but a significant decrease ($p<0.05$) after 25 days. There were no significant changes in Na^+-K^+ATPase activity for animals groups injected with caffeine and nifedipine (Sel+Caf+Nif) after 15 and 25 days but significant decrease ($p<0.05$) of treatment appeared after 5 days.

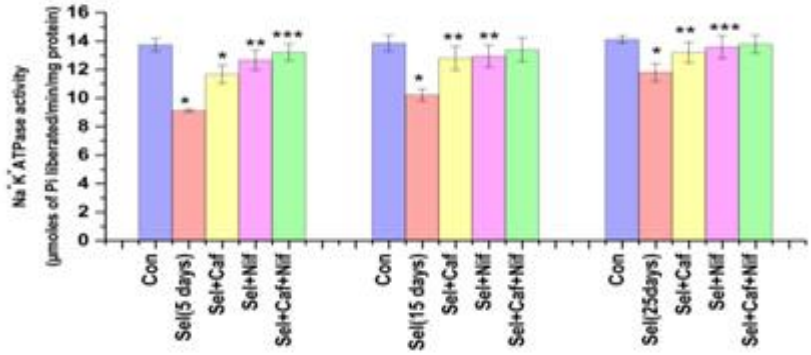

Figure (1) Na^+-K^+ATPase activity in rat lens membrane (µmoles of Pi liberated/min/mg protein) to control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups after 5, 15 and 25 days of selenite injection. ***p<0.05 (S), **p<0.01 (H.S), *p<0.001 (V. H.S)

Figure (2) illustrated the total soluble lens protein of rats groups injected with selenite and treated with caffeine and/or nifedipine compared to their control after 5 days, 15 days and 25 days. Total soluble lens protein was decreased (V.H.S, P<0.001) for selenite groups after 5, 15 and 25 days. Sel+Caf groups revealed a highly significant decrease ($p<0.01$) after 5 and 15 days of selenite injection then a significant decrease ($p<0.05$) after 25 days. For nifedipine groups, the total soluble protein was highly significant decrease ($p<0.01$) after 5 days but significant decrease ($p<0.05$) only after 15 and 25 days. There were not any significant changes in total soluble lens protein for all animals groups injected with caffeine and nifedipine (Sel+ Caf+Nif) after 5,15 and 25 days.

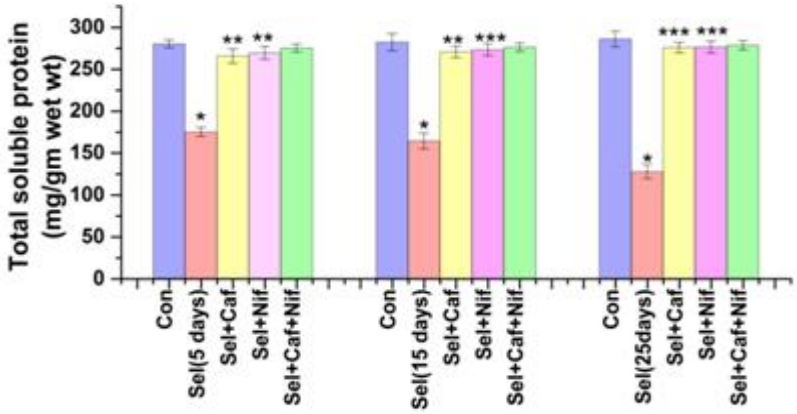

Figure (2)Total soluble protein of lens (mg/gm wet wt) to control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups after 5, 15 and 25 days of selenite injection. ***p<0.05 (S), **p<0.01 (H.S), *p<0.001 (V. H.S)

Figure (3) illustrated the calcium content for all groups. For control rats, the calcium content value ranges from 0.8±0.32 to 0.90±0.20 mg/mg proteins. After all periods of injection of sodium selenite (5, 15 and 25 days), there was a marked increase (V.H.S, P<0.001) of Ca++ content. After caffeine treatment, there was very highly significant increase after 5 days and highly significant increase appeared after 15 days and 25 days. Nifedipine injection leads to statistically highly significant increase (p<0.01) after 5 days and 15 days but a significant increase (p<0.05) after 25 days. There were no significant changes in Ca^{++} content for animals groups injected with caffeine and nifedipine (Sel+Caf+Nif) after 15 and 25 days but significant increase (p<0.05) of treatment appeared after 5 days.

