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The main aim of this investigation on the experimental cataract models is to know the sequential changes occurring in the lens epithelia during the progression of lenticular opacity by carrying out histomorphological, micrometrical, electron microscopical and biochemical studies. It is also to know the role and involvement of these inducing factors in mechanism of cataractogenesis and to find out “whether the progression of opacity is dose or duration dependent or not?” The prerequisite to attain this goal is information on the dose or duration depend effects of sodium selenite, UV radiation and hydrogen peroxide.

Several studies on different experimental models and on human cataract have now suggested that the damage occurred to lens epithelia has important role in cataract development. But, a number of unresolved issues require further investigation. In particular the sequential effects on cellular integrity in all the three regions of LEp during dose or duration dependent changes. In this chapter the results obtained were described in terms of alterations in the morphology of nucleus, cytoplasm and cellular organisation (integrity) in all the three regions of LEp and accordingly changes in the levels of protein, sulphhydryl and the protective enzymes.

4.1 HISTOMORPHOLOGICAL AND ULTRASTRUCTURAL STUDY

The results obtained by light microscope and fluorescent microscope is discussed in following terms.

4.1.1 Nucleus and Cytoplasm

Various abnormal forms of nucleus include faintly stained enlarged, pyknotic, leaking, vacuolated and micronuclei are observed in the cataractous lens epithelia. Occurrence of these forms were increased with progression of opacity. The
The abnormality is more prominent in the shape and nuclear membranes. The blebbing of nucleus was observed in the cataractous lens epithelia which may involve either the outer membrane or inner membrane or both. Such blebs lead to the production of micronuclei or a discontinuous nuclear area with fragments of membrane and nucleoplasm (Broglio and Worgul, 1982). The fluorescent microscopical observations have suggested that such blebs were DNA positive. The condensation of nucleus leads to formation of smaller fragments which can be seen under fluorescent and light microscope.

During H$_2$O$_2$ treatment the nucleus become pyknotic initially and later on it leaks nuclear material. In selenite and UV radiation induced cataractous LEp, few nuclei have shown liquidization of chromatin material and rupturing of the nuclear membrane which subsequently leads to dispersion of nuclear material into the cytoplasm. The further enlargement of faintly stained enlarged nuclei results into the throughout dispersion of chromatin material into the entire cell. Such events do not lead to the formation of micronuclei. An EM studies of the H$_2$O$_2$ treated LEp had shown distinct mechanism of nuclear degeneration. First the space between the two nuclear membranes enlarges towards the inner side, inpushing the nuclear material towards the center and later on such nucleus shows fragmentation in a form of several beaded structures of chromatin. Some time, such nuclei also becomes enlarged and its chromatin material also becomes diffused.

The morphological changes observed in the nucleus following the treatment differ considerably from normal denucleation process of the cell which is characterised by a simple break-up of the nuclear membrane and a disruption of
PLATE : 50. A Schematic diagram showing conclusive findings on nuclear morphology of lens epithelial cells.

1. Typical double membrane bounded nucleus with regularly distributed chromatin material.
2. Blebbing of membranes, inside as well as outside the nucleus.
3. Vesicle or vacuole formation.
4. Further condensation and fragmentation.
5. Aggregation of heterochromatin and its adhesion to inner membrane.
6. Blebbed nucleus containing heterochromatin material.
7,8,9, Sequential degenerative changes of the nucleus
7. Heterochromatin aggregation at few places, enlargement and decondensation of nucleus.
8. Shrinking of nuclear size and aggregation of heterochromatin at one place.
10. Nuclear edentation.
11. Enlargement of nucleus and decondensation of chromatin.
12. Pyknotic or highly condensed nucleus showing beaded appearance of chromatin and its further dispersal in the cytoplasm.
nuclear sap into the cytoplasm. However, the formation of faintly stained enlarged nuclei, rupturing of nuclear membrane and subsequent leaking of nuclear material observed in LEp suggests that the normal denucleation process has been accelerated due to treatment or absence of control mechanism. In case of CR, it may therefore be inappropriate to regard this event as interphase cell death, since ultimate fate of the cell is unclear (Worgul and Merriam, 1980). Occurrence of such abnormal form of nucleus in the LEp may lead to the cell death which may further lead to decrease in the cell density and formation of acellular foci. It is evident by densitometric studies and micro-photographs. However, such condition in PR indicate the reduced cellular activity.

The observed morphological changes in the nucleus reflect the alteration in the DNA. DNA single strand and double strand breaks are reported in the selenite cataract (Reddy et al., 1998), UV induced cataract (Reddy et al., 1998) and H₂O₂ induced cataract (Kleiman et al., 1990). Although LEp contains enzymes that can rapidly repair single stranded DNA breaks (Spector et al., 1989), such lesions may not be repaired or incorrectly repaired and may have the following possibilities (Jose, 1978):

1. Lesions may be excised and replaced by the correct nucleotides and such cells can perform normal functions.
2. Lesions are critical or overwhelming so that cell may die.
3. Lesions may not be repaired.
4. Lesions may be incorrectly repaired.

