Evaluation of the effect of ellagic acid in the prevention of selenite-induced apoptosis in epithelial cells of the ocular lens

1. INTRODUCTION

Apoptosis, the most common form of programmed cell death, is characterized by a conserved sequence of morphological, cytological, and biochemical events (Kerr et al., 1972; Song and Steller, 1999; Hengartner, 2000; Hacker, 2000; Kornbluth and White 2005). The lenticular epithelium, the most metabolically-active cell layer in the lens, is the initial cellular layer to be exposed to environmental stress and oxidative insult. This single layer of lenticular epithelial cells is essential for maintaining metabolic homeostasis and transparency of the entire lens (Spector, 1991). Under normal physiological conditions, most of these cells have a relatively long life span. However, if these conditions are altered or disturbed by factors such as oxidative stress, the lenticular epithelial cell may lose its viability, possibly resulting in opacification of the lens (Li et al., 1995a). Previous studies have demonstrated that external factors can cause cataract, under both in vivo and in vitro conditions, with oxidative stress being the underlying mechanism (Spector, 1995; Gupta et al., 2003; Dong et al., 2003; Geraldine et al., 2006). It has also been suggested that apoptosis of lenticular epithelial cells may be an early and critical event during cataractgenesis induced by these external factors (Kato et al., 1997; Michael et al., 1998; Takamura et al., 2003; Kim et al., 2010). Hence, maintaining the integrity of the lenticular epithelium and survival capability of epithelial cells even under adverse conditions is critical to the maintenance of lenticular transparency.

A key feature of apoptosis is the activation of a unique family of cysteine-dependent aspartate-specific proteases called caspases. The mammalian genome is
reported to encode fourteen distinct caspases, of which seven are believed to be involved in apoptosis and hence termed ‘apoptotic’ caspases (Petrilli et al., 2007; Chowdhury et al., 2008). Caspases are synthesized as inactive proenzymes, which work in a precisely-controlled proteolytic cascade to activate themselves and one another (Yuan et al., 1993; Nicholson and Thornberry, 1997; Bergmann et al., 1998; Budihardjo et al., 1999; Wolf and Green, 1999; Lamkanfi et al., 2007; Salvesen and Riedl, 2008). The apoptotic caspases are generally classified as initiators (also called apical) or effectors (also known as executioners), depending on their position in the proteolytic hierarchy. Initiator caspases are activated through dimerization facilitated at multi-protein complexes. Activation of caspase-9, the initiator caspase of the intrinsic pathway, involves its recruitment to the apoptosome by Apaf-1-Cyt c complex, while the apical caspase of the extrinsic apoptotic pathway, caspase-8, is activated within the death-inducing signaling complex (DISC) (Zou et al., 1997; Ashkenazi and Dixit, 1998; Rodriguez and Lazebnik, 1999; Boatright and Salvesen, 2003; Riedl and Shi, 2004). On the other hand, activation of effector caspases, such as caspase-3 and -7, occurs upon their cleavage at specific internal aspartic acid residues by initiator caspases (Boatright and Salvesen, 2003; Riedl and Shi, 2004). Downstream of this activational cascade, caspases cleave a variety of regulatory and structural proteins and important enzymes, ultimately leading to death of cells. Vaculova and Zhivotovsky (2008) have reported that such cleavage occurred in known endogenous caspase targets, such as the DNA repair enzyme, poly ADP-ribose phosphorylase (PARP) and in cytoskeletal proteins. The morphological manifestations of apoptosis include cellular detachment and shrinkage, nuclear condensation and segmentation, membrane blebbing, and disassembly into apoptotic bodies that are often engulfed by neighbouring cells or phagocytes (Hengartner, 2000; Hacker, 2000).

Cataract formation is reported to be associated with the functional abnormality of lenticular epithelial cells (Hightower et al., 1994; Li et al., 1995a). Data have been
presented indicating involvement of apoptosis in age-related cataract, in general, and in posterior capsular opacification, in particular (Li et al., 1995a; Kato et al., 1997). Further, it has been reported that apoptosis of lenticular epithelial cells can be induced by ultraviolet radiation, hydrogen peroxide and hyperglycemia (Li and Spector, 1996; Takamura et al., 2003). Moreover, Tamada et al. (2000) have reported that selenite can induce apoptosis in rat lens during the process of cataractogenesis. In the experiments described in the present chapter, an attempt was made to evaluate the modulatory influence of ellagic acid in selenite-induced apoptosis in epithelial cells of the ocular lens. This was achieved by evaluation of caspase activation, cleavage of PARP and cytoskeletal proteins. To confirm the apoptotic nature of the epithelial cells in selenite-induced cataractous rat lenses and the putative protective influence of ellagic acid, the lenticular epithelial cells were also examined by transmission electron microscopy (TEM) to detect morphologic alterations.