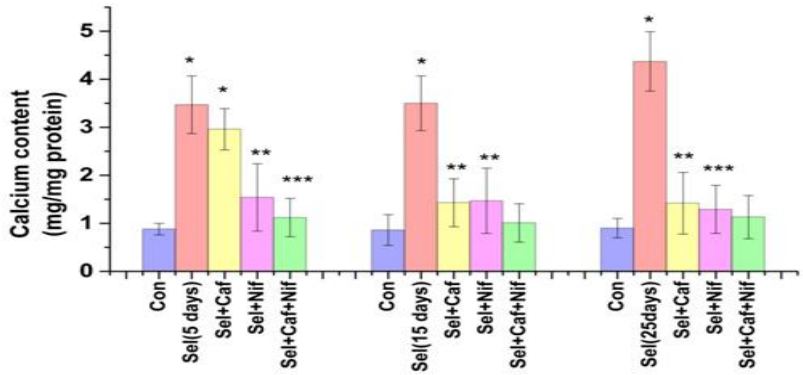

Figure (3) Calcium content of rat lens (mg/mg protein) to control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups after 5, 15 and 25 days of selenite injection. ***p<0.05 (S), **p<0.01 (H.S), *p<0.001 (V. H.S)

Figure (4) showed the electrophoretic mobility of soluble lens protein of control rats, injected with sodium selenite, caffeine treatment, nifedipine treatment and both of caffeine and nifedipine treatment after 5,15 and 25 days. To analysis of peaks that corresponding to different fractions of soluble lens protein, fitting to the pattern of each group was done.

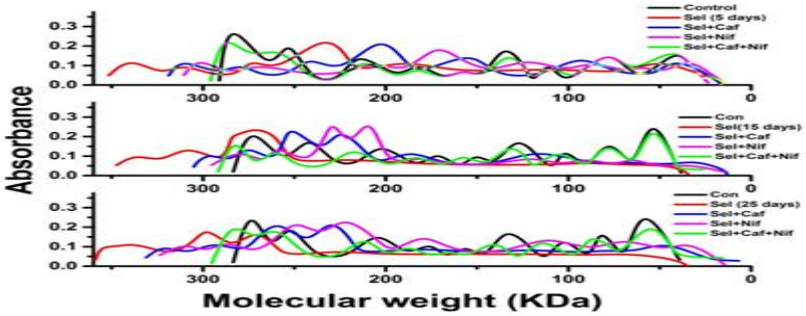

Figure (4) Electrophoretic mobility of soluble lens protein after 5 days (top), 15days (middle) and 25 days (bottom) of selenite injection for control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups.

Figure (5) showed the fitting analysis of the electrophoretic pattern for Con group, Sel group, Sel+Caf, Sel+Nif and Sel+Caf+Nif after 5 days of selenite injection. The control pattern was distinguished by the presence of 9 peaks that covered the molecular weight range 40-283 kDa. After selenite injection, there were a reduction in the peaks to 6 peaks and a shift to high molecular weight to be covering the range 51-339 kDa. The peaks were highly statistically significant ($p<0.001$) than their corresponding peaks in the control in the intensity and the area under peaks as shown in table (1) that illustrated the molecular weight of all peaks, area under peak and absorbance of each peak to evaluate the effects of selenite injection and treatments after 5 days. Sel+ Caf group revealed the appearance of 7 peaks covered the range 40-310 kDa that highly significant ($p<0.01$) compared to control. Sel+Nif group revealed the existing of 8 peaks covered the molecular weight range 33-302 KDa. Sel+Caf+Nif group pattern characterize by 9 peaks and no significant, changes observed for most of peaks in their molecular weight and absorbance as showed in table (1).