In the later cases, such cells can not carry out its normal functions; metabolism and may not secrete proper capsular proteins. If such cell divides, the daughter cells will be abnormal. They may not differentiate properly (Roy and Spector, 1976). Such
changes in LEp are more important because repair synthesis is slow in the lens requiring at least 21 hours for its completion (Jose, 1978). As PR is most active region of LEp and from here the terminal differentiation process starts, the observed changes are more important for the differentiation process. The DNA changes which is taking place in the PR cell can lead to the formation of fiber cells with abnormal proteins and/or enzymes or can not differentiate properly and in both the cases may lead to opacification. Although the cells of ER may show DNA repair mechanism (Jose and Yielding, 1977), but genes for specific proteins of fiber cells may be repaired incorrectly or not repaired, cause abnormal mRNA and ultimately improper proteins are produced, which may lead to lens opacification. The damage to the DNA of CR cells may also alter critical homeostasis genes which may further lead to altered normal functioning of the underlying fibre cells (Kleiman et al., 1990).

The cellular changes include cytoplasmic vacuolization, occurrence of intercellular space and swollen cells in the LEp of experimental models. Such changes are also evident in electron microscopic observations. The cytoplasmic vacuolization indicates loss of cytoplasmic contents or hydration of the cells. The formation of intercellular space indicates disturbances in cell-cell interaction and cell aggregation which are very important in the structure and functions of LEp and maintinance of transparency. The occurrence of the intercellular spaces may also indicate the abnormal or deficient cation pumping.

4.1.2 Cell density and Cell death

The results of the densitometric studies indicates the reduced cell density in all the three regions of LEp during all types of treatment. The cell density is considered to be an important parameter in the ability of the cells to tolerate
oxidative stress (Fagerholm and Philipson, 1981). The low numbers of cells in the LEp can be explained due to,

1. Inherited low numbers of cell
2. Cell degeneration and cell death
3. Reduced mitotic activity
4. Various combination of 1,2 and 3.

Various types of abnormal nuclear forms, their occurrence and other cytoplasmic aberrations indicate degeneration of cell which subsequently cause the cell death. Mainly two types of cell death is observed, in first, the nucleus becomes faintly stained and it further leads to disruption of chromatin material into the cells due to breaking of nuclear membrane while in the second type, the nucleus becomes condensed, blebbled and ultimately becomes fragmented which is evident by both LM and FM observations. Both the types of cell death is observed in all types of treatment but in selenite treatment mainly first type of cell death is observed while in $H_2O_2$ treatment second type of cell death is observed. The UV irradiated LEp shows both the types of cell death. The decondensation of nucleus and later death seems to be necrotic type of cell death while condensation of nucleus may reflect apoptotic cell death. Both types of cell death are reported in LEp during various types of treatment. The observed extensive effects on mitochondria suggests effects on mitochondrial DNA. Such effects on mitochondria can cause enhanced apoptotic response (Kessel and Sun, 1999). During oxidative stress and in human cataract, apoptosis has been found to be the major route of cell death (Spector et al., 1995a). In rat also 10% of nuclei were reported to be apoptotic after threshold dose of ultraviolet radiation (Soderberg et al., 1998). Various types of abnormal and degenerating nuclear forms may indicate mutagenic effects on DNA.
Such mutagenic and cytotoxic effects follow inactivation of critical enzymes, transport and metabolic process and finally it leads to cell damage (Hightower, 1995). If the metabolic system of LEp cells involved in the fighting against oxidative stress caused by selenite, H₂O₂ and UV radiation are compromised than it may lead to cell death (Giblin et al., 1990).

The vulnerability of the cells mostly depend upon the stage of the cell in the cell cycle and/or its stage of differentiation (Worgul and Rothstein, 1975). As the cells of PR are mitotically active, they are expected to be more easily damaged than the quiescent cells of the CR. However, the densitometric study indicates that cell death is observed in both the regions and probably it is more prominent in the CR than in the PR. This may be due to constant mitotic activity of the cells of PR by which the numbers of cells are maintained. The cells of CR which are arrested in the G₀ phase are sufficiently vulnerable to the treatment (Worgul and Rothstein, 1975; Lindgren and Riley, 1973) and due to lack of mitotic activity in the CR the cell density is most affected. The cell death in the LEp leads to formation of acellular foci, increased intercellular space and so cell-cell interaction is reduced and cells become more or less isolated. Such isolated cells are more susceptible to oxidative stress than the contiguous cells (Giblin et al., 1985). According to Andley and coworkers (1999), cells either repair DNA damage and proceed in the cell cycle or do not repair and are eliminated by cell death. This may be the cause of the rapid decrease in cell density during high dose or long duration of treatment. After further treatment the cells of LEp therefore, may arrange into the patches of low and quite high density and superimposition or multilayering.