2. MATERIALS AND METHODS

2.1. Experimental animals and morphological examination

The rat pups, grouping of animals and treatment regimen used in the experiments described in this chapter were as described in the General Materials and Methods (Page 27). At the end of the experimental period, morphological examination was performed on the 16th postnatal day by a slitlamp biomicroscope at 12X magnification and the findings were recorded. The animals were then sacrificed by cervical dislocation. The lenses from each rat were dissected out for further analysis.

2.2 Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of gene expression

Lenses for this experiment, after removal from rat eyes, were immediately homogenised in Trizol (Sigma-Aldrich, St. Louis, MO, USA) reagent (1 ml/100 mg tissue) and RNA was isolated per the manufacturer’s protocol. RNAs were quantified
using a spectrophotometer at 260 nm and checked for integrity of the RNA by assessing 18S and 28S band intensities using agarose gel electrophoresis.

RT-PCR was performed using a one-step RT-PCR kit (Qiagen, Germany), per the manufacturer’s instructions; 1 μg of template RNA and 0.6 μM of each of the forward and reverse primers specific to the caspase 3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were added to the reaction mixture (Table 4.1). Amplification was done in an Eppendorf thermal cycler (Germany). The RT-PCR conditions were as described in Chapter II (Page 46). After completion of the PCR reaction, a 10 μl portion of the PCR product was electrophoresed in a 2% agarose gel. The ethidium bromide-stained gel was photographed with a DS-34 type Polaroid camera and the band was scanned with an imaging densitometer (Bio-Rad, Model GS-670). The GAPDH gene was used as an internal standard for the RT-PCR reaction. To quantitate the transcript level, the ratio of the study gene product to the GAPDH gene product was calculated.

2.3 Western blot analysis

The rat lenses that had been removed were then homogenized in 10 times their mass of 50 mM phosphate buffer (pH 7.4), and centrifuged at 14,000xg for 15 minutes. The supernatant obtained was used for immunoblot analysis. Proteins subjected to SDS-PAGE were electrophoretically transferred to a nitrocellulose membrane using a semidry blotting apparatus (Bio-Rad, USA). Blotting was done at 25 V for 1 hour. Blocking was done with 5 % non-fat dry milk in Tris buffer saline (pH 7.5) with 0.1 % (v/v) Tween 20 for 2 hours. Specific antibodies against caspase-3 (1:1000 dilution; Sigma, St. Louis, USA), caspase-8 (1:500 dilution; Sigma, St. Louis, USA), caspase-9 (1:1000 dilution; Sigma, St. Louis, USA), PARP (1:250 dilution; Santa Cruz, CA, USA) vimentin (1:50 dilution; Sigma, St. Louis, USA), spectrin (1:200 dilution; Sigma, St. Louis, USA) and β-actin (1:3000 dilution; Sigma, St. Louis, USA) were
Table: 4.1. Primer sequences and expected product sizes for the genes amplified in samples from lenses of Wistar rats

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence 5'-3'</th>
<th>PCR cycle #</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-3 - up</td>
<td>GGA CCT GTG GAC CTG AAA AA’</td>
<td>30</td>
<td>463</td>
</tr>
<tr>
<td>Caspase-3 - down</td>
<td>TAC CCC ACT CCC AGT CAT TC’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH - up</td>
<td>TCAAGAAGGTTGGTGAAGCAGGC</td>
<td>30</td>
<td>207</td>
</tr>
<tr>
<td>GAPDH - down</td>
<td>GGTCACCACCACCTGTGCTGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(control gene)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GAPDH** - Glyceraldehyde-3-phosphate dehydrogenase
used. Immunoreactivity was visualized using alkaline phosphatase conjugated with anti-mouse IgG or anti-rabbit IgG secondary antibodies and 5-bromo 4-chloro 3-indolyl phosphate/nitroblue tetrazolium chloride (BCIP/NBT [Genei, Bangalore, India]).

2.4 Transmission electron microscopic studies

The TEM studies adopting resin embedding were performed essentially as described by Havat (1981) and Hess and Moore (1993).