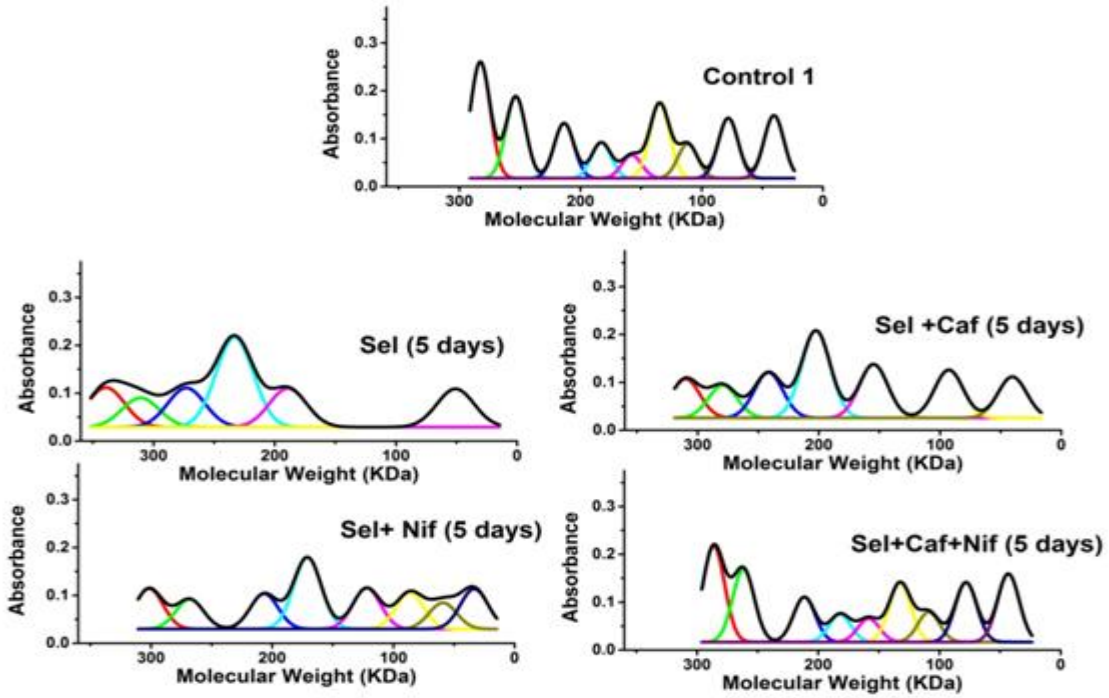

Figure (5) electrophoretic mobility of soluble lens protein after deconvolution to control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups after 5 days of selenite injection.

Table (1) Estimated peaks from electrophoretic pattern indicating the molecular weight and area under curve with the absorbance for the different studied groups after 5 days of selenite injection.

