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THE POTENTIAL EFFECT OF GINKGO BILOBA EXTRACT ON DEVELOPMENT OF CATARACT IN SELENITE INDUCED CATARACT RAT PUPS

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ABSTRACT: Present study focuses on the isolation of flavonoids from *Ginkgo biloba* to investigate the role of different concentrations of *Ginkgo biloba* (Gb) extract (40, 60, 100 mg/kg body weight) in the prophylaxes or delay the development of cataract in the selenite induced cataract rats. Seventy five of neonatal rat pups were obtained from the animal house of the Research Institute of Ophthalmology, Giza, Egypt. All rats fed on basal diet. Eight rat mothers having rat pups aging 10±21 days were included in this study. Each rat mother and their pups were housed in one cage and served as groups from one to eight. The rat pups in the experimental groups 2, 4, 6 and 8 received a single subcutaneous injection of sodium selenite (30µmol/kg bw) while groups (1and 3) were injected with normal saline (0.3ml). Catalase activity and total antioxidants were determined in plasma. Malondialdehyde, lipid profiles; total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C); the liver functions (aspartate transaminase; AST and alanine aminotransferase ALT), kidney functions (urea and creatinine) were also measured in serum.
Our results show that *Ginkgo biloba* leaves contains high amounts of narengin (193.15 ppm), rutin (108.49 ppm), hisperdin (107.21ppm) and quercitrin (94.86 ppm). Ophthalmic examinations revealed three rats only developed cataract with a 25% of lenses (stage 1), while no opacification in 75% of the group treated with100mg/kg bw Gb. Biochemical estimation found significantly increased in the level of MDA, and significantly decreased in catalase activity and total antioxidant in cataractous group. After rats treated with *Ginkgo biloba*, data showed gradually higher level in catalase activity and total antioxidant and lower level in MDA as increased in the concentrations of *Ginkgo biloba* extract. The best results obvious clearly in cataract group treated with100mg/kg bw *Ginkgo biloba*. Our results concluded that *Ginkgo biloba* in a dose of concentrations 100mg/kg bw *Ginkgo biloba* inhibited cataract formation, and *Ginkgo biloba* supplementation protected the eye from cataracts development.

Key words: *Ginkgo biloba* extract, selenite induced cataract, flavonoids, catalase, MDA, total antioxidant, liver and kidney functions.

INTRODUCTION

Ginkgo biloba is one of the most widely used herbs in the world, and is known for its numerous health benefits [1]. The pharmacological activity of *Ginkgo biloba* extracts was attributed to synergistic action of flavonoids such as terpene and trilactones [2]. Clinical studies indicated use of the ginkgo extract in the treatment of poor circulation, impotence, heart diseases, chronic cerebral insufficiency, short term memory loss, diabetes, depression and dementia [3-5]. Also, acting as a powerful antioxidant [6,7], Platelet aggregation factor antagonism [8] and it used for treatment bronchial asthma [7]. In addition, *Ginkgo biloba* extracts were found to protect rats against different eye diseases such as age related macular degeneration [9], diabetic retinopathy [10], glaucoma[11] and radiation cataract [12]. *Gingko biloba* leaves contain is full of flavonoids, which act as antioxidants. These flavonoids are known to help with retinal problems [13] and possibly even cataracts [14].

The major causes of blindness are cataract, glaucoma, age related macular degeneration, corneal opacities, diabetic retinopathy and

trauma. Among these alternations, cataract is the foremost cause of blindness globally and is responsible for 50, 51% of total blindness [15]. Although modern method of cataract surgery is an efficient means to treat cataract, it has its own complication. Therefore an alternative medicine treatment for the control/delay of cataract occurrences could make a great impact in these parts of the world. Recognition of the role played by natural antioxidant in delaying the onset of cataract has opened new avenues for treatment of cataract [16]. Significant research has been dedicated to investigate the means to make natural antioxidant more efficient and use them as therapeutics for cataract. Recent investigations have shown that phytochemicals antioxidants can scavenge free radicals and prevent various diseases like cataract.

Because there is sufficient evidence that oxidative stress plays a role in the mechanisms of cataractogenesis, there is an increasing interest in developing suitable antioxidant nutrients, both of synthetic and plant origin that could be effective in a delaying or preventing the formation of cataract [17]. Selenite cataract is associated with oxidative stress and protein insolubilization in the lens.

