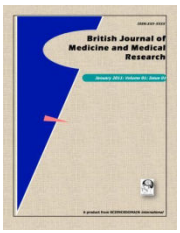

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Biochemical and Ultrastructural Changes in lens during Aging of Wistar Albino Rats

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Research Article

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ABSTRACT

Aims: To correlate the age-related structural changes in lens with its contents of amino acids and minerals involved in its functional activity (sodium, calcium, magnesium and copper).
Study design: Experimental study.
Place and Duration of Study: Department of Zoology, Faculty of Science, Mansoura University, Egypt, between June 2009 and July 2011.
Methodology: One hundred male and female albino rats of the Wistar strain (*Rattus norvegicus*) aging 1, 6, 18, 30 & 42 months were used during experimentation. At the mentioned ages, the animals were sacrificed by diethyl ether and ocular regions were dissected and lens separated. Amino acids content were determined in both lenses of five individuals. In the other five individuals, unilateral ectomized lenses were subjected for chemical determination of calcium, sodium, cupper and magnesium. The rest lenses on the other side were fixed in 10% phosphate buffered formalin followed by 2.5% glutaraldhyde for scanning electron microscopic investigation. Statistically analysis was carried out by the help of SPSS software.
Results: The estimated amino acids were altered in both sexes with advancement of aging. The lens amino acids; taurine, prolline, serine, threonine, methionine, lysine and arginine were markedly increased during aging comparing with apparent depletion of asparate, valine and methionine. The alterations in amino acid contents were parallel with increased accumulation of calcium, sodium and cupper and a depletion of magnesium content. Scanning Electron Microscopic (SEM) observations exhibited sequences of changes of the structures of lens fibers and their pattern of attachments during the advancement of aging. In the advanced aged group, the lens fibers become fragile and widely separated as a result of loosely attached of the interfibrillar junctions of the ball and

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socket especially at 30 & 42 month old rats.
Conclusion: Aging led to alterations of lens fibers at ultrastructural levels associated with diversity of changes in their amino acids contents parallel with increase accumulation of sodium, calcium and cupper and a decrease of magnesium content. The observed findings may be attributed to the increase of advanced glycation end products initiated oxidative stress, impairing lens structure and function.

Keywords: Aging; lens; amino acids; minerals; sodium; calcium; cupper; magnesium; SEM.

1. INTRODUCTION

The aging human lens has been the subject of intense research over the past 20 years. The fact that the incidence of cataract rises exponentially with age after 50 years provides the driving influence for much of the effort, but the unique accessibility, homogeneity, and basic simplicity of structure of the organ itself makes it a fruitful system for fundamental studies of tissue growth, development, and differentiation (McAvoy and Chamberlain, 1990; Duncan et al., 1997).

The ocular lenses in most animal species contain a unique set of proteins having extraordinary stability and transparency throughout life (Wang and Spector, 1994) and a low oxygen concentration that minimizes oxidative damage (Eaton, 1991). Three types of ionic channels have been identified in the lens. The smallest appears to be a calcium channel and the larger ones are associated with sodium and potassium movements (Duncan and Jacob, 1984).

Cataract is the major cause of blindness in the world (West and Valmadrid, 1995; Kuszak et al., 2000) with incidence up to 50 millions in the world (Bunce et al., 1990; Minassian and Mehra, 1990; Santana and Waiswo, 2011) and more widespread in developing countries (Sperduto, 2000, WHO, 2005).

Aging of the lens is associated with progressive changes in the physical and chemical properties of its crystalline structural protein. Highly reactive oxygen species can be generated in the eye through photochemical pathways (Linetsky et al., 1999) or Fenton-type reactions (Fu et al., 1998) and oxidative stress altering lens protein structure including crystalline cross linking, aggregation, loss of solubility, conformational alterations, fragmentation, and enzyme inactivation. Normal young lenses maintain optimal activity of antioxidant enzymes and high concentrations of ascorbate and glutathione and hence minimize the alterations wrought by excessive oxidation. If this balance was altered during aging, the oxidation of lenticular proteins may lead to senile cataract (Spector, 1995).

One of the most causes of senile cataract is the formation of advanced glycation end products (AGEs) which formed by the nonenzymatic reaction of reducing sugars, ascorbate and other carbohydrates with amino acids, lipids and nucleic acids and through lipid peroxidation as well (Vlassara and Palace, 2002; Peppa and Vlassara, 2005; Luevano-Contreras and Chapman-Novakofski, (2010). Accumulation of proteins containing high levels of AGEs has been noted with diabetic complications and age-related ocular alterations (Kaji et al., 2007, 2010; Kaji, 2009).

On the other hand, the structure of lens fibers changes significantly both with maturation and senescence. Many studies have shown that the lens fibers in the adult and juvenile nuclear regions undergo considerable age-related compaction (Brown et al., 1988; Taylor et al., 1996; Al-Ghoul and Costello, 1997). It is well established that variations in fiber structure adversely affect the optical properties of the lens (Kuszak et al., 2000). The transparency and high refractive index of the lens are achieved by the precise architecture of the fiber cells and the homeostasis of the lens proteins in terms of their concentration, stability, and supramolecular organization (Santana and Waiswo, 2011).