		Con	Sel (5days)	Sel + Caf	Sel + Nif	Sel + Caf + Nif
Peak 1	**Mwt**	282.62 ±5	339.21 ±6*	309.93 ±8**	301.51 ±7**	286.17 ±7
	AC	4.98 ±0.2	3.19 ±0.1*	2.41 ±0.5*	2.43 ±0.4*	4.51 ±0.6
	Abs	0.26 ±0.02	0.11 ±0.01*	0.11 ±0.02*	0.11 ±0.01*	0.22 ±0.03
Peak 2	**Mwt**	255.34 ±7	310.59 ±6*	279.16 ±4*	268.02 ±10**	261.67 ±6
	AC	3.50 ±0.3	2.35 ±0.4*	2.01±0.3*	1.79 ±0.1*	3.41 ±0.2
	Abs	0.19 ±0.02	0.19 ±0.02	0.09 ±0.02*	0.09 ±0.02	0.18 ±0.02
Peak 3	**Mwt**	213.49 ±6	272.89 ±3*	241.46 ±4*	206.56 ±7**	211.42 ±6
	AC	2.36 ±0.2	3.15 ±0.2*	2.80 ±0.1**	2.12 ±0.3*	2.12 ±0.2**
	Abs	0.13 ±0.01	0.11 ±0.01*	0.12 ±0.02	0.10 ±0.02*	0.11 ±0.01*
Peak 4	**Mwt**	182.80 ±3	233.04 ±5*	202.36 ±4**	174.93 ±4**	182.06 ±3
	AC	1.53 ±0.4	7.22 ±0.6*	5.40 ±0.4*	4.29±0.2*	1.41 ±0.3
	Abs	0.09 ±0.01	0.22 ±0.02*	0.21 ±0.01*	0.18 ±0.02*	0.07±0.02
Peak 5	**Mwt**	157.64 ±4	189.07 ±3*	154.84 ±3	122.01 ±3*	158.39 ±3
	AC	0.99 ±0.1	3.12 ±0.2*	3.32 ±0.2*	2.46 ±0.2*	1.10 ±0.2
	Abs	0.06 ±0.01	0.11 ±0.01*	0.14 ±0.01*	0.12 ±0.01*	0.07 ±0.01
Peak 6	**Mwt**	134.63 ±5	50.81 ±3*	92.72 ±6*	86.45 ±6*	132.48±4
	AC	3.19 ±0.3	3.08 ±0.2	2.97±0.4	2.17 ±0.3*	2.87 ±0.4
	Abs	0.18 ±0.02	0.11 ±0.03*	0.13 ±0.01**	0.11 ±0.01*	0.14 ±0.02
Peak 7	**Mwt**	111.53 ±5		40.34 ±6*	59.15 ±3*	108.81 ±4
	AC	1.47 ±0.2		2.54 ±0.3*	1.58 ±0.2	1.40 ±0.2
	Abs	0.09 ±0.02		0.11±0.02***	0.08 ±0.02	0.08 ±0.01
Peak 8	**Mwt**	78.04 ±2			33.33 ±4*	78.04 ±3
	AC	2.58 ±0.3			2.40 ±0.2	2.70 ±0.2
	Abs	0.14 ±0.01			0.11 ±0.01*	0.14 ±0.01
Peak 9	**Mwt**	40.34 ±3				43.14 ±
	AC	2.69 ±0.4				3.21 ±0.2**
	Abs	0.15 ±0.01				0.15 ±0.02

***p<0.05 (S), **p<0.01 (H.S), *p<0.001 (V. H.S)

Figure (6) showed the fitting analysis of the electrophoretic pattern for Con group, Sel group, Sel+Caf, Sel+Nif and Sel+Caf+Nif after 15 days of selenite injection. Table (2) showed the estimated peaks from electrophoretic pattern indicating the molecular weight and area under the curve with the absorbance for the different studied groups after 15 days of selenite injection. After 15 days of selenite injection, the protein fraction had a very highly significant ($p<0.001$) shift to high molecular weight and also absorbance of all peaks and reduction of protein fraction to 5 peaks. After treatment of animals with caffeine or nifedipine, there was some kind of repair of the shift of the molecular weight. The protein fraction in Sel+Caf+Nif group after 15 days revealed the restoration of most peaks to their control value.

Table (2) Estimated peaks from electrophoretic pattern indicating the molecular weight and area under curve with the absorbance for the different studied groups after 15 days of selenite injection