Materials and methods

1. Materials

a. Plant materials

Ginkgo biloba leaveswere collected from tree cultivated in Orman garden, Giza, Egypt during summer seasons (2009-2010). All leaves were washed then air dried in shade at room temperature for 5–7 days, grinded to fine powder and kept for different experiments.

b. Chemicals

All chemicals were of analytical grade. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits for determination of serum levels of lipid profiles, liver and kidney functions were purchased from (Bio-Diagnostic Company).

c. Experimental diet

The basal diet, was formulated according to A.O.A.C. [18]

Experimental animal design:

Seventy five of neonatal rat pups of Wistar strain were obtained from the animal house of Research Institute of Ophthalmology, Giza, Egypt. The animals were housed in stainless steel cages and kept under normal healthy laboratory condition. All rats fed on basal diet; the basal diet was formulated according to A.O.A.C. [18]. Eight rat mothers having rat pups aging 10±21days were included in this study. Each rat mother and their pups were housed in one cage and served as groups from one to eight. Group one served as normal control group (8 pups). Group two: selenite-induced cataract (16 pups). Group three: normal control group treated with 40mg/kg body weight *Ginkgo biloba* (7 pups) and group four: selenite-induced cataract treated with 40mg/kg body weight *Gingko biloba* (6 pups). Group five: normal control group treated with 60mg/kg body weight *Gingko biloba* (7 pups). Group six: selenite-induced cataract treated with 60mg/kg body weight *Gingko biloba* (13 pups). Group seven: normal control group treated with 100mg/kg body weight *Gingko biloba* (6 pups) and group eight: selenite-induced cataract treated with 100mg/kg body weight *Gingko biloba* (12 pups).

Gingko biloba extract was treated as once a day orally in all groups. The rat pups in the experimental groups (2, 4, 6 and 8) received a single subcutaneous injection of sodium selenite (30µmol/kg) according to the method described by Orhan *et al*. [19], while groups (13) were injected with normal saline (0.3 ml). The ophthalmic investigation to follow up cataract progression was under observation all time of the experiment.

a) Slit lamp biomicroscopic examination and lenticular opacification:

Both eyes of all rat pups were dilated using 2% tropicamide solution eye drops. The progression of cataract was observed by slit lamp after 4 days. Cataract could be seen obviously by the naked eye in cataractous group. The progression of cataract in all cataract groups was under

observation till the end of the experiment. Slit lamp biomicroscope examination (Zeiss, japan) was carried out at regular intervals and the stages were designated according to the method described by Kawara and Obazawa [20]. Briefly, lenses were examined on alternate days and opacities observed were graded into five stages. A mature cataract was observed as a dense opacity in both cortex and nucleus. The rat's eyes were examined for detecting symptoms of cataract, all rats lenses were examined by the ophthalmoscope.

e. Blood sampling:

At the end of the experiment (two months), all rats were fasted overnight, anesthetized, and blood was collected from eye can thus (Retro-orbital blood collection). Blood samples were collected using heparinized capillary tubes into two separated tubes. Whole blood is then centrifuged at 3500 r.p.m for 10 minutes to separate plasma for determination of catalase (CAT) activity, total antioxidants (TAO). The second tube was used to separate serum by centrifuging at 3500 r.p.m for 10 minutes to determined malondialdehyde (MDA), liver and kidney functions. All samples were kept in a deep freezer under -20°C until used.

2. Methods:

A. Proximate analysis of *Ginkgo biloba* leaves

The moisture, total nitrogen, crude fiber, ash and total lipid content of *Ginkgo biloba* leaves were determined according to the methods of A.O.A.C. [18]. Also total carbohydrate was calculated by difference according to the A.O.A.C. [18].

b. Identification of total flavonoid compounds in *Ginkgo biloba* leaves by high performance liquid chromatography (HPLC):