2.4.1. Reagents

**Perfusion fluid** (glutaraldehyde 1%, paraformaldehyde 1% and phosphate buffer 0.1M [3.205 g of sodium dihydrogen phosphate and 20.65 g of disodium hydrogen phosphate] were added and made up to 1000 ml in double distilled water). **Phosphate buffer (0.2 M).** **Wash buffer** (5 g of sucrose in 100 ml of 0.1 M phosphate buffer). **Primary fixative (2.5% glutaraldehyde).** **Osmium tetroxide** (a stock solution of 2% osmium tetroxide [O₄SO₄] was prepared by dissolving 1 g in 50 ml of double distilled water and stored at 4°C; it was diluted 1:1 using 0.2 M phosphate buffer [pH 7.4] just before use; the solutions were kept in a tightly-stoppered amber-colored bottle covered by black paper and stored at 4°C in the dark). **Spurr’s mixture** (8 ml of 2-nonen-1–ylsuccinic anhydride [NSA], 1 ml of 4-vinylcyclohexanediocxide, 10 ml of resin and 0.1 ml of 2-dimethylaminoethanol [DMAE]). **Toluidine blue O, uranyl acetate, lead citrate.**

2.4.2. Procedure

Entire lenses from 16-day-old rats were dissected out and immersed in 2.5% glutaraldehyde in 50 mM cacodylate buffer (pH 7.2; containing 4% sucrose and 2 mM CaCl₂) for 2 hours, and were then transferred to 10% buffered formalin for 48 hours. The processing and preparation of lenses were done in sequence: fixation, processing and staining. **Fixation:** Transverse slices of lenses were fixed overnight in
2.5% glutaraldehyde prepared in cacodylate buffer (pH 7.4). The tissues were then rinsed in wash buffer and post-fixed in 1% osmium tetroxide. Processing: The dehydration was performed through ascending grades of alcohol, i.e. 30%, 50%, 70%, 80%, 90% and absolute alcohol, respectively. The tissue was cleared in propylene oxide. Impregnation was performed at room temperature using propylene oxide and Spurr’s mixture (Sigma, USA) at increasing concentrations, using a slow speed rotary shaker. Embedding was done in a flat embedding mold with tissues oriented in such a manner as to obtain. The embedded molds were kept in an incubator at 60°C for 48 hours and cooled. Semi-thin sections of 1μm thickness were obtained using Leica (Germany) ultramicrotome and ultra-thin sections of 50-70 μm (silver to gray) were cut using the same microtome. Staining: Semi-thin sections of 1 μm thickness were stained using toluidine blue O. Ultrathin sections were placed in copper grids and stained with uranyl acetate and lead citrate. Semi-thin sections were examined in a Carl Zeiss (Germany) Axio 2 Plus research microscope. Images were captured through a CCD camera in a computer and processed using Carl Zeiss Axiovision software. Areas in TBO-stained sections were chosen for obtaining ultrathin sections. Sections were examined in a Phillips 201C (Holland) transmission electron microscope and electron micrographs (TEM) at x1500 to x50000 magnifications were obtained.

2.5 Statistical analysis

Statistical analysis was performed with SPSS software package for Windows (Version 11.5; SPSS Corporation, Chicago, IL). Differences between all experimental groups were assessed by one-way ANOVA. Post-hoc testing was performed for intergroup comparisons using the least significance difference test. The experiments were performed at least three times with duplicate samples. \( P \) values < 0.05 were considered statistically significant.
3 RESULTS

3.1 Effect of ellagic acid on caspases

In the present study, the expression of caspase-3 was examined both at the gene transcriptional level and protein level. The levels of caspase 3 (Fig. 4.1) gene transcripts were found to be significantly \((P < 0.05)\) higher in samples from Group II (selenite-challenged, untreated) rat lenses than those in samples from Group I (normal) rat lenses and were at near normal levels in samples from Group III (selenite-challenged, ellagic acid-treated) rat lenses.

The proteolytic processing of procaspase-3 was determined by immunoblot analysis. Western blotting, using a monoclonal antibody to caspase-3, revealed a band at 32 kDa, which represented uncleaved caspase; this was most intense in the samples from lenses of normal rats. In samples from Group II rat lenses, an additional band was present at 17 kDa, possibly representing a fragment of cleaved caspase enzyme. However, in samples from Group III rat lenses, no such cleavage was observed (Fig. 4.2).