There is no available work correlated amino acids content with lens fibers ultrastructure and mineral content during aging processes of albino rats. The aim of the present study was to investigate the effect of aging on lens fibers ultrastructures parallel with metabolic changes of their amino acids contents and their inclusions of sodium, calcium, magnesium and copper involved in its functional activity.

2. MATERIALS AND METHODS

One hundred male and female albino rats of the Wistar strain (*Rattus norvegicus*) aging 1, 6,18, 30 & 42 months, 10 individual per each sex were used during experimentation. The animals were obtained from Breeding Farm, Ministry of Health, Cairo, Egypt and maintained in aerated room with controlled temperature (21±2ºC), 50±5% humidity and 12 hour light and dark cycle. Free excess of food and water were allowed *adlibitum.* Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiment.

2.1 Amino Acid Analysis

Five male and female rats at the mentioned ages were anesthetized with diethyl ether and sacrificed by cervical dislocation and the eye globes were excised. The lenses were then dissected, rinsed with ice-cold saline and preserved deep-frozen under saline. Both lenses of each individual rat were processed together to constitute a single value. Each lens of the five individuals per sex was hydrolyzed by 6M hydrochloric acid (24 hours, 70ºC). Sensitive amino acids (especially tryptophane and cysteine) will be partially destroyed. Gas phase hydrolysis with the addition of other acids (e.g. propionic acid, methansulphonic acid) can be used to shorten the hydrolysis time and improve the yield of sensitive amino acids. The samples were washed in hot dilute detergent solution at neutral pH and rinsed in warm tap water and then distilled water. Any pulpy protein in the column was squeezed out and extracted several times with petroleum ether, followed by 95% ethyl alcohol and allowed to dry in a watch glass. The samples were dried under vacuum, redissolved in 10 to 100 µl 0.2 M sodium citrate buffer, pH 2.0, and loaded on the amino acid analyzer equipped with a cation exchange column (Amersham Pharmacia Biotech), which was equilibrated in 0.2 M sodium citrate buffer, pH 2.0. Elution was performed with a gradient of pH and ionic strength as instructed by the manufacturer. Detection of the modified amino acids was achieved calorimetrically at 440 nm for proline and hydroxyproline and at 570 nm for all other amino acids (Niece et al., 1991).

2.2 Measurements of Lens Mineral Content

In the other five individuals of both sexes, unilateral ectomy of lens was carried out to determine the sodium, calcium, cupper and magnesium content. Each lens was first blotted on moistened filter paper, reweighing, placed in Stoppard tubes and then digested with 0.2ml 30% nitric acid for overnight and then completes the solution till 5ml with bi-distilled water. Ion concentrations were measured by atomic absorption spectroscopy according to Delamere et al., (1983).

2.3 SEM Investigation

The other lenses were fixed for 24 hr at room temperature in 10% phosphate buffered formalin, followed by washing in buffer and further fixed for 3–5 days in 2.5% glutaraldhyde (in 0.12 M sodium cacodylate buffer, ph 7.2) at room temperature with fresh fixative changes daily. After overnight washing in 0.2 M sodium cacodylate buffer, the lenses were dehydrated in ascending grades of ethyl alcohol till 100%. Drying was done in a carbon dioxide critical point drying apparatus. The samples were mounted on aluminum stubs using adhesive colloidal silver plaster, placed in sputter coated with gold and examined in a JEOL JSM 35 c scanning electron microscope (Kuszak et al., 1983).

2.4 Statistical Analysis

Statistical analysis was done using SPSS MANOVA with comparisons of the multi-variables between the control and the other experimental groups and within the same group. Values were determined and significance at $P<0.05$.

3. RESULTS

3.1 Amino Acids Content

Data from table (1), two sets of amino acids are determined, non-proteogenic amino acids and this include only taurine amino acid and the other set is proteogenic amino acids. The table illustrates the changes of lens amino acids in aging groups into three categories. The first includes the amino acids; glycine, alanine, histidine, phenylalanine, tyrosine, isoleucine and glutamine which exhibit slight steady increase during aging. The second was markedly increased and include taurine, prolline, serine, threonine, methionine, lysine and arginine. The third group includes asparate, valine and methionine showed apparent reduction during aging.

3.2 Trace Element Content

From table (2), the estimated lens mineral content of calcium, sodium and cupper were markedly increased during aging, however, the magnesium content was decreased.