		Con	Sel (15 days)	Sel+ Caf	Sel + Nif	Sel + Caf + Nif
Peak 1	Mwt	272.14 ±9	337.15 ±7*	296.65 ±4*	280.56 ±5**	281.96 ±8**
	AC	3.71 ±0.2	2.44 ±0.2*	2.01 ±0.2*	1.49 ±0.1*	3.49 ±0.2**
	Abs	0.20 ±0.01	0.09 ±0.03*	0.09 ±0.02*	0.11 ±0.03*	0.15 ±0.04
Peak 2	Mwt	242.86 ±7	308.52 ±5*	275.69 ±4*	250.20 ±4**	260.35 ±6*
	AC	2.99 ±0.4	3.66 ±0.4*	2.88 ±0.3**	1.83 ±0.5*	1.66 ±0.4*
	Abs	0.17 ±0.01	0.13 ±0.01*	0.13 ±0.01*	0.12 ±0.01*	0.10 ±0.01*
Peak 3	Mwt	202.36 ±7	270.82 ±6*	251.93 ±6*	228.92 ±5**	210.70 ±6
	AC	2.40 ±0.3	7.68 ±0.2*	5.49 ±0.4*	4.38 ±0.5*	1.80 ±0.4*
	Abs	0.14 ±0.02	0.23 ±0.02*	0.23 ±0.01*	0.24 ±0.03*	0.12 ±0.02
Peak 4	Mwt	172.32±9	218.44 ±5*	224.71 ±7*	210.02 ±4*	182.21 ±8
	AC	1.64 ±0.4	1.98 ±0.3*	4.97 ±0.4*	4.38 ±0.2*	1.55 ±0.4
	Abs	0.10 ±0.01	0.08±0.01	0.20 ±0.01*	0.24 ±0.03*	0.08 ±0.02
Peak 5	Mwt	149.97 ±4	87.11 ±3*	187.01±4*	181.40±4*	154.05 ±3
	AC	1.57 ±0.2	1.29 ±0.3***	2.26 ±0.3*	0.93 ±0.3*	1.23 ±0.2
	Abs	0.10 ±0.01	0.06±0.01*	0.11±0.02	0.08 ±0.02	0.09 ±0.01
Peak 6	Mwt	127.62 ±3		116.48±2*	119.95 ±2*	131.82 ±3
	AC	2.95 ±0.2		2.34 ±0.1*	0.97 ±0.2*	2.13 ±0.2*
	Abs	0.17 ±0.01		0.11 ±0.01*	0.08 ±0.01*	0.15 ±0.2
Peak 7	Mwt	101.05 ±4		32.67 ±3*	89.92 ±4	106.00±5
	AC	1.91 ±0.3		1.12 ±0.3*	0.82±0.4	1.59 ±0.4
	Abs	0.11 ±0.02		0.07 ±0.02	0.08 ±0.02	0.11 ±0.02
Peak 8	Mwt	77.38 ±4			64.10 ±3*	77.38 ±3
	AC	2.59 ±0.4			0.80 ±0.5*	2.40 ±0.5
	Abs	0.15 ±0.03			0.07 ±0.03**	0.14 ±0.02
Peak 9	Mwt	53.62 ±3				53.62 ±5
	AC	4.43 ±0.5				3.88 ±0.4
	Abs	0.24 ±0.02				0.21 ±0.03