Immunoblot analysis was also performed to detect the activation of caspase 8 in the samples from lenses of selenite-challenged, untreated (Group II) rats. Western blot analysis of these samples appeared to confirm the activation of caspase-8 since there was a reduction in procaspase-8 (55 kDa) with the simultaneous appearance of its cleaved product, a 42 kDa fraction (Fig. 4.3).

To further understand the involvement of the mitochondrial apoptotic pathway, the level of caspase 9 protein was also determined since it has been reported that, upon activation, procaspase-9 (45 kDa) cleaves into a fragment of approximately 35 kDa (Li et al. 1997). This was clearly evident in the present investigation in samples from lenses of selenite-challenged, untreated (Group II) rats. However, in samples from
Fig. 4.1. (a) RT-PCR for caspase-3 gene in lenses of Wistar rats, visualized on an ethidium bromide-stained agarose gel. M - 100 bp DNA ladder, GI - Group I (normal rats), GII - Group II (selenite-challenged, untreated rats), GIII - Group III (selenite-challenged, ellagic acid-treated rats) (b) The results (the corresponding band intensity) depicted are normalized to control levels. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as loading control. Data are mean values (experiments run in triplicate) of ratios of intensity for gene of interest divided by that for GAPDH. * Group I vs Group II values \((P< 0.05)\); # Group II vs Group III values \((P< 0.05)\).
Fig. 4.2. (a) 10% SDS-PAGE.

(b) Blots demonstrating expression of caspase-3 in the lenses of normal, selenite-challenged, untreated and selenite-challenged, ellagic acid treated groups. The β-actin antibody was used as loading control.

M- protein molecular weight marker; GI - Group I (normal rats); GII - Group II (selenite-challenged, untreated rats); GIII - Group III (selenite-challenged, ellagic acid-treated rats)
lenses of selenite-challenged rats treated with ellagic acid, there did not appear to be any activation of either caspase-8 or caspase-9 (Fig. 4.3).

3.2 **Effect of ellagic acid on cleavage of poly ADP ribose phosphorylase (PARP)**

The DNA repairing enzyme, PARP, which serves as a substrate for caspase-3, is cleaved following activation of caspase-3. Such cleavage of PARP was noted in samples from lenses of selenite-challenged, untreated rats; immunoblot analysis revealed the presence of 89 kDa and 25 kDa fragments, in addition to its native protein (116 kDa). Treatment with ellagic acid to selenite-challenged rats prevented such an enzymatic activation of caspase-3, which was evident by the absence of cleaved products (Fig. 4.4) in samples from lenses of this group of rats.

3.3 **Effect of ellagic acid on the state of cytoskeletal proteins**

Western blot analysis was performed to detect alterations in the cytoskeletal proteins, α-spectrin and vimentin, which serve as substrates for caspase-3. In selenite-challenged, untreated (Group II) rat lenses, the mean band intensity of vimentin and of spectrin was found to be only 65% and 18%, respectively, of that noted in normal (Group I) rat lenses (Fig. 4.5). Moreover, cleaved products of vimentin (52 kDa) and spectrin (150 kDa and 120 kDa) were also observed in samples from the lenses of these selenite-challenged, untreated rats. No such cleaved products were observed in samples from lenses of selenite-challenged, ellagic acid-treated rats, and vimentin and spectrin appeared to be intact, similar to that observed in samples from lenses of normal rats. Densitometric analyses of the band intensities also confirmed the above finding.

3.4 **Confirmation of structural changes through TEM study**

TEM studies, adopting the resin embedding method, were performed on samples from lenses of rats of Groups I, II and III. Ultrathin sections of the lenses were examined to detect putative changes in the epithelial cells. Central zone epithelial cells from the lenses of selenite-challenged, untreated (Group II) rats revealed separation
Fig. 4.3 Immunoblots demonstrating cleavage of caspase-8 and caspase-9 in the lenses of selenite-challenged, untreated rats.
GI - Group I (normal rats); GII - Group II (selenite-challenged, untreated rats); GIII - Group III (selenite-challenged, ellagic acid-treated rats)