Table 1. Amino acids contents of lens during aging of Wistar albino rats

Amino Acids (µg/100mg)	1		6		18		30		42		*F value*
	♂	♀	♂	♀	♂	♂	♀	♀	♂	♀	
Taurine	0.45±0.02	0.57±0.10	0.89±0.10	1.04±0.09	1.41±0.26	0.87±0.06	0.89±0.15	1.30±0.23	0.91±0.03	0.83±0.08	4.61
Glycine	1.60±0.12	1.18±0.05	2.26±0.10	2.28±0.07	2.88±0.06	1.22±0.04	1.13±0.16	2.20±0.06	1.80±0.09	1.75±0.08	16.59
leucine	1.67±0.12	1.12±0.05	1.18±0.05	2.26±0.10	2.28±0.07	1.22±0.04	1.13±0.16	2.88±0,06	2.20±0.05	1.80±0.09	42.87
Proline	1.82±0.09	1.18±0.11	2.11±0.15	2.37±0.08	2.65±0.18	2.15±0.25	1.99±0.09	2.65±0.14	2.08±0,12	2.32±0.07	8.41
Alanine	1.52±0.25	1.13±0.03	2.15±0.04	2.23±0.17	3.02±0.14	1.03±0,10	0.96±0.04	2.38±0.15	2.01±0,17	1.46±0.16	26.10
Serine	0.96±0.14	0.71±0.21	1.78±o.15	2.16±0.13	2.93 ±0.09	2.44±0.09	2.10±0.07	2.61±0.15	1.89±0.25	1.84±0.15	21.14
Phenylalanine	1.52±0.07	1.88±0,10	1.33±0.27	1.56±0.12	3.65±0.20	1.24±0.25	1.37±0.15	2.86±0.30	2.06±0.27	1.53±0.18	13.78
Threonine	0.45±0.05	0.55±0.07	0.44±0.04	0.63±0,04	0.87±0.03	0.50±0.07	0.59±0.13	1.15±0.04	0.77±0.10	0.75±0.07	5.19
Tyrosine	2.23±0.15	1.66±o.18	1.95±0.19	2.54±0.19	3.35±0.24	1.74±0.18	1.75±0.19	2.72±0.18	2.23±0.12	1.45±0.17	11.72
Valine	0.45±0.09	0.57±0.13	0.53±0.10	0.74±0.14	0.58±0.09	0.32±0.09	0.52±0.06	0.54±0.03	0.46±0.08	0.37±0.03	2.52
Methionine	4.04±0.25	5.06±0.22	4.69±0.21	5.62±0.30	5.08±0.05	3.05±0.09	3.31±0.27	4.65±0.18	3.53±0.15	3.29±0.18	19.73
Lysine	1.52±0.20	1.90±0.27	1.76±0.23	2.48±0.23	3.36±0.22	1.34±0.20	1.26±0.13	3.10±0.36	2.17±0.12	2.11±0.11	9.35
Asparatic acid	2.21±0,16	2.70±0.17	1.92±0.23	2.38±0.13	3.36±0.14	1.23±0.11	1.17±0.08	2.84±0.06	2.08±0.15	2.03±0.06	24.13
Arginine	1.82±0.09	1.49±0.15	2.11±0.06	2.52±0.09	3.41±0.14	1.86±0.06	1.75±0.08	2.99±0.29	2.20±0.05	2.14±0.38	11.25
Isoleucine	1.03±0.14	0.84±0.07	1.19±0.10	1.41±0.12	1.92±0.10	1.16±0.05	1.04±0.05	1.68±0.08	1.24±0.04	1.18±0.04	14.89
Histidine	0.58±0.08	0.31±0.09	0.45±0.04	0.76±0.04	0.60±0.05	0.32±0.03	0.20±0.02	0.53±0.03	0.66±0.05	0.38±0.03	18.30
glutamine	1.82±0.08	1.49±0.03	2.11±0.06	2.23±0.04	3.20±0.04	1.46±0.13	1.84±0.08	3.10±0.06	1.95±0.10	2.01±0.09	60.02

Each result represent the mean ± SE of five replicates. Significant at P<0.05

Table 2. Calcium, sodium, cupper and Magnesium content (µg/g dried lens) in aged lens

Ages (Months)	Ca		Na		Cu		Mg	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
1M	27.88 ± 2.40	30.21 ± 2.22	330 ± 11.63	380 ± 12.22	6.70 ± 0.31	8.50 ± 0.14	790 ± 13.14	652 ± 12.21
6 M	28.6 ± 2.34	35.21 ± 2.19	384 ± 12.35	370 ± 14.51	13.20 ± 0.18	9.00 ± 0.11	624 ± 12.65	357 ± 11.63
18 M	40.12 ± 3.38	38.01± 2.12	451 ± 14.02	400 ± 13.87	14.80 ± 0.23	13.20 ± 0.21	432 ± 14.09	377 ± 15.01
30 M	48.52 ± 3.45	43.78 ± 3.34	652 ± 11.98	455 ± 15.02	15.50 ± 0.30	14.30 ± 0.13	377 ± 12.87	405 ± 13.02
42 M	62.77 ± 2.2	78.42 ± 4.33	653± 12.13	620 ±13.24	21.00 ± 0.18	16.90 ±0.10	290 ± 12.3	330 ± 11.9
F-test	**63.97**		**115.55**		**114.31**		**97.71**	
P<0.05	**S**		**S**		**S**		**S**	

Each result represent the mean ± SE of five replicates. Significant at P<0.05

3.3 SEM Observations

Examination of the lenses of all groups demonstrated presence of a thin capsule on the outer surface of the lens covering the underlying single layer of lens epithelium. Inner to the epithelium, large number of concentric layers of densely packed lens fibers could be seen and representing the superficial and deep cortical fibers. At the center of the lens, a group of straight fibers were passing along the antero-posterior axis representing the embryonic nucleus. Fibers appeared as tightly joined parallel ribbon-like structures with minimal intercellular spaces in between. Each fiber had the shape of a polygon or hexagon with two wide parallel sides and four other smaller ones (Figs. 1A & 1B).