Flavonoid compounds of *Ginkgo biloba* leaves were extracted according to method of Mattila *et al*. [21] as follow: Five g of sample were mixed with methanol and centrifuged at 1000 r.p.m (Sanyo, centrifuge, model- Harrier 18/80) for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml were collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with auto samplling injector, solvent degasser, ultraviolet (UV) detector set at 330nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1ml/min. Flavonoid standard was obtained from sigma Co. and dissolved in a mobile phase and injected into HPLC. Retention time and peak area was used to calculation of phenolic compounds concentration by the data analysis of HEWLLET Packared software.

c. Extraction of *Ginkgo biloba* leaves:

The extract of *Ginkgo biloba* leaves was obtained by grinding dried plant material leaves. This was taken in a round-bottomed flask and 80% methanol added to cover the material to form an extract. This extract refluxed in a water bath at 65°C for 24h.The extract was decanted, filtered and concentrated to remove the solvent by using rotary evaporation.

d. Preparation of sample extracts for the protecting doses:

Ginkgo biloba extract was prepared with a concentration 40, 60, 100 mg/Kg B.W. and dissolved in 5% DMSO for oral uptake by using stomach tube. These preparations were freshly prepared.

e. Biochemical analysis:

Catalase activity (CAT) was determined according to the method described by Aebi [22]; total antioxidants capacity (TAO) was measured according to the method of Koracevic *et al*. [23]; malondialdehyde (MDA) was determined to the method described by Satoh [24]. Serum total cholesterol (TC) was determined according to the method described by Allain *et al*. [25], triacylglycerols (TAG) was analysed according to the method described by Fossati and Prencipe [26], high density lipoprotein cholesterol (HDL-C) was measured by using method of Lopes-Virella *et al*. [27], and low density lipoprotein-cholesterol (LDL-C) was estimated by Tietz [28]. Urea and creatinine levels was analyzed according to the method of Fawcett and Soctt

[29]; serum (aspartate transaminase; AST and alanine aminotransferase ALT), was determined according to the method of Reitman and Frankel [30].

Statistical analysis:

SPSS package (version 10) was used for data analysis. Data were analyzed using one way ANOVA, mean and standard error were descriptive measures of data. Least significant difference (LSD) multiple comparison tests were then carried out. P values were significant if <0.05.

Results:

1. **Proximate analysis of**

Table 1: The proximate analysis of *Ginkgo biloba* leaves:

Constituents %	Proximate analysis
Moisture	79.18%
Ash	16.44%
Crude protein	3.93%
Total lipid	4.86%
Crude fiber	13.59%
Total carbohydrate	74.77%

The percentage of moisture content of *Ginkgo biloba* leaves was 79.18% while, the ash content was found to be 16.44%. The crude protein content of *Ginkgo biloba* leaves was 3.93% and the total lipids was 4.86%. The percentage of crude fiber content of *Ginkgo biloba* leaves was 13.59% while total carbohydrate (calculated by difference) in *Ginkgo biloba* leaves was 74.77 %.

2. **Flavonoid compounds in *Ginkgo biloba* leaves:**

Table 2: Flavonoid compounds in *Ginkgo biloba* leaves determined by high performance liquid chromatography (HPLC).

Flavonoid compounds	Concentration (ppm)	Flavonoid compounds	Concentration (ppm)
Narengin	139.15	Luteolin	7.83
Rutin	108.49	Narenginin	0.97
Hisperdin	107.21	Kampferol	1.59
Rosmarinic	2.19	Hispertin	1.08
Quercitrin	94.86	Apegnin	10.26
Quercetin	5.97	7-Hydroxy flavon	0.39

Table (2) shows, *Ginkgo biloba* leaves contain high amounts of narengin, rutin, hisperdin and quercitrin. Flavonoid found in a moderate amount such as apegnin, luteolin and quercetin and in low amount such as rosmarinic, kampferol, hispertin, narenginin and 7-Hydroxy flavon.

Slit lamp examination and degree of lenses opacification:

The results of slit lamp examinations in Table (3) and Figure (1) shows the white clouds covering the whole eyes of cataract group (Fig. b) as compared to treated and normal control groups (Fig. a) and indicated that 100% in selenite-induced cataract group developed bilateral cataract (stage 5) while all lenses were clear in control rat pups. In selenite-induced cataract group treated with 40mg/kg body weight *Ginkgo biloba* (Fig. c), data showed lowered maturation of selenite induced cataract to 33.3%, while 66.67% of the lenses still displayed stage 4. The data in Table (3) shows the percentage of cataract treated with 60mg/kg body weight *Ginkgo biloba* was 39% (5 rats) while 61% (8 rats) of the lenses displayed stage 3 (Fig. d). Whereas, in selenite-induced cataract group treated with 100mg/kg body weight (Fig. e), three rats only developed a bilateral cataract with a 25% of lenses (stage 1), while 75% of that group was improved after treatment with *Ginkgo biloba* extract.