***p<0.05 (S), **p<0.01 (H.S), *p<0.001 (V. H.S)

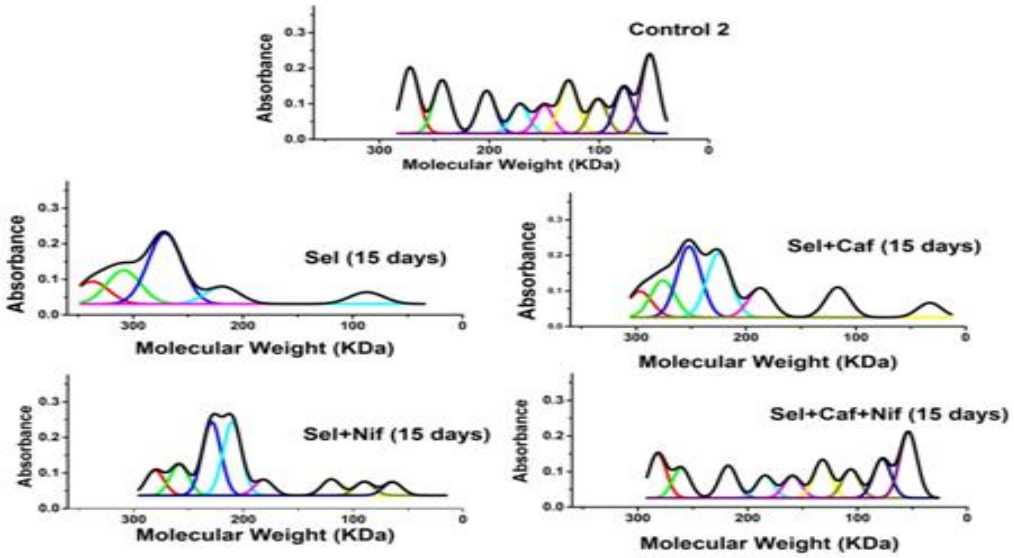

Figure (6) electrophoretic mobility of soluble lens protein after deconvolution to control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups after 15 days of selenite injection.

Figure (7) showed the fitting analysis of the electrophoretic pattern for Con group, Sel group, Sel+Caf, Sel+Nif and Sel+Caf+Nif after 25 days of selenite injection. Table (3) showed the estimated peaks from electrophoretic pattern indicating the molecular weight and area under the curve with the absorbance for the different studied groups after 25 days of selenite injection. The most important observation was the loss of soluble proteins to their mobility in selenite group after 25 days and appears in some sort of repair in Sel+Caf+Nif group.

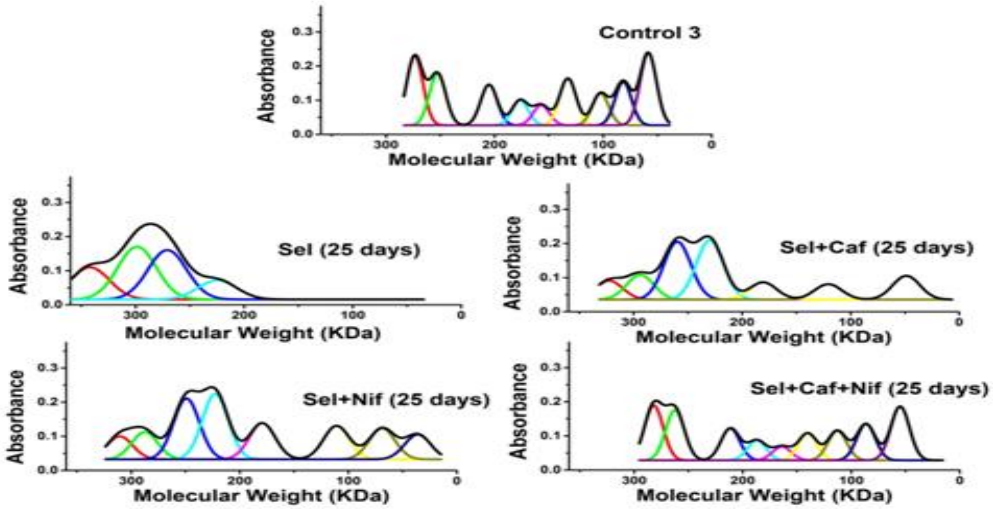

Figure (7) electrophoretic mobility of soluble lens protein after deconvolution to control (Con), selenite (Sel), selenite+caffeine (Sel+Caf), selenite+nifedipine (Sel+Nif) and caffeine+nifedipine (Sel+Caf+Nif) groups after 25 days of selenite injection.

Table (3) Estimated peaks from electrophoretic pattern indicating the molecular weight and area under curve with the absorbance for the different studied groups after 25 days of selenite injection.