At 6 month old rat, the lens fibers appear as tightly joined parallel ribbon-like structures with minimal intercellular spaces in between the long axis of the fibers is parallel to the surface of the lens. Lens fibers are of larger diameter and their distribution is more regular in the form of straight lines parallel to the linear holes. The adjacent fibers are interconnected by numerous ball and socket junctions on each planar surface. The projections from the acute vertices of the hexagonal fibers appear at relatively regular intervals. Since the fibers are tightly packed with very little extrafibrillar space, the adjacent fibers must have a complementary surface. The regularity of the depressions and the existence of complementary projections are Strong (Fig. 1 C-F).

At 18 moths-old, the lens fibers showed a wavy lateral cell membrane with secondary branching of the balls and irregular diameter. The flaps are present in zigzag-like lines accompanied by linearly arranged sockets. Widened spaces between lens fibers are originated as a result of free attachment between balls and sockets (Fig. 2 A-D).

At 30 months old, the fibers have inter-fibrillar associations which are limited to well-defined rows of projections localized along the edge of the fiber. The interfibrillar junctions of the ball and socket are loosely associated (Fig. 3 A-F).

At 42 week old rats, abnormal fiber structure with widened separation between lens fibers. There are successive layers of fibers with irregular branching at their lateral inter-digitations (Fig. 4 A-D).