Table 3: Grading of cataract on the basis of slit lamp examinations

Groups	Number of rats	Stage of cataract*					%of cataract	% of cataract improvement
		1	2	3	4	5		
Normal control	8	-	-	-	-	-	0%	-
Cataract	16	-	-	-	-	16	100%	-
Cataract treated with 40 mg/kg b.w	6	-	-	-	4	-	66.7%	33.3%
Cataract treated with 60 mg/kg b.w	13	-	-	8	-	-	61%	39.0%
Cataract treated with 100mg/kg b.w	12	3	-	-	-	-	25%	75%

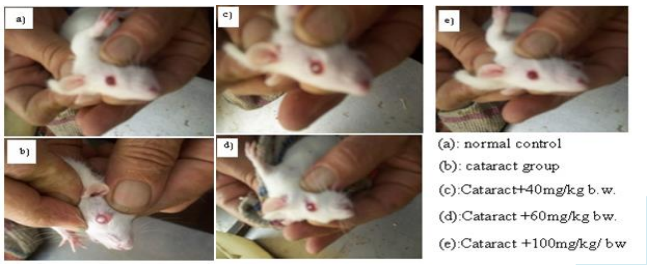

Figure 1: Anticataract effect of different concentrations *Ginkgo biloba* extract against cataract formation.

Results of biochemical assessment:

Data in Table (4) show the mean values±S.E. of catalase activity (CAT U/l), malondialdehyde (MDA nmol/ml) and total antioxidant (TAO mmol/l) in different groups. Cataract group shows significant decrease in the levels of CAT activities and TAO, while significant increase in the level of MAD was also noticed comparing to normal control values. In normal control and cataract treated with 40mg/kg b.w Gb groups, our data revealed not significant change in the levels of CAT activity, MDA and TAO in both groups comparing to normal control group. On the other hand, in cataract group treated with 40mg/kg b.w Gb, the percentage was decreased to -19.21%, -7.52% and -9.6% concerning to CAT, MDA and TAO respectively comparing to normal control. Also in normal control and cataract groups treated with 60mg/kg b.w Gb, there was no significant change in the levels of catalase activity and TAO comparing to normal control group, but the level of MDA revealed significant decrease in cataract group treated with 60mg/kg b.w Gb. The percentage was decreased to -37.33% comparing to normal control group and decreased to -53.42% comparing to cataractous group. The best results obvious clearly in cataract treated with 100mg/kg b.w Gb, since the results of CAT and TAO were improved rather than other groups after rats treated with 100mg/kg b.w GB in addition to decrease in the level of MDA to-60.66%. The mean values of CAT and TAO were increased and MDA was decreased gradually according to the increased in concentrations of Gb extract.

Data in Table (5) show the mean values±S.E. of serum total cholesterol (TC mg/dl), triacylglycerol (TAG mg/dl), high density lipoprotein-cholesterol (HDL-c mg/dl) and low density lipoprotein-cholesterol (LDL-c mg/dl), in different groups. As shown in this table, all lipid parameters in cataract group were significantly decreased comparing to the data in normal control group. But all lipid parameters in all groups' revealed not significant change comparing to the data in normal control group except, the level of TC in cataract groups treated with 60 mg and both normal control and cataract groups treated with 100mg/kg b.w Gb. Also, the level of LDL-C shows significant decrease comparing to normal control group. Our data also noticed gradually improvement in the lipid profiles according to *Ginkgo biloba* extract concentration.