Decrease in the cellular density of LEp is reported in various types of experimental cataract (Sidjanin et al., 1993; Zigman et al., 1995) and in human
during aging (Holzman et al., 1989; Vasavada et al., 1991). The loss of cells in lens epithelial layers particularly in the CR by apoptosis or other mechanisms of cell death does not seem to play a major role in the age related cataractogenesis (Harocopoulos et al., 1998). Yet it is likely that such cells are a subfraction of all the cells suffering from treatment and the process is an extreme reflection of the damage in the remaining cells (Broglio and Worgul, 1985). However, our data suggests that the time required for opacification seems to be related to the time required for significant cell death. The viability and/or behaviour of the epithelial cells is important for maintaining the transparency of the lens throughout the life span (Uga et al., 1988). Flake like opacities reported in the rat lens epithelia can be explained by the nonuniform distribution of the apoptotic bodies in the epithelium (Michael et al., 2000).

4.1.3 Superimposition and Multilayering of Cells

The results indicate the occurrence of the superimposed cells during the treatments. However, the results suggest that they are not dose or duration dependent. Similar type of the superimposition is reported from our laboratory in human cataract (Vasavada et al., 1991). Superimposition seems to be different phenomenon than the multilayering. In superimposition, the cells are found scattered or in patches of few cells and are found throughout the LEp irrespective of CR or PR while multilayering starts from PR and gradually spreads to CR. Superimposition may seems to be the initial stage of the multilayering. The area showing multilayering or superimposition indicates area of higher cellular activity or cell proliferation. On the other hand in other area of the same LEp, atrophy and degenerative changes were observed. So in the same LEp two distinct regions with different cellular activity are found. Such type of observations were reported in
immature cataract and in senile cataract of human (Samuels, 1947; Lyda and Waugh, 1954; Vasavada et al., 1991).

The change in intercellular spaces, infolding of the plasma membranes and initial changes in superimposition all precedes DNA synthesis (Reddan et al., 1970). The initiation of mitosis in the cultured lens is preceded by and dependent upon characteristic and well-defined periods of RNA and protein synthesis. The cells with intensive RER were also observed during the treatment under the EM, which supports such a hypothesis. Changes in tissue organization have been suggested to play an important role in the control of cellular proliferation and may be a reflection of trigger mechanism of cell division required for multilayering or superimposition. It would certainly seem that the phenomenon of contact inhibition (Abercrombie and Ambrose, 1962) or cellular communication (Loewenstein and Kanno, 1964) play a role in the control of cell division, the triggering of the cell cycle should be accompanied by changes in cell-to-cell relationships (Reddan et al., 1970) which is best observed in LM and EM studies. The observed degenerative changes may have initiated by degeneration of the underlying cortical cells rather than by any external stimulus and it is proposed for multilayered epithelial proliferation in mice with cataract Fraser gene (Drenchkhahn, 1978) and in rats treated with lipidosis induced drugs (Gorthy, 1979). Our observations also suggest a decreased connection between the fibers and epithelium. Even failure of compactness of the deeper lens fiber may produce spurt in the mitosis (Shun-Shin et al., 1991). It is also possible that due to subthreshold exposure of oxidants, epithelial cells may not die but might be stimulated to create a multilayered shield to protect against the external stimulus (Michael et al., 2000). The multilayering or superimposition of cells in PR seems to be more important because such cells may not enter into the differentiation because of disturbed meridional row arrangement.
An extensive hyperplasia, hypertrophy and migration of lens epithelial cells may lead to the production of highly dense area called plaque. Such plaque contains some eosinophilic fibrous material and were observed in some of the sodium selenite treated LEp. In mature cataracts also, epithelial cell population divides into area with very high and very low density. Harocopos and coworkers (1998) have suggested that lens epithelial cell density is increased in the area directly over the opaque area of the lens and speculated that this change in density may have resulted from increased proliferation. Such an area with superimposition may represent the cells showing DNA repair mechanism. Recently epidermal growth factors (EGF) found in aqueous humor and the presence of prolyl-4-hydroxylase in lens epithelial cells proliferation on the inner surface of the lens capsule suggests that these cells are involved in the production of procollagen and fibrosis during capsular injury and its repair (Majima et al., 1998; Saika et al., 1998) during this type of cataractogenesis. It may also be possible that the epithelial cells which causes PCO are the cells of PR which can not enter into the process of differentiation due to disturbed meridional row arrangement. So the superimposition or multilayering of cells in the PR atleast suggest the extent of improper differentiation of PR cells into the lens fiber cells. The effect or role of superimposition in the CR is not known in the opacification but it can lead to disturbed cellular architecture or integrity in LEp which is required for LEp-fiber relationship.