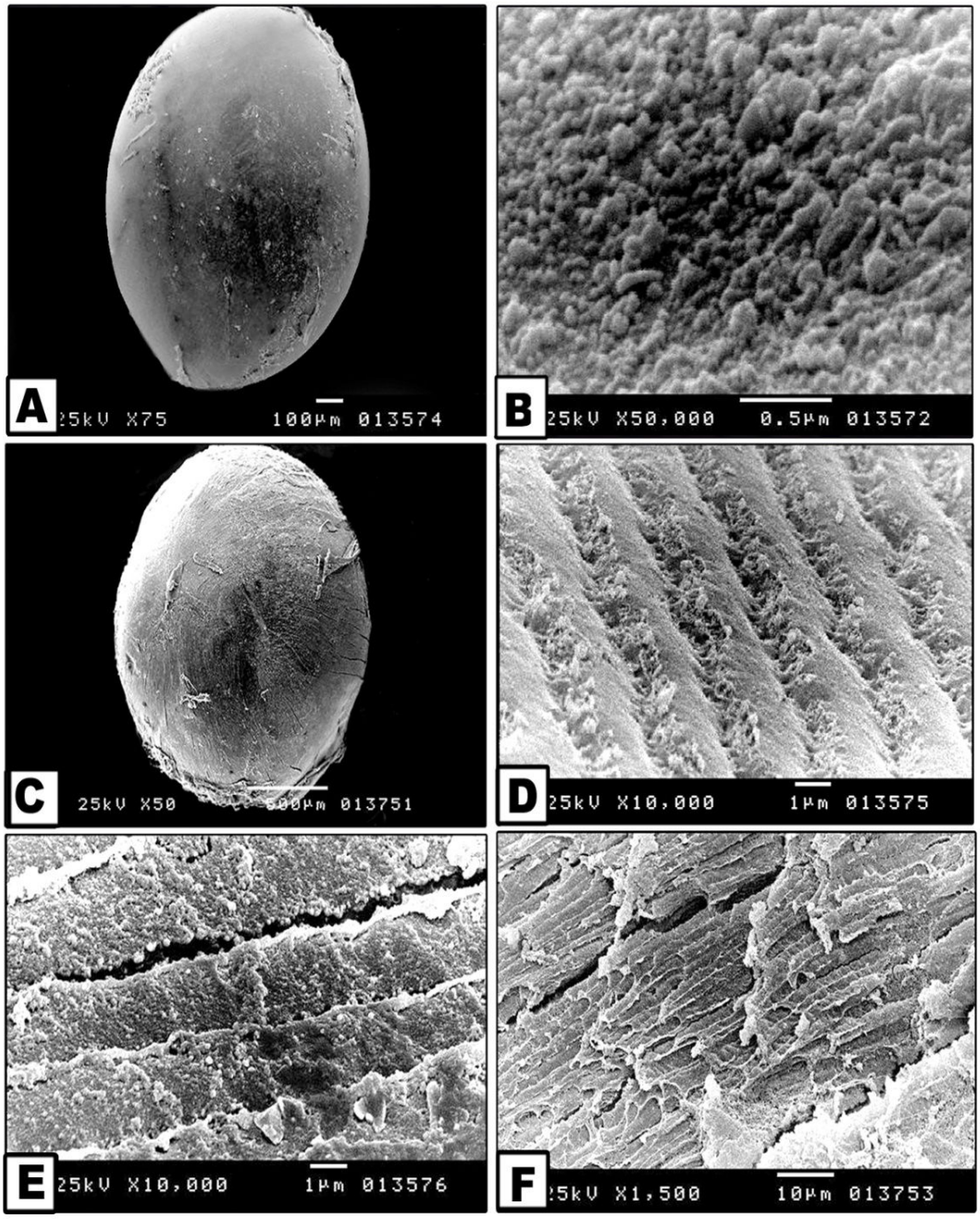

Fig. 1 (A-F). SEMs of lens at one (Fig. A&B) and 6 months-aged rat (Fig. C&F).
A&B. One-month old showing small uniform lens fibers.
C-F. Six month old showing hexagonal lens fibers regularly oriented and tightly backed.

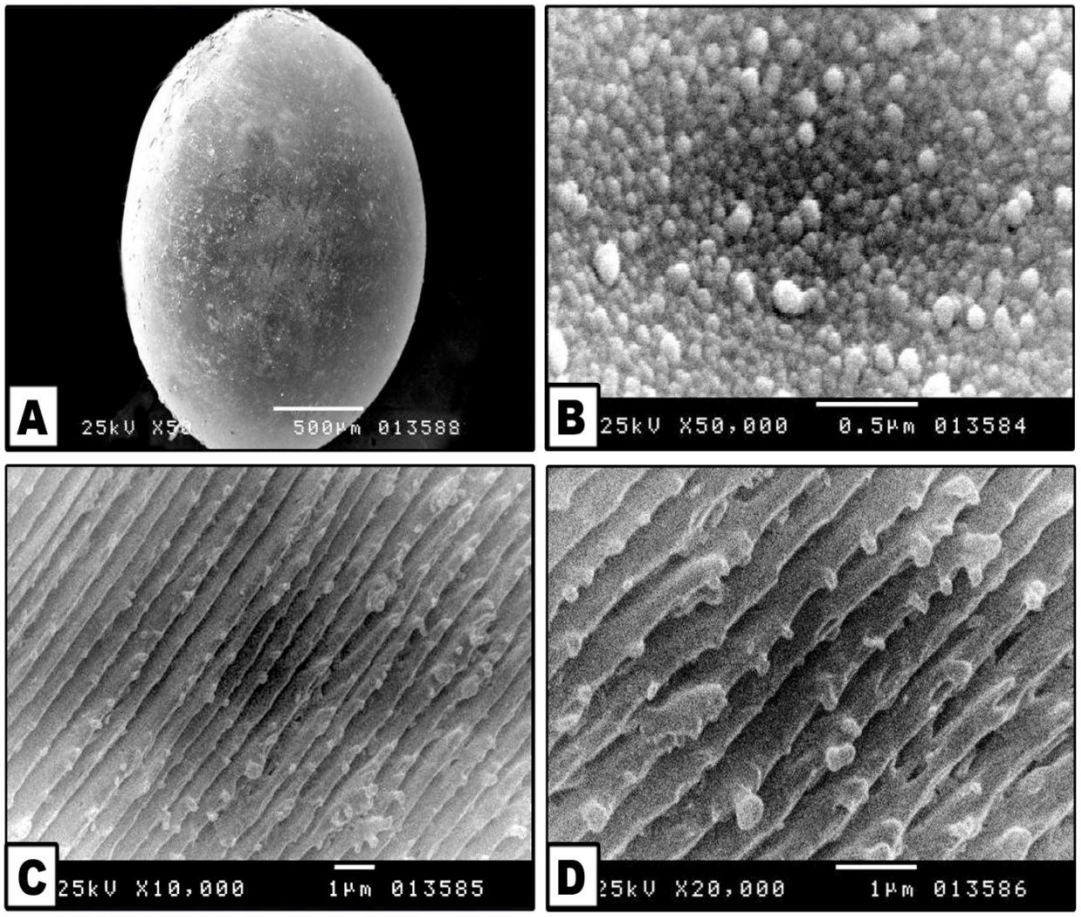

Fig. 2 (A-D). SEMs of lens at 18 months-old rat showing narrow spaces between lens fibers are originated as a result of free attachment between ball and socket.