Data in Table (6) show that the mean values± S.E. of serum urea (mg/dl), creatinine (mg/dl), serum aspartate transaminase (AST) and alanine aminotransferase (ALT) levels in different groups. In cataract group, data shows an increased in the percentage level of urea, creatinine, AST and ALT (33.34%, 34.42%. 33.57% and 75.75%) respectively when compared to normal control group Moreover, there was no significant change in the level of urea, creatinine, AST and ALT comparing to normal control group but highly significant change comparing to cataract group. Furthermore the percentage was decreased to -27.79%, -35.98%, -38.36% and -36.68% concerning to urea, creatinine, AST and ALT comparing to cataract group.

Table 4: Activities of catalase activity (CAT U/l), malondialdehyde (MDA nmol/ml) and total antioxidants (TAO mmol/l), in normal control, selenite induced cataract, and different groups treated and untreated with different concentration of *ginkgo biloba*.

Parameters	Groups	CAT activity U/l	MDA nmol/ml	TAO mmol/l
Normal Control	mean	185.67 ±4.77 cd	3.59 ±0.18 b	0.73 ±0.031 ab
Cataract	mean	132.33	4.83	0.48
	±S.E.	± 13.78 e	±0.77 a	±0.034 c
	%Change	-28.73	34.54	-34.25
Normal Control treated with 40 mg/kg /b.w Gb	mean	194.83	3.18	0.72
	±S.E.	±12.47 bcd	±0.13 cb	±0.055 ab
	%Change	4.93	-11.42	1.37
	%Change	47.23	-34.16	50
Cataract treated with 40 mg/kg /b.w Gb	mean	150.00	3.32	0.66
	±S.E.	±6.19 de	±0.33 cb	±0.068 bc
	%Change	-19.21	-7.52	-9.6
	%Change*	13.35	-31.26	37.5
Normal Control treated with 60 mg/kg/ b.w Gb	mean	212.50	3.09	0.76
	±S.E.	±28.2 bc	±0.51 cb	±0.055 ab
	%Change	14.5	-13.93	4.11
	%Change	60.58	-36.02	58.33
Cataract treated with 60 mg/kg/ b.w Gb	mean	211.7	2.25	0.85
	±S.E.	±16.67 bc	±0.25 cd	±0.053 ab
	%Change	14.02	-37.33	16.44
	%Change*	59.98	-53.42	77.08
Normal Control treated with 100 mg/kg /b.w Gb	mean	233.33	3.23	0.79
	±S.E.	±15.83 ab	±0.36 bc	±0.070 ab
	%Change	25.67	-10.03	8.22
	%Change	76.32	-33.13	64.59
Cataract treated with 100 mg/kg /b.w Gb	mean	278.00	1.90	0.88
	±S.E.	±23.53 a	±0.31 d	±0.14 a
	%Change	49.73	-47.08	20.55
	%Change*	110.08	-60.66	83.33

Different litters mean significant. % Change: Percentage change from normal control and % Change*: Percentage change from cataract group.

Table 5: Serum of triacylglycerol (TAG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-c) as well as low density lipoprotein-cholesterol (LDL-c), in normal control, selenite induced cataract, and different groups treated and untreated with different concentration of *ginkgo biloba*.

Parameters	Groups	TC mg/dl	TAG mg/dl	HDL-c mg/dl	LDL-c mg/dl
Normal Control	mean	109.50± 3.25 b	95.80 ±4.32 bc	38.38 ±2.10 ab	44.27 ±3.16 bc
Cataract	mean	121.50±	115.82	28.83	57.23
	±S.E.	7.05 a	±2.98 a	±2.87 c	±3.40 a
	%Change	10.96	20.90	-24.88	29.27
Normal Control treated with 40 mg/kg /b.w Gb	mean	104.83±	93.80	38.78	40.52
	±S.E.	3.36 bc	±4.75 cb	±1.39 ab	±3.4 dbc
	%Change	-4.26	-2.09	1.04	-8.47
	%Change	-13.72	-19.01	34.51	-29.19
Cataract treated with 40 mg/kg /b.w Gb	mean	109.67±	103.71	33.83	46.89
	±S.E.	3.77 b	±8.95 ab	±3.17 bc	±4.89 b
	%Change	0.16	8.26	-11.86	5.92
	%Change*	-9.74	-10.46	17.34	-18.07
Normal Control treated with 60 mg/kg/ b.w Gb	mean	100.7	88.03	40.18	38.53
	±S.E.	±2.56 bc	±6.05 bc	±4.00 ab	±3.19bcd
	%Change	-8.04	-8.11	4.69	-12.97
	%Change	-17.12	-23.99	39.37	-32.67
Cataract treated with 60 mg/kg/ b.w Gb	mean	96.38	90.73 ±8.51	38.33	35.76 ±3.34
	±S.E.	±3.81 dc	bc	±3.89 ab	cd
	%Change	-11.98	-5.29	-0.130	-19.22
	%Change*	-20.67	-21.66	32.95	-37.52
Normal Control treated with 100 mg/kg /b.w Gb	mean	87.00	80.60	44.26	37.67
	±S.E.	±2.96de	±3.91 c	±2.58 a	±3.04bcd
	%Change	-20.55	-15.87	15.32	-14.91
	%Change	-28.39	-30.41	53.52	-34.18
Cataract treated with 100 mg/kg /b.w Gb	mean	82.17	83.70	39.43	32.41
	±S.E.	±1.62 e	±3.44 c	±3.41 ab	±2.27 d
	%Change	-24.96	-12.63	2.74	-26.79
	%Change*	-32.37	-27.72	36.77	-43.37