Post-irradiational mitosis is reported to be absolutely required for the development of cataract particularly in the pre-equatorial or germinative zone of LEp (Merriam and Worgul, 1983). Changes in RNA and DNA synthesis are essential prelures to the initiation of DNA synthesis and mitosis in LEp (Bagchi et al., 1968; Reddan et al., 1970). The pattern of macromolecular synthesis relative to mitosis
may also leads to enhanced rate at which fibers are laid down, which may contain defective gene or mRNA and hence the defective proteins or metabolism. Such phenomenon can cause cortical opacity observed in the $\text{H}_2\text{O}_2$ and UV radiation cataract. It is also reported that the drop in glutathione is a secondary effect in sugar cataract formation because without mitosis and subsequent fiber formation, cataract will not develop. Such a requirement of mitosis for the development of cataract is proved by the experiments on squirrel and hypophysectomised frogs. Same phenomenon may be occurring here.

4.1.4 Effects on Different Regions of LEp

Central region (CR) of lens epithelia is reported to be first affected during hyperglycaemic cataract (Robison et al., 1990). Our observations also suggests that during all the types of treatments first changes were observed in CR. The noncycling cells of CR suffer damage but it is not expressed because they are ordinarily nonmitotic, but if they are induced to divide by any type of injury then they can participate in the cataract development (Worgul and Merriam, 1980). Our results suggests superimposition of cells which shows more mitotic activity in the CR, so, during cataract development they may participate in terms of increased mitotic activity atleast in some regions. However, the response required for such higher cellular activity is under question. Now, it is reported that, if only CR is affected with rest of the cells shielded then opacity will not develop (Merriam and Worgul, 1983). On the other hand, it is also reported that germinative zone atleast “its part” must be exposed to irradiation to develop cataract (Worgul and Rothstein, 1975). In the $\text{in vivo}$ condition also light can indeed reach some areas of the germinative zone (Coroneo et al., 1991). Therefore, superimposition of cells may start first in CR or may start together in CR and PR but the influence required for such superimposition may be coming from PR cells. The PR of LEp is responsible for all growth and
differentiation in the lens, because this is the region from where the cells enters into
the radiating columns of ER. If PR is altered, which is observed in our experiment
then cells entering into the radiating columns will vary in size or its speed can
change. Such cells interfere with the ordinary mechanism leading to alignment of
rows. It can produce fibers which were incapable of the precise orientation which is
necessary for the transparency of the lens. Such phenomenon is also reported in
the X-irradiation cataract (Worgul and Rothstein, 1975; 1977).

Meridional row architecture is considered to be very important for the
maintainance of transparency. The data suggests disorganisation in meridional
rows, which is found to be dose dependent. The cataracts of congenital origin, those
produced by alkylating agents, inflammation and aging are associated with
meridional row arrangement (Worgul and Rothstein, 1975; Merriam and Worgul,
1983). However, the reasons for such misalignment is not known (Worgul and
Rothstein, 1975; 1977). Cytopathology observed in the PR precedes
cataractogenesis and meridional row disorganisation but one can not eliminate the
possibility of some factors within the rows themselves becoming altered and
secondarily cause the misalignment (Merriam and Worgul, 1983). The cells of PR
which are not able to differentiate, remains in their place having lost their capacity to
migrate can also be responsible for such misalignment (Worgul and Merriam, 1980).
The ER is also associated with alteration in the levels of expression of several
genes including those of crystallin and unique gene products (Berthoud et al., 1999).
Therefore, abnormal cells of ER can form abnormal fiber or they may continue their
displacement posteriorly and can accumulate in the posterior region of cortex
causing alteration in lens cytoarchitecture and posterior subcapsular cataract (PCO)
(Eshanghian and Streeton, 1980). The later case may be due to loss of control
mechanisms. Loss of regulatory control is reported to be of common occurrence in
genetically transformed cells (Berwald and Sacks, 1965). The occurrence of such a phenomenon in cataract development provides further evidence of the relationship between DNA damage and cataractogenesis (Jose, 1978).