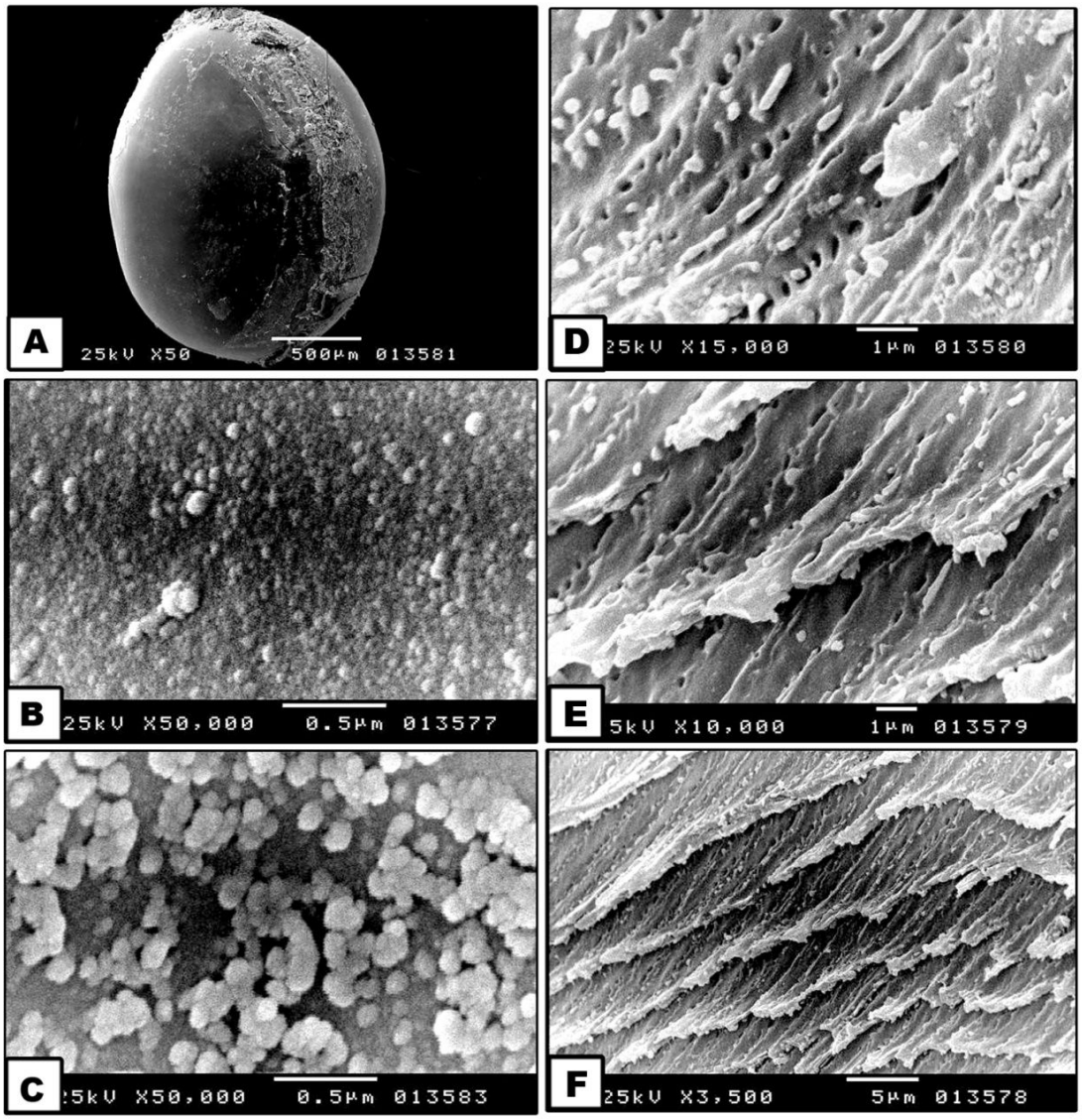

Fig. 3 (A-F). SEMs of lens at 30 months-old rat showing partial loose of lens fibers. Widened spaces are originated as a result of free attachment between ball and socket.