		Con	Sel	Sel + Caf	Sel + Nif	Sel + Caf + Nif
Peak 1	**Mwt**	273.55 ±8	342.02 ±6*	322.47 ±5*	310.59 ±7*	281.96 ±8
	AC	3.86 ±0.2	4.20 ±0.1*	1.72 ±0.2*	2.01 ±0.2*	3.47 ±0.1*
	Abs	0.23 ±0.04	0.11 ±0.02*	0.09 ±0.02*	0.10 ±0.03*	0.19 ±0.03
Peak 2	**Mwt**	252.59 ±9	298.71 ±6*	293.84 ±7*	287.57 ±9*	262.41 ±10
	AC	2.87 ±0.2	6.91 ±0.4*	2.26 ±0.2**	2.40 ±0.4**	3.18 ±0.2***
	Abs	0.18 ±0.01	0.17 ±0.01	0.11 ±0.01*	0.11 ±0.01*	0.18 ±0.02
Peak 3	**Mwt**	205.16 ±7	270.82 ±4*	259.61 ±6*	249.13 ±7*	211.43 ±6
	AC	2.17 ±0.2	6.46 ±0.3*	5.31 ±0.2*	5.34 ±0.3*	2.13 ±0.2
	Abs	0.15 ±0.01	0.16 ±0.02	0.20 ±0.01*	0.21 ±0.01*	0.12 ±0.02
Peak 4	**Mwt**	176.54 ±10	224.71 ±6*	229.58 ±8*	222.56 ±7*	187.01 ±8***
	AC	1.39 ±0.1	2.51 ±0.2*	5.45 ±0.2*	5.75 ±0.3*	1.35 ±0.3***
	Abs	0.10 ±0.01	0.07 ±0.01	0.21 ±0.03*	0.22 ±0.03*	0.09 ±0.01
Peak 5	**Mwt**	156.98 ±7		48.67±6*	179.34 ±4*	163.91 ±6
	AC	1.12 ±0.2		2.18 ±0.2*	3.18 ±0.3*	0.96 ±0.3
	Abs	0.09 ±0.01		0.09 ±0.01	0.14 ±0.02*	0.07 ±0.02
Peak 6	**Mwt**	132.48 ±6		180.66 ±4*	110.87 ±7*	140.16 ±4**
	AC	2.61 ±0.2		1.58 ±0.2*	2.64 ±0.2	1.70 ±0.2*
	Abs	0.16 ±0.01		0.08 ±0.01*	0.13 ±0.02	0.11 ±0.03**
Peak 7	**Mwt**	102.46 ±5		120.60 ±4*	68.96 ±2*	112.93 ±5*
	AC	1.77 ±0.2		1.41 ±0.3**	2.72 ±0.2*	1.87 ±0.3
	Abs	0.12 ±0.02		0.11 ±0.01	0.12 ±0.01	0.12 ±0.01
Peak 8	**Mwt**	81.50 ±3			36.13 ±2*	86.45 ±4
	AC	2.42 ±0.3			2.18 ±0.2**	2.30 ±0.3
	Abs	0.15 ±0.02			0.11 ±0.02**	0.14 ±0.02
Peak 9	**Mwt**	58.49 ±2				57.02 ±3
	AC	4.03 ±0.2				3.41 ±0.2*
	Abs	0.24 ±0.01				0.19 ±0.01*

***p<0.05 (S), **p<0.01 (H.S), *p<0.001 (V. H.S)

Discussion

Opacification of the eye lens called cataract and it leads to absence of vision. The highly concentrations of proteins and antioxidants that found in the lens are very important to maintain transparency. Cataract was resulted from a mutation of lens proteins especially crystalline. That crystalline are essential in keeping the transparency of the lens [18].

Most human age-related metabolic sicknesses and disarranges are because of oxidative damage, which assumes a key part in age-related metabolic changes, for example, cataract. Being an avascular organ, the lens is helped by an effective glutathione redox cycle to oppose oxidative stress [19]. The human visual lens needs to keep up its transparency and tensile properties more than quite a few years to supporting typical vision. It is by and large acknowledged that oxidative stress is a critical variable in the pathogenesis of cataract in experimental animal models [20,21].

Cataract resulted due to sodium selenite injection has attributes like those seen clinically in humans, for instance, cytoskeletal decrease, concealment of mitosis and diminished rate of epithelial cell differentiation, and calpain-induced proteolysis. Selenite-induced cataract has turned out to be a fast and helpful animal model for cataract [22]. A many biochemical procedures, for example, oxidative stress, modified epithelial metabolism system, increase of calcium concentration, crystalline precipitation and cytoskeletal loss happen amid the advancement of selenite-induced cataract [23].