4.2 BIOCHEMICAL STUDY

4.2.1 Proteins

The lens epithelial cells, similar to the lens fiber cells contain α-, β- and γ-crystallins. There are temporal and spatial differences in the distribution of α-crystallins on one hand and β- and γ-crystallins on the other in the lens epithelial cells. The α-crystallins are present in all the epithelial cells while β- and γ-crystallins are restricted to the non-dividing elongating cells at the equatorial region (Duncan, 1981; Harding, 1991). β-crystallins are detected immediately with cessation of cell division as the cells enter the equatorial region while γ-crystallins are detected later in the elongation process (Bloemendal, 1981). Considerable differences exists in the noncrystallin proteins of the fiber cells and the epithelial cells of the lens. The lens epithelial cells includes various membrane proteins such as some of components of the cytoskeletal structure, enzymes like glyceraldehyde-3-phosphate dehydrogenase and various ATPase like Na⁺-K⁺-ATPase and Ca²⁺-ATPase. α-crystallin are also associated with lens membranes (Ifeanyi and Takemoto, 1990; Zhang and Augusteyn, 1994). The lens epithelial cell contain an extensive cytoskeletal network as compared to the fiber cells. It is represented by thin filaments, intermediate filaments and beaded chain filaments. Actin, vimentin, keratin, tubulin, α-actinin and spectrin are found scattered in cytoplasm. The actin and intermediate filaments are aggregated along the apical surface of the cells, specially at epithelial-fiber junctional interface suggestive of an important role for the cytoskeleton in lens cell differentiation (Byers and Porter, 1964). Large number of
enzymes are present in the lens. They play an important role in metabolism, transport, transparency and defence of the lens. The lens epithelial cells are characterised by the presence of many enzymes, often in manifold concentration relative to the fiber cells; some others are exclusive to the lens epithelium.

During all types of treatment water soluble proteins were found to be decreasing progressively. The decrease was highest in \( \text{H}_2\text{O}_2 \) treatment, along with decrease in WSP, an increase in the levels of urea soluble proteins was found. UDSP shows no change during selenite treatment while during UV and \( \text{H}_2\text{O}_2 \) treatment it shows decrease. No change was observed in the UDIP while TP has shown decrease in all groups.

Five possibilities for the decrease in WSP in LEp are,

1. Leakage of peptides into the aqueous humor
2. Decreased protein synthesis
3. Increased breakdown
4. Insolubilization
5. Decreased cell density.

Leakage of low molecular weight proteins were reported during cataractogenesis (Augusteyn, 1981). Similarly the reported damage in plasma membrane of the lens fibers are also possible for LEp (Watanable and Shearer, 1989), because LEp is a first target for these three types of treatments. Such events can lead to leakage of proteins into aqueous humor. Such an increase of WSP in aqueous humor is reported by Watanable and Shearer (1989) and Watanable et al., (1990). Decreased protein synthesis is also an important factor. The pyknotic and other abnormal forms of nuclei observed in various types of treatment suggests
inactivation of nucleus, so no production of mRNA and decreased protein synthesis. The decreased amino acid transport may also affect protein synthesis. As the mRNA for β and γ-crystallin are produced in LEp, stabilized and expressed only during the fiber formation (Duncan, 1981). The degenerative changes observed in the cells can also affect such mRNA and hence the production of β- and γ- crystallin. The fibers possessing deficient β- and γ-crystallin can lead to the development of cortical cataract. An extensive crosslinking of WSP and formation of disulphide links and further insolubilization of protein is reported in the lens. The decrease in the levels of –SH groups and an increase in USP in LEp during all type of treatments suggest its occurrence in LEp. As LEp possess highest amount of α-crystallin in WSP fractions and as it contains least amount of –SH groups present it seems to be quite stable. But these observations suggest drastic decrease in the WSP. So, levels of α-crystallins may also decrease along with the decrease in the levels of cytosolic water soluble enzymes. The decreasing α-crystallin can also decrease its chaperon like activity to other proteins, so other proteins becomes susceptible. Particularly effects on integrity of other key proteins involved in the structure of cytoskeleton leads to opacification (Weinreb et al., 2000). Such things are more important in the elongation compartment because it may lead to cortical opacities.

Increased calcium level and increased proteolysis with the development of various cataract, both in vivo and in organ culture is reported including senile cataracts (Duncan et al., 1994; Harding, 1991; David and Shearer, 1984). The observed cell death and particularly apoptic changes and therefore the loss metabolic competent cells may disturb the osmoregulation of the epithelium and therefore with the protein and water balance of the underlying fiber. The local degeneration of water and ion homeostasis in the epithelium may lead to an
extracellular accumulation of calcium (Vrensen et al., 1995). Both proteasome and calpain are reported as a calcium dependent proteolytic enzyme. Such a proteolytic capabilities are considered as secondary defence system which can avert or delay the accumulation of damaged proteins (Taylor, 1993). Rat lens contains relatively high level of calpain, so part of all these different types of cataracts in rats is probably activation of calpain (David and Shearer, 1986).

LEp also response to increased Ca\(^{2+}\) from external or internal stores which leads to increased proteolytic activity by calpain, proteasome or by both and it is important in the development of cataract (Karlson et al., 1999). LEp may modulate growth of the lens abnormalities in Ca\(^{2+}\) regulation (Duncan et al., 1994). The \(\alpha\)-crystallin which is found in the WSP of LEp can become turbid in presence of 1-10 mM Ca\(^{2+}\) ion which increases with increasing Ca\(^{2+}\) concentration (Bando and Obazawa, 1989). Lens or LEp HMP (proteosome) can degrade mildly photooxidized lens proteins but proteins which are extensively damaged are not degraded and such proteins may accumulate in LEp and results in the increase of USP fraction. Similarly it is also possible with calpain. The increased Ca\(^{2+}\) can also inhibit Na\(^{+}\)-K\(^{+}\)-ATPase which has varied effects on the osmoregulation of lens (Hightower and Hind, 1982) and ultimately decreases the total protein in LEp.