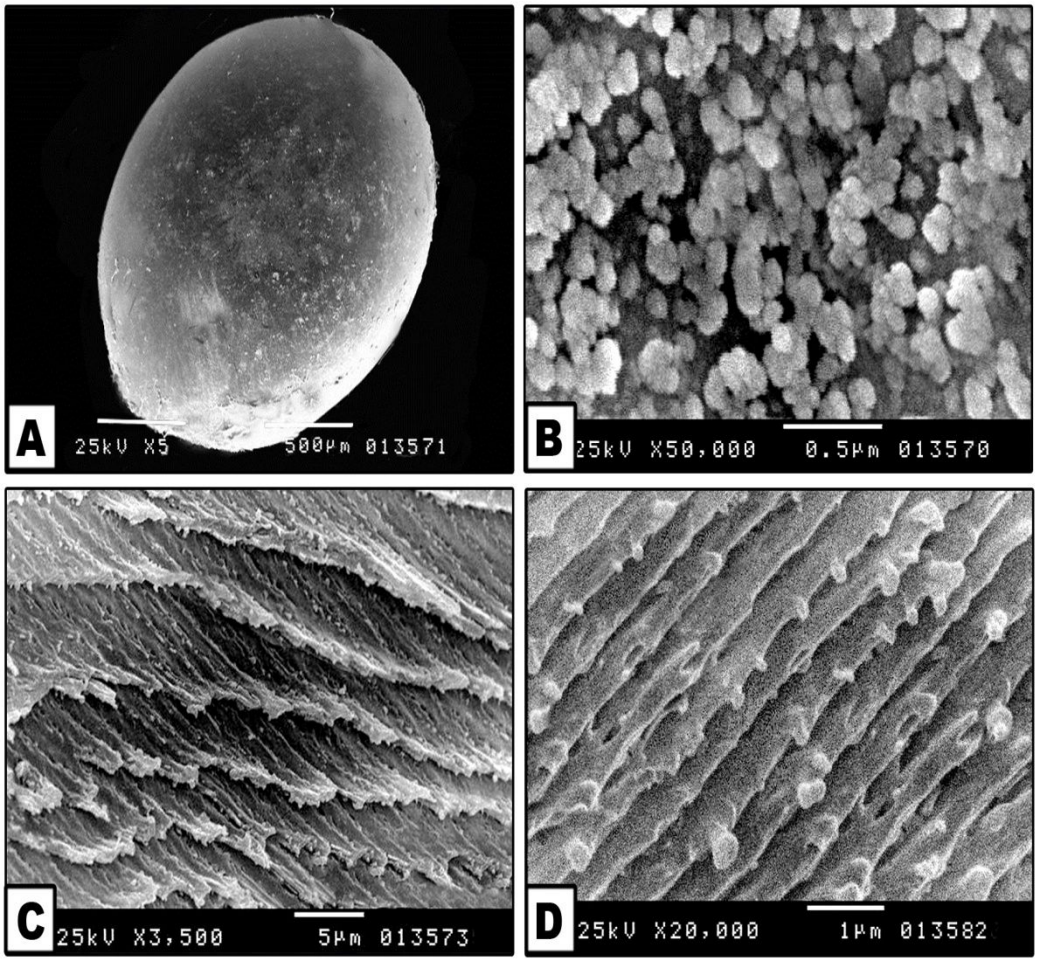

Fig. 4 (A-D). SEMs of lens at 42 months-old rat showing widened spaces between lens fibers are originated as a result of free attachment between ball and socket.

4. DISCUSSION

Although earlier work was done in lens amino acids content, yet no more extensive illustrations concerning aging and sex of individuals as well as parallel with lens fibers ultrastructure and their mineral contents. Whatever is the underlying mechanism, alterations in amino acids profile with aging of rats especially at 42 week old, has been considered to be the ultimate factor in lens opacification. Except asparate, valine and methionine, the estimated amino acid increased steady during aging. The amino acids prolline, serine, threonine, methionine, lysine and arginine attained highest accumulation level.

The present findings are inline with the work of Srivastava and Srivastava (2003) and Hains and Truscott (2007) who showed that post translational modifications and accumulation of large amount of insoluble proteins derived from soluble protein due to aggregation is the major mechanism in cataractogenesis. Lens cytoskeletal proteins comprise of 2–4% of the

total lens proteins, which include vimentin, tubulin, spectrin, actin etc (Song et al., 2008). The transparency of the crystalline lens has been attributed to the complex, ordered arrangement of its components at both microscopic and molecular levels (Taylor et al., 1996). Maintenance of crystalline profile is essential for lens transparency and the alteration in elution profile in cataract induced group may be due to the proteolytic degradation of crystallins (Rooban et al., 2011). These findings are consistent with the earlier reports (Yan et al., 2003) which indicated that proteolysis had resulted in an increase in αH-crystalline and decrease of αL-, βH-, and βL-crystallins and loss of many polypeptides from the soluble, insoluble, and intrinsic membrane fraction.

The estimated amino acids, namely, lysine, arginine, prolline and histidine were markedly increased during aging in both male and female rats. These amino acids are most likely to form carbonyl derivatives as a result of direct metal-catalyzed oxidation (Stadtman and Berlett, 1997). Different explanations about the progressive decrease of protein sulfhydryls have been observed generally during the development of diabetic and senile cataracts (Hum and Augusteyn 1987; Duhaiman 1995; Boscia et al., 2000). Sulfhydryl oxidation is thought to be one of the main pathological events leading to molecular aggregation, protein precipitation and lens opacification (Perry et al., 1987; Swamy and Abraham 1987; Takemoto, 1997).

There were other explanations of senile cataract either through inhibition of protein synthesis and increased osmotic pressure (Barber, 1968) and or elevated H_20_2 in aqueous humor which enhanced extensive oxidation of lens protein (Spector, 1995).

The observed alterations in amino acid metabolism in lens during aging of rats were parallel with abnormal accumulation of sodium, calcium and cupper, especially at 42 week-old. Several studies explained that the accumulation of the mentioned minerals during aging suspected to be the monitors of inducing severe damage of lens and progress of cataract.

Similar raised calcium content was detected in lens of mature senile cataract in patients (Duncan and Jacob, 1984; Ringvold et al., 1988) as well as in lens of diabetic rats (Cekic and Bardak, 1998).

In lenses with highly localized opacities, the calcium distribution was not uniform and was highest in regions that scattered most light (Duncan and Jacob, 1984).

Concerning the observed increase of cuppor accumulation during aging of lenses, similar findings were reported by Aydin et al., (2005), in lens of diabetic patients presumably has a greater association with the development of lens opacification in diabetics.