Fig. 4.4 Immunoblot demonstrating expression of poly ADP ribose phosphorylase (PARP) in the lenses of experimental groups of rats.
GI - Group I (normal rats); GII - Group II (selenite-challenged, untreated rats); GIII - Group III (selenite-challenged, ellagic acid-treated rats)
Fig. 4.5  (a) 10% SDS-PAGE
(b) blots demonstrating expression of α-spectrin and vimentin in the lenses of experimental groups of rats. The β-actin antibody was used as loading control.
(c) Bar graphs of mean normalised densitometric readings
M- protein molecular weight marker; GI - Group I (normal rats); GII - Group II (selenite-challenged, untreated rats) GIII - Group III (selenite-challenged, ellagic acid-treated rats);
* Group II vs. Groups I values ($P<0.05$);  #Group III vs. Groups II values ($P<0.05$).
(wide white gap) of basal membrane and lenticular capsule. Prominent changes were observed in the cellular organelles such as swelling of mitochondria, marked concentrations of polyribosomes with their accompanying rough endoplasmic reticulum and intracellular vacuoles in ultrathin sections of lenses from selenite-challenged, untreated rats (Fig. 4.6a). However, in ultrathin sections of lenses from selenite-challenged, ellagic acid-treated rats, there were few such alterations (Fig. 4.6b).

4. DISCUSSION

Apoptosis is considered to be a common cellular mechanism underlying oxidative stress-induced non-congenital cataract in humans and animals (Li et al., 1995a). Tamada et al. (2000) reported that during selenite-induced cataractogenesis, apoptosis was also induced in rat lenses through the activation of caspase-3. Caspase-3 is reported to play a central role in regulating and executing apoptosis in mammalian cells; the activation of this protease was shown to be an early and essential step in multiple apoptotic signaling pathways triggered by different apoptotic signals (Tewari et al., 1995; Nicholson and Thornberry, 1997). In the present study, it was observed that the gene transcript level of caspase-3 was increased in samples from lenses of selenite-challenged untreated rats. To validate these data, an immunoblot analysis was made with specific antibody to detect caspase-3 protein. In samples from lenses of selenite-challenged, untreated (Group II) rats, the band intensity of caspase-3 was lower than that seen in samples from lenses of normal Group I rats; in addition, a proteolytic fragment of active caspase-3 (17 kDa) was noted (Fig. 4.2). However, the anti-caspase-3 antibody could not detect such a proteolytic fragment (active form) in samples from lenses of selenite-challenged, ellagic acid-treated (Group III) rats.

The participation of the mitochondrial-mediated pathway in the death of lenticular epithelial cells has also been reported in various cataract models induced by ultraviolet irradiation (Michael et al., 1998) hydrogen peroxide (Yao et al., 2008) and calcimycin (Li et al., 1995b). Hence, in the present investigation, an attempt was made
Fig. 4.6. Low-magnification comparison of central zone lenticular epithelial cells from selenite-challenged, untreated (a), selenite-challenged, ellagic acid-treated (b) and normal (c) rats. The selenite-challenged, untreated lenticular epithelial cells show separation (wide white gap) of basal membrane and lenticular capsule, intracellular vacuoles and membrane bound vesicles containing cellular fragments are seen in the apoptotic cells. In contrast, ellagic acid-treated rat lenticular epithelial cells show normal membrane contacts between basal membrane and lenticular capsule. The highly-magnified right panel illustrates the epithelial cells containing, (d) the elongated mitochondria and vacuoles in selenite-challenged, untreated rat lenses, (e) selenite-challenged, ellagic acid-treated cells have a typical number of organelles such as mitochondria, rough endoplasmic reticulum and cytoskeletal elements as compared to, (f) normal rat lenses.
to assess the activation of caspase-9 in selenite-induced cataractogenesis and also the ability of ellagic acid in modulating its activation. Normally, the protease, caspase-9, is found to occur in an inactive form as procaspase-9 but is activated during apoptosis upon the release of mitochondrial factors into the cytosol. Procaspase-9 (approximately 45 kDa), upon activation, is reported to cleave into fragments of approximately 35 kDa (Li et al., 1997; Zhivotovsky et al., 1999). In the present set of experiments, a similar activation of procaspase-9 was noted in samples from lenses of selenite-challenged, untreated rats which showed cleaved products of 35 kDa fragments. However, ellagic acid treatment appeared to prevent such activation, since in samples from lenses of selenite-challenged, ellagic acid-treated rats, only procaspase-9 was detected (similar to that seen in material from lenses of normal Group I rats).

In order to demonstrate the involvement of the death receptor-mediated pathway, Western blot analysis was performed to detect the activation of caspase-8. Caspase-8 lies at the apex of an apoptotic cascade and initiates proteolytic activation of downstream caspase family members, resulting in apoptosis (Scaffidi et al., 1997; 1999). Andersson et al. (2000), while observing the caspase and proteosome activity during staurosporin-induced apoptosis in bovine lenticular epithelial cells, noted increased activity of caspase-8 while observing activation of caspase-3. In the present study, caspase-8 was found activated in samples from lenses of selenite-challenged, untreated rats. However, ellagic acid appeared to prevent the activation of caspase-8, since 42 kDa fragments was not observed in samples from lenses of selenite-challenged, ellagic acid-treated rats.