Different litters mean significant. % Change: Percentage change from normal control and % Change*: Percentage change from cataract group.

Table 6: Serum of urea (mg/dl), creatinine (mg/dl) and aspartate transaminase (AST U/l) and alanine aminotransferase (ALT U/l), level in normal control, selenite induced cataract, and different groups treated and untreated with different concentration of *ginkgo biloba*.

Parameters	Groups	Urea mg/dl	Creatinine mg /dl	AST U/l	ALT U/l
Normal Control	mean	26.78 ±2.0 cd	0.523 ±0.044 bc	44.00 ±7.99 b	29.50 ±1.06 bc
Cataract	mean	35.77	0.703	77.33	40.00
	±S.E.	±1.64 a	±0.109 a	±9.92 a	±3.26 a
	%Cha nge	33.57	34.42	75.75	35.59
Normal Control treated with 40 mg/kg /b.w Gb	mean	26.40	0.512	55.00	30.67
	±S.E.	±1.16 dce	±0.039 bc	±7.89 ab	±1.50 bc
	%Change	-1.42	-2.10	25.00	3.97
	%Change	-26.19	-27.17	-28.88	-23.32
Cataract treated with 40 mg/kg /b.w Gb	mean	32.37	0.598	67.33	34.00
	±S.E.	±3.15 ab	±0.047 ab	±7.45 ab	±1.32 b
	%Change	20.87	14.34	53.02	15.25
	%Change*	-9.51	-14.94	-12.93	-15.00
Normal Control treated with 60 mg/kg/ b.w Gb	mean	23.01	0.490	45.33	29.66
	±S.E.	±1.12 ed	±0.032 bc	±9.64 b	±3.57 bc
	%Change	-14.08	-6.31	3.02	0.54
	%Change	-35.67	-30.29	-41.38	-25.85
Cataract treated with 60 mg/kg/ b.w Gb	mean	29.58	0.540	63.00	26.67
	±S.E.	±1.69 bc	±0.042 cb	±11.6 ab	±0.56 c
	%Change	10.45	3.25	43.18	-9.59
	%Change*	-17.31	-23.19	-15.53	-33.33
Normal Control treated with 100 mg/kg /b.w Gb	mean	21.40	0.445	43.67	29.00
	±S.E.	±0.95 e	±0.023 c	±8.29 b	±2.28 bc
	%Change	-20.09	-14.91	-0.75	-1.69
	%Change	-40.17	-36.69	-43.52	-27.5
Cataract treated with 100 mg/kg /b.w Gb	mean	25.83	0.450	47.67	25.33
	±S.E.	±2.02 dce	±0.026 bc	±10.99 b	±1.52 c
	%Change	-3.55	-13.96	8.34	-14.14
	%Change*	-27.79	-35.98	-38.36	-36.68

Different litters mean significant. % Change: Percentage change from normal control and % Change*: Percentage change from cataract group.