Other important reason for the decrease in WSP is unfolding of the peptide chains due to binding of GSH. Observed increase in USP and concomitant decrease in GSH level supports such phenomenon. Due to such unfolding of the proteins, its chances for crosslinking increases.

The decrease in the proteins, due to decreased protein synthesis, is not only restricted to the crystallins (WSP) but it is a general phenomenon affecting other proteins of the lens including the cytoskeleton and the membrane proteins (Ozaki et
al., 1985; Tagliavini et al., 1986; Garadi et al., 1984; Matsushima et al., 1997) (USP, UDSP). The present results have shown an increase in the USP, which may be due to conversion of WSP into USP. However the cytoskeleton and membrane proteins are very important in the maintainance of lens transparency (Ireland and Maisal, 1982; Tagliavini et al., 1986; Bloemendal, 1991; Carter et al., 1995; Bettelheim et al., 1995) The decreasing cytoskeleton and membrane proteins may affect the cellular architecture which is evident as the formation of intercellular space and superimposition of the cells.

4.2.2 Sulphydryl Groups (-SH Groups)

The lens and lens epithelia contain two forms of sulphydryl groups, protein bound and non-protein sulphydryl groups. The major roles of sulphydryl groups in the lens are:

1. Disulphides and –SH groups have multifactorial role in protein structures and its functions (Thomton, 1981).
2. Sulphydryl groups located near the active site of an enzyme are important in the activity of enzyme, e.g. the sulphydryls are important in Na+/H+ exchange (Grinstein et al., 1985) and organic anion transport (Spector, 1982; Tse et al., 1984).
3. Disulphide bonds on other hand have been implicated in regulatory functions as well as surving a catalytic role (Pontremoli et al., 1967; Gilbert, 1982). Recent evidences suggest its dynamic role in transport processes (Turner and George, 1984).
4. The sulphydryl groups and disulphides have some role in hormone action (Rasmussen et al., 1960; Sen, 1985). Therefore, the lens and particularly the
LEp, -SH are critical for lens epithelial cell communication and lens transparency.

The gradual decrease was observed in protein bound soluble, nonprotein soluble and total sulphydryl groups. However, in certain low dose or low duration of treatment, the observed decrease is nonsignificant. In such cases during high dose or duration it shows severe decline. In \( \text{H}_2\text{O}_2 \) treatment the decrease was almost constant. Along with the decrease in soluble sulphydryls concomitant increase in the levels of insoluble sulphydryls was found. Significant decrease was found in the levels of total sulphhydrals in the lens epithelia of \( \text{H}_2\text{O}_2 \) and UV treated lenses while the decrease is nonsignificant in the sodium selenite treatment. The decrease in total soluble sulphydryl is the manifestation of decline in both protein bound and nonprotein soluble sulphydryls.

The observed decrease in the levels of protein bound -SH may be due to crosslinking of -SH groups and the formation of disulphide bonds (PSSP) and/or due to binding of GSH (PSSG). Such phenomenon may be responsible for the increased insoluble protein levels in the lens epithelia. SH oxidation and protein insolubilization are believed to be associated with the structural deformation of proteins. Altered plasma membrane integrity and increased leakage could be the other reasons for loss of SH groups (Cooper et al., 1986).

Most of the SH groups are photolysed by either direct effect of sunlight (Zigler and Goosey, 1984) or \( \text{H}_2\text{O}_2 \) (Linetsky and Ortworth, 1995). Chromatophores present in the natural normal and cataractous lenses are capable of initiating photooxidative process involving endogenous thiols and ascorbic acid. This observation may be pertinent to UVR induced cataract. Actually, the thiols lead the production of \( \text{H}_2\text{O}_2 \) which may contribute to thiol loss. On the other hand selenium is
a strong sulphur oxidant. Selenite in particularly causes oxidation of membrane –SH in the lens epithelia which may effect several important enzymes and transport system (Hightower and McCready, 1991). However selenium cataract does not appear to be caused by extensive –SH oxidation and cannot be attributed exclusively to the GSH loss (David and Shearer, 1984). An observed increase in the level of insoluble protein bound SH is found during the treatment may be due to the fact that not all –SH is to be oxidized before the soluble protein transforms into an insoluble form (Kemoto and Iwata, 1978). α-crystallin of LEp may provide chaperon like activity and protect other lens proteins from oxidative stress induced aggregation and also provide some protection to their thiol groups (Wang and Spector, 1995). The decrease in the levels of sulphhydryl groups particularly NPSH and PBSH in the LEp, entire lens cannot withstand against the oxidative environment.