In the present study a single subcutaneous injection of sodium selenite 30 nmol/g body weights induce changes in protein profiles of the lens, increased level of calcium. In addition, SDS-PAGE results showed changes in soluble proteins profiles during the progress of selenite induced cataract. The high molecular weight aggregates is suitable to scatter light and lead to the loss of lens transparency. The regular, order space of the cells and proteins is the major factor of transparency of the lens. Disturbance of this order for instance due to protein aggregation. Membrane degeneration or fluctuations in protein distribution was resulted in local change of refractive index [24]. The well established concept that intraocular generation of oxygen-free radicals initiates oxidative stress, resulting in cataract formation. The levels of soluble proteins in cataract lenses were reduced significantly ($P<0.001$) but gradually from 5 to 15 days. These reductions in total soluble protein in cataract lenses of different stages of maturation have also been reported by Anthrayose and Shshidhar [25] which is consistent with the present observation. The reduction in soluble proteins in the lens during cataractogensesis could be due to the leaking of the lens fibre membrane which is supported

by an increase in protein concentration that exist in the aqueous humour. "Osmotic swelling" resulted from accumulation of proteogenic amino acids also affect the leakage of soluble protein from the lens.

Calcium homeostasis has been recognized to be critical for lens structural integrity and transparency, with elevated levels of lens calcium and the subsequent stimulation of calpain activity reported causing cataract or lens opacification [26]. The increased level of calcium in cataract lens is due to increase lipid peroxidation which has altered the membrane permeability of the lens leading increased intracellular calcium levels and in turn leads to cataract formation.

When protein synthesis was inhibited, the Na^+-K^+ATPase α1 protein abundance remained the same but Na^+-K^+ATPase activity was reduced [27]. A slow rate of protein turnover in lens fibers may leave the Na^+-K^+ATPase polypeptide vulnerable to modifications that contribute to low Na^+-K^+ATPase activity as indicated in our study.

Guided by the results, subcutaneous injection of 0.1 mg/kg nifedipine and 5.15 µmole caffeine prevented selenite-induced cataract. Caffeine and nifedipine group showed near to normal appearance of the bands of soluble lens proteins in gel electrophoresis and there are no significant different in bands area or absorbance compared with control.

Caffeine has a superior antioxidant potential. It scavenges reactive oxygen species (ROS). This could be quite important in the lens. Additionally it could favorably influence the lens metabolism by inhibiting cyclic adenosyl monophosphate phosphodiesterase and therefore maintaining adequate levels of cAMP. Also caffeine preserves GSH concentration levels in the lens and protects cells from apoptosis [12,28] . Caffeine is used with significant success in treating diseases associated with neural and muscular dysfunctions, such as the Alzheimer's and Parkinson's diseases that their pathogenesis is related to oxidative damage. Thus there is a similarity of these diseases with cataract in the formation of lipid peroxidation products such as malondialdehyde and the presence of DNA degradation products in the neural tissues, similar to that of the lens. Caffeine either is acting as antagonist of adenosine to help overcome the neural transmission defects. Adenosine modulated nerve transmission by binding to the G-protein-linked receptors [29].

An interesting finding in this study that nifedipine is more effective in delaying selenite-induced cataract. Nifedipine slow the progression of cataracts through mechanisms other than blockade of L-type Ca^{++} channels. Nifedipine not only block Ca^{++} influx into lens cells but also attenuate the oxidative stress. Nifedipine might exhibit an inhibitory effect on cataracts as an anti-oxidant drug. Also it has been demonstrated that nifedipine had a potent effect in inhibiting sarcolemmal membrane lipid peroxidation [10].

In conclusion according to oxidative hypothesis of cataract formation, reactive oxygen species can damage lens proteins and lens membrane. Therefore, nutrients with antioxidant capabilities can potentially delay against these changes such as caffeine but blockade of L-type Ca^{++} channels in the lens are needed as nifedipine to complete protection from cataractogenesis.

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