Discussion:

It has been well known that oxidative stress is one of the possible causes of cataract. The present study was designed to determine the possible protective effects of *Ginkgo biloba* against oxidative damage induced by sodium selenite. 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 [31]. 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 excess oxidative stress was previously reported to induce extensive oxidative modifications on lens proteins especially *α*-crystalline protein, a major protein component of the lens resulting in the enhanced lens opacity. *Ginkgo biloba*extract is considered an alternative medicine for the treatment and / or the prevention of different eye diseases. Intraperitoneal injection of EGb 761 can enhance the antioxidant ability of retina and partially inhibit the apoptosis of photoreceptors [32]. *Ginkgo biloba* has also been shown to prevent diabetic retinopathy in diabetic rats. *Ginkgo biloba* may act as a neuroprotective and prevent damage to retinal ganglion cells, this plant extract would be an interesting component for prevention and treatment of ocular diseases and other major neurodegenerative retinal pathologies [33].

Ophthalmic examination at the end of the present study, noticed lenticular opacification (stage 4; 66.7%) of the group that had received a single subcutaneous injection of sodium selenite and treated with 40 mg/kg body weight Gb. And lenticular opacification (stage3; 61%) in the group that had received a single subcutaneous injection of sodium selenite and treated with 60 mg/kg body weight Gb. While lenticular opacification (stage 1; 25%) in three rat pubs only in selenite induced cataract treated with 100mg /kg body weight Gb was noticed. As shown in Table [3] *Ginkgo biloba* extract had effective to reduce the stages of cataract formation from stage (4) in the group treated with 40 mg/kg body weight Gb to stage (1) in the group treated with 100mg/kg bw, also reduced the percentage from 66.7% to 25% in rats of that groups. This is proved the protective effect of *Ginkgo biloba* against cataract formation. Ertekin *et al*. [12] suggested that, *Ginkgo biloba* is an antioxidant that protects the rat lens from radiation-induced cataracts. These data suggested also *Ginkgo biloba* is very important to protect the lens from cataract formation and development. Also, Figure (1) proved an improvement in the lens cataract formation as increased in the concentrations of *Ginkgo biloba* extract for delay the progress of cataract formation.

At the present study, we extract flavonoids in ethyl alcohol to get the higher amount of

flavonoids from *Ginkgo biloba* leaves. Qualitative and quantitative analysis of different extracts of *Ginkgo biloba*leaves [ethyl acetate, 70% ethanol, and water extracts] were studied by Ahmed *et al*.[34], who found that, quantitative analysis ethanolic extract of *Ginkgo biloba* leaves showed a higher amounts of flavonoids and phenolic compounds than other extracts.

In the present study the phenolic compounds present in *Ginkgo biloba* extract were identified by HPLC. The observed findings in this study could be well because of the ameliorative action of the bioactive compound narengin, rutin, hisperdin, quercitrin, apegnin, luteolin, quercetin, rosmarinic, kampferol, hispertin, narenginin and 7-Hydroxy flavone in extract as shown in Table (2). Several pharmacological actions of the flavonoids may be useful in the prevention or treatment of ocular disease. From different studies it was found that, flavonoid consumption has reduced risk of developing cataract [35,36]. Flavonoid isolated from green and black tea [37], grape seeds [38], garlic and cactus pear juice [39,40], broccoli, [41].

As shown in this study, there is a high amount of narengin, rutin, hispertin in the *Ginkgo biloba* extract. Naringin has shown protective effects against oxidative damage in rats [6]. Our results confirmed with Muthenna *et al*. [42]; Sasikala *et al*. [43] who suggested the therapeutic potential of rutin, a bioflavonoid, against selenite-induced cataract. Whoever, Majumdar and Srirangam [44] suggested that hesperidin can be used in ocular diseases because of its varied pharmacological actions. We found an appreciable amount in hesperidinand Hispertinin *Ginkgo biloba*extract. The efficient of hesperidinand Hispertinin this study were reported to stop the progression of cataract formation besides their effect on vascular permeability and ocular blood flow as reported by Majumdar and Srirangam [44]. Both hesperidin and hesperetin demonstrate strong antioxidant properties [45]. This antioxidant activity is through their ability to quench oxidative radical chain reactions. Our results also demonstrated the anticataractogenic effect of luteolin and quercetin by virtue of its antioxidant property as reported by Roobane*t al*. [46] and Ramana, *et al*. [47]. Quercetin reduced the risk of cataract formation through the maintenance of characteristic osmotic ion equilibrium and protein levels of the lens and by affecting multiple pathways pertinent to eye lens opacification, including oxidative stress, nonenzymaticglycation, the polyol pathway, lens calpain proteases, and epithelial cell signaling [48]. As shown in the data of HPLC, *Ginkgo biloba* contains flavonoids such as apegnin, rosmarinic, kampferol, hispertin, narenginin and 7-Hydroxy flavone that are potent free radical scavengers and can protect against cataract by reducing the injury caused by oxidation on the lens of the eye.