Caspase-3 (CPP 32) cleaves a variety of proteins, such as cytoskeletal protein and a nuclear protein, PARP, which is often found, degraded in apoptotic cells. Caspase-3 is believed to act on PARP, a DNA-repair enzyme whose expression is triggered by DNA strand breaks. It has been reported that 116 kDa PARP is degraded during apoptosis into distinct 89 kDa and 25 kDa fragments by caspase-3.
(Kaufmann et al., 1993; Lazebnik et al., 1994). Hence, to confirm the occurrence of apoptosis in samples of lenticular epithelial cells during selenite-cataractogenesis, and to test the ability of ellagic acid to prevent this, the specific degradation of PARP was looked for by Western blot. Degraded products of PARP were clearly evident in samples from lenses of selenite-challenged, untreated (Group II) rats, denoting the occurrence of apoptosis. However, ellagic acid-treatment appeared to prevent such degradation of PARP, since PARP remained as a single peptide in samples from lenses of selenite-challenged, ellagic acid-treated (Group III) rats and from lenses of normal (Group I) rats.

During apoptosis, activation of caspases can lead to cleavage of cytoskeletal proteins such as spectrin and vimentin (Deveraux and Reed, 1999). The full-length α-spectrin (a 240-kDa polypeptide) was reported to be cleaved by caspase-3 into 150 and 120 kDa polypeptide fragments (Nath et al., 1996a) whereas, proteolysis by calpain results in cleavage products of 150 and 145 kDa size (Nath et al., 1996b). In the present investigation, putative cleavage products 150 and 120 kDa polypeptides, were detected in samples from lenses of selenite-challenged, untreated (Group II) rats; however such cleavage products were not observed in samples from lenses of selenite-challenged, ellagic acid-treated (Group III) rats.

Vimentin, an intermediate filament of the cytoskeleton, is reported to be degraded by calpain in lens-cell network (Roy et al., 1983). Its degradation has also been observed in tamoxifen (a cytotoxic drug)-induced apoptotic skin fibroblasts and in ionizing-radiation-induced apoptosis in prostate epithelial tumor cells (Hashimoto et al., 1998; Prasad et al., 1998). In the present set of experiments, degradation of vimentin was noted in lenticular samples from selenite-challenged, untreated (Group II) rats, whereas such degradation was not observed in lenticular samples from selenite-challenged, ellagic acid-treated (Group III) rat lenses.
Ultrastructural changes consistent with apoptotic morphology were observed in samples from selenite-challenged, untreated (Group II) rat lenses. Nuclear chromatin condensation and peripheral migration of chromatin were noted in lenticular epithelial cells. Such nuclear changes were rarely observed in samples from selenite-challenged, ellagic acid-treated (Group III) rat lenses. TEM examination of central zone lenticular epithelial cells from Group II rats revealed a separation (wide white gap) between the basal membrane and the lenticular capsule. In addition, prominent changes were observed in cellular organelles, such as mitochondrial swelling, marked concentrations of polyribosomes with their accompanying rough endoplasmic reticulum and intracellular vacuoles, in samples from selenite-challenged, untreated (Group II) rat lenses. However, samples from selenite-challenged, ellagic acid-treated (Group III) rat lenses revealed that normal contact was maintained between the basal membrane and the lenticular capsule; similarly, cellular organelles revealed few alterations.

5. CONCLUSION

The results of the present investigation suggest that ellagic acid prevents selenite-induced apoptosis in lenticular epithelial cells of Wistar rats. The activation of caspase-3, that plays a central role in executing apoptosis, was confirmed by its cleavage to its active form (17 kDa), and also by the proteolysis of PARP and cytoskeletal proteins resulting in degraded fragments; these changes were evident in samples from lenses of selenite-challenged, untreated rats. These samples also revealed the activation of caspase-9 and caspase-8, suggests the involvement of both the mitochondrial-mediated and death receptor-mediated pathways in apoptosis. However, the activation of caspases and the cascade of events leading to cell death, appeared to be prevented in samples from lenses of selenite-challenged, ellagic acid-treated rats. The results of the studies on various lenticular enzymes mediating apoptosis were confirmed by morphological studies on lenticular epithelial cells.