Glutathione (GSH) forms a major part of nonprotein sulphhydryls in the lens (Spector, 1995). Mammalian lens contains high concentration of GSH, the highest being in the epithelium (Reddy et al., 1990). The epithelium contains five times more concentration of GSH than in the cortex (Reddy and Giblin, 1984). In the lens, lens epithelium and cortex are the site for synthesis and active metabolism of GSH (Kinoshita and Merola, 1958) and possesses a mechanism leading to the formation of reduced glutathione (GSH) from oxidized glutathione (GSSG) through the action of glutathione reductase. The lens epithelium has two sources for the GSH. First, is de novo GSH synthesis from circulating and aqueous humor amino acids which can be only a minor source of the millimolar concentration of GSH in the LEp (Mackie et al., 1997). Second one is the major source, the transport of intact GSH from the circulation and aqueous humor (Zlokovic et al., 1994; Mackie et al., 1996). GSH transport in the LEp is carrier dependent and partially depend on Na⁺ which
suggests the existence of two systems, a Na\(^+\) dependent (coupled) and Na\(^+\) independent or facilitated transport (Kannan et al., 1998). The effect to the membrane -SH, can also affect the transport of GSH in the LEp.

The -SH group of GSH is responsible for much of the biological activities of GSH. GSH performs various functions in the lens and lens epithelia which are,

1. Detoxification of noxious electrophils of extralenticular origin by formation of mercapturic acid, so it helps in the maintainance of the transport process of the lens by acting as an antioxidant (Rathbun and Hanson, 1979; Giblin et al., 1981).

2. Oxidation of hydroperoxides largely of intramolecular origin by GPx-GR pathway. GSH can scavenge all such free radicals at 10\(^{-4}\)M concentration (Giblin et al., 1981; Verma et al., 1977).

3. Protection of critical -SH groups, particularly membrane -SH groups involved in cation transport and permeability (Epstein and Kinoshita, 1970a,b).

4. Reduction of lenticular protein disulphide links (Rathbun, 1980).

5. GSH acts as a coenzyme for the activity of enzymes e.g., two enzyme glyoxalase system (Van Heyningen et al., 1954).

The drastic decrease in the concentration of GSH in the LEp during all the three types of treatments may be due to its transport from circulation or aqueous humor or due to its reduced synthesis. Besides its other causative factor may be the leakage into the optic fluids, degradation of GSH or due to decrease in the activity of glutathione reduction cycle system. Leakage and degradation of GSH is ruled out (Reddy, 1990) but reduction in the activity of enzymes like γ-GCS and GR may be an important factor (Kalaria, 1997). The direct effect of or other ROS on GSH in
the absence of protective enzymes can cause a chain reaction facilitating the formation of oxidized glutathione (GSSG) and mixed disulphides (Winterbourn and Metodiewa, 1994; Park and Thomas, 1988). Selenite is also known to bind with GSH and causes GSH depletion.

The contribution of lens epithelial GSH is important since the interior of lens contains less reduced GSH and less metabolic activity. Due to low level of GSH, the lens is dependent on the epithelia for GSH to protect itself. So, more susceptible to oxidative insult (Spector, 1982). Due to decrease in the levels of GSH, cells can not maintain the critical SH groups including membrane -SH and Na⁺ - K⁺ - ATPase. It has adverse effect on lens transport and it further causes membrane permeability (Spector et al., 1995b) and such lenses are susceptible to oxidative damage resulting in an inactivation of the Na⁺/K⁺ pump, thus leading to ionic changes and cataract development (Reddy et al., 1988). The depletion of GSH also leads to the decreased amino acid accumulation in the LEp (Pillion and Leibach, 1975), which may further affect the synthesis of crystallins and important protective enzymes. The decreased amino acid accumulation may itself decrease the synthesis of GSH. Although elevated GSH do not appear to protect the LEp from H₂O₂ insult (Spector et al., 1987) but, critical concentration of GSH may be required for the short term protection of these membrane -SH groups (Hightower et al., 1989). Therefore GSH has to be reduced to a certain critical level before any change to occur (Epstein and Kinoshita, 1970a). The decreased level of GSH can also cause increased mitotic activity and disorganisation of meridional rows (Calvin et al., 1991). The protein thiol also become oxidized only when the levels of GSH in the lens drops below the critical level (Reddy, 1990). The decreasing amount of GSH and GSH redox cycle enzymes leads to extensive nuclear and cellular damage because of secondarily generated hydroperoxide from H₂O₂ (Flohe, 1979; 1982). So an overall concept:
The decrease in -SH groups including GSH disrupts the redox homeostasis of cell which further disturbs the oxidative defence of the LEp and lens which further leads to membrane permeability and finally to the development of cataract. The observed degree of variation in epithelial morphology and opacification is dependent on the extent of -SH oxidation and protein insolubilization, which was found to be progressive and dose or duration dependent.