It turns out that *Ginkgo* increase the oxidation enzymes superoxide dismutase and glutathione peroxidase in the eye [12]. Simultaneous determination of catalase, total antioxidants and malondialdehyde levels were carried out in this study. Data in cataract group revealed a significant decrease in the percentage of plasma catalase activity and total antioxidant (-28.73%, -34.25%), respectively, while a percentage increase in the level of MDA (34.54%) in serum and lens (34.54%) comparing to control group. The data revealed an improvement in the selenite to induced cataract groups (40, 60, 100mg/kg bw Gb). Significantly increased in plasma CAT activities, TAO and tended to reduce MDA level in that group as the concentration of the extract increased comparing to control group. Our results showed that *Ginkgo biloba* extracts caused a significant increase in the activity of catalase and total antioxidants in the blood of treated groups as the concentration of the extract increased. The best results obvious clearly in the group treated with 100mg/kg bw Gb. This was accompanied by a simultaneous decrease in the levels of MDA in plasma. Our results suggest that *Ginkgo biloba* supplementation in rat pubs treated with 100mg/kg bw Gb may delay the cataract formation and may enhance antioxidant defense. These results may be applied in the future for the prevention and treatment of cataracts by using *Ginkgo biloba*. The results also suggest that *Ginkgo biloba* can prevent or retard experimental selenite-induced cataractogenesis in Wistar rats, *Ginkgo biloba*extract is rich in flavonoids and commonly used natural supplement and its main properties are

protection against free radical damage and lipid peroxidation. As reported previously flavonoids prevents experimental selenite-induced cataractogenesis in rat pups and treatment abated selenite induced oxidative stress and cataractogenesis by maintaining antioxidant status, reducing ROS generation and lipid peroxidation in the lens possibly by inhibiting lipid peroxidation as reported by Muthenna*et al*. [42], Sasikala *et al*. [43], Rooban *et al*. [46].

The relationships between cataract lipids have been discussed earlier rats [49]. In the present study TC, TAG, and LDL-C were significantly increased in cataract group as compared to normal control group. The percentage increased to10.96%, 20.9% and 29.27% respectively. However, treatment with *Ginkgo biloba* extract significantly reduced in their levels gradually according to increase in *Ginkgo biloba* extract concentrations. The percentage changes were decreased severe in the group treated with 100mg/kg bw Gb as compared to cataract group (Table 5). Our results confirmed with the previous studies Dubey *et al*. [50] and Yao *et al*. [51] and suggested that *Ginkgo biloba* can modulate lipids and have a protective role of cataract formation. Hesperidin-flavonoid in *Ginkgo biloba* - has been reported to possess hypo-lipidemic as reported by Monforte *et al*. [52].

The results of kidney functions (serum of urea and creatinine and liver enzymes aspartate transaminase and alanine aminotransferase (Table 6) were revealed more or less normal values after rat treated with *Ginkgo biloba* 100mg/kg bw Gb. Treatment with *Ginkgo biloba* produced amelioration in liver and kidney functions and its strongest effect was observed at a dose of 100mg/kg bw *Ginkgo biloba*. The results showed that *G. biloba extract* is a potential effect against cataract development, and its protective role is dose of extract dependent.

Its anti-cataract potential may be attributed to the presence of high phenolics and flavonoids in *Ginkgo biloba*. It can be concluded that oral consumption of *Ginkgo biloba* following selenite injection effective in preventing cataractogenesis in selenite model by enhance antioxidant enzyme defense of blood and protect the lens from the oxidative stress, reducing the intensity of lipid peroxidation, decreasing oxidative stress and inhibiting free radical generation and can delay the onset and/or prevent the progression of cataract.

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