In human, the opacification of the lens cortex in man is accompanied by an extremely high concentration of sodium (van Heyningen, 1976; Duncan et al., 1984), calcium (Hightower, 1985; Gekic 1998) and copper (Srivastava et al., 1992; Cumurcu et al., 2006; Yildirim et al., 2007). These changes in ion concentrations are thought to be brought about by functional disruption of the lens epithelium.

The distribution of free calcium in the lens varied with age and was correlated with a change in the sensitivity of the lens to cataract and a change in lens birefringence. Three types of ionic channels have been identified in the lens. The smallest appears to be a calcium channel; the larger current fluctuations are associated with sodium and potassium movements. A marked decrease in protein synthesis and net leakage of proteins was

closely associated more strongly with an increase in calcium than with an increase in sodium (Duncan and Jacob, 1984).

Also, the sodium ions that leaks into the lens is presumably pumped out by the sodium pump, the Na,K-ATPase. The sodium pump transports potassium in the opposite direction, maintaining a high lens potassium concentration as it balances the continual passive outward potassium leak. In human cortical cataract, the lens sodium and potassium pump-leak balance fails (Shinohara and Piatigorsky, 1977; Delmere and Tamiya, 2009).

These changes in electrolyte composition are deleterious to the lens and contribute to the loss of cortical transparency. For example, the osmotic disturbance resulting from the altered sodium and potassium levels causes the lens to accumulate water, leading to cell swelling and damage. Protein synthesis patterns also may be disturbed by altered lens sodium and potassium levels (Shinohara and Piatigorsky, 1977).

In cataractous lenses from human and animal models, a high lens calcium level is almost always associated with a high sodium and low potassium content (Hightower and Hind, 1982; Duncan and Jacob, 1984).

Calvin et al., (1992) suggested the presence of osmotic stress prior to cataract stage 1 (developing floriform). Increased lens hydration was first apparent in stage 1, coincident with a marked elevation of Ca^{2+}, further increase in Na^{+} and decrease in K^{+}. These trends persisted in the stage 2 cataract (completed floriform). Subsequently the changes of lens hydration and cation contents during the advanced cataract stages 3 (degenerate floriform) and 4 (amorphous translucent) were found to suggest substantial influx of extracellular fluid into the affected lenses.

Proteins rich in early glycation products were less capable of competing for copper ions in the presence of other ligands (e.g., glycine and calcein), suggesting that glycated proteins might have diminished stability constants of their copper chelates compared to control counterparts. Oxidative damage on proteins documented by protein carbonyl content and amino acid analysis indicates the involvement of Fenton chemistry upon metal chelation (Argirova and Ortwerth, 2003).

Edward et al., (2011), report the pattern of deposition of copper in the Descemet membrane of the cornea and the anterior lens capsule in multiple myeloma, associated with hypercupremia.

On the other hand, aging lenses of rat was associated with a reduction of magnesium content. The present data are similar to the work of Mibu et al., (1994), who observed a close association of development of cataract in the human and animal models with an increase in vitro in sodium and calcium content and a decrease in magnesium cation content. This change was associated with increases in the level of malondialdehyde, a breakdown product of lipid peroxide which accompanies the development of lens opacity. Ringvold et al., (1988), indicated a possible connection between increased level of magnesium aqueous/serum ratio and the presence of cataract in patients. Light and scanning electron microscopy of the optical surface of intraocular lens of patients revealed multiple fine granular deposits varying in size and shape that were positive for alizarin red confirmed the presence of calcium and phosphorous (calcium apatite),sodium, aluminum , magnesium and potassium (Fodor et al., 2011).

Furthermore, dramatic changes of estimated of amino acid contents and minerals were concomitantly parallel with alteration of lens fibrils which loose their normal integrity and become widened from each other manifesting degenerative phases of ball and socket. The architectural pattern model was disorganized with aging manifesting abnormal lens structure.

Similar findings were reported by Al-khudari et al., (2007), in lens of aging New Zealand White rabbits which undergo a greater degree of age-related compaction. Atilano et al., (2005), attributed aging to the declining of mitochondrial function including high loads of damage and mutation in mtDNA.

The observed disorganization of lens fibers may be attributed to liberation of Reactive Oxygen Species (ROS), the major risk factor in the pathogenesis of age-related cataract (Ozmen et al., 2002). ROS is mostly generated within the mitochondria in lens epithelium and the superficial fiber cells, which are highly reactive and can damage macromolecules in living cells, such as lipids, proteins, and nucleic acids, causing mutagenesis and cell death (Conlon et al., 2003; Huang et al., 2008) and consequently apoptosis of lens epithelial cells influencing in the pathogenesis of cataracts (Yao et al., 2008; Zhang et al., 2010).

5. CONCLUSION

From the present research it is concluded that during advancement of aging of rat several abnormal metabolic processes take place in the lens. These metabolic processes in the lens facilitate the impairing of its functions and results in increased accumulation of sodium, calcium and copper and a decrease of magnesium. These metabolic processes in the lens also contributes in the increase of amino acid content in both sex especially the lens amino acids, namely, taurine, prolline, serine, threonine, methionine, lysine and arginine comparable with degeneration of lens fibers. These changes may be resulted from increase lipid peroxidation and formation of advanced glycation end products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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