4.2.3 Glutathione Reductase (GR)

The GR is important to keep the cellular concentration of the reduced GSH high by catalysing the conversion of GSSG to GSH. So it maintains proper reducing environment of lens and lens epithelia. GR is primary located in the LEp (Raghavachari et al., 1999).

Decrease in the activity of GR enzyme was found during all types of treatments. Constant decrease was found in the GR activity during H₂O₂ treatment while after low dose of selenite and low duration exposure of UV radiation its decrease remains nonsignificant, but, after high dose treatment of selenite and high duration of UV radiation, it shows drastic decrease in GR activity.

Various reasons for the decreasing activity of GR were suggested by various researchers. According to Rogers and Augusteyn (1978) GR is denatured because of alteration in the salt and water balance. Ohrloff er al. (1984) and Harding (1973) has reported labilation of GR in human cataracts. Other important reason for the inactivation of GR enzyme may be due to impaired concentration of cofactor NADPH (Srivastava et al., 1973).

Decreased activity of GR evokes changes in the LEp as GR provides reducing capacity for the synthesis of DNA (Holmgren, 1981; Vani, 1995; Thampi,
Thus, from the above inferences, it may be speculated that the diminished activity of GR could affect GSH and secondarily to the lens proteins leading to the accumulation of GSSG and H$_2$O$_2$. The LEp cells are killed when they possess deficient GR activity (Giblin et al., 1990). Decrease in GR and further inability of LEp to detoxify H$_2$O$_2$ and disturbances of the cytoskeleton leads to focal weakening of cell surface (Ikebe et al., 1989).

4.2.4 Superoxide Dismutase (SOD)

SOD, catalase and glutathione peroxide (GPx), these three enzymes are known to occupy a prominent position in the defence system of the lens against harmful oxidants such as superoxide anion (O$_2^{•−}$) and hydrogen peroxide (H$_2$O$_2$). The superoxide anion, a powerful oxidant is converted to H$_2$O$_2$ by the enzyme SOD. Thus, the role of SOD is to protect the lens from deleterious effects of O$_2^{•−}$. In the lens, major activity of SOD is located in the lens, epithelium (Bhuyan and Bhuyan, 1978).

Decrease in the activity of SOD enzyme was recorded in all types of treatment. Although in the low dose or low duration treatment decrease was less and nonsignificant.

The observed decrease in the activity of SOD may be due to inactivation which is reported in the rat lenses and inactive molecules were also reported (Dovrat and Gershon, 1981). SOD is inhibited by low H$_2$O$_2$ level (Bhuyan and Bhuyan, 1978), therefore it may be assumed that the remaining SOD activity is further decreased by the elevated H$_2$O$_2$, so that the concentration of O$_2^{•−}$ rises. This explains the large number of post-translational oxidative modifications that were proved by protein structure (Ohrioff et al., 1984).
Decrease in the activity of SOD can increase the level of \( \text{O}_2^{2-} \) by 2-3 fold (Bhuyan and Bhuyan, 1978). Photolysis in the presence of SOD results in a marked increase in \( \text{H}_2\text{O}_2 \) formation, inspite of the fact that SOD converts into \( \text{H}_2\text{O}_2 \) only 50% of the \( \text{O}_2^{2-} \) with which it reacts (McCormick and Thomason, 1978). These provides firm evidence for the photogeneration of \( \text{O}_2^{2-} \) and further indicates that only a fraction of this species is converted into \( \text{H}_2\text{O}_2 \) in the presence of SOD (McCormick and Thomason, 1978; Inoue et al., 1982).

4.2.5 Catalase

Two enzymes which can metabolize \( \text{H}_2\text{O}_2 \) are GPx (Pirie, 1965) and catalase (Bhuyan and Bhuyan, 1978). Catalase can metabolize \( \text{H}_2\text{O}_2 \) by a catalytic reaction producing \( \text{H}_2\text{O} \) and \( \text{O}_2 \) (Sies et al., 1973). This reaction is depend on \( \text{H}_2\text{O}_2 \) concentration becoming more effective with the increase in \( \text{H}_2\text{O}_2 \) concentration, due to the higher Km value (Spector et al., 1996). Thus, catalase regulates endogeneous \( \text{H}_2\text{O}_2 \) when it increases above the physiological level (Bhuyan and Bhuyan, 1979). GSH redox cycle is the primary defence of the lens and LEp but at steady state level of \( \text{H}_2\text{O}_2 \), catalase becomes important in detoxifying a brief burst of \( \text{H}_2\text{O}_2 \) (Giblin et al., 1990). Catalase also inhibits the formation of hydroxyl radical (OH* ) (Halliwell and Gutteridge, 1984). Catalase is also primarily located in the LEp and the peripheral region of LEp contains its extensive amount (Reddan et al., 1996a; Young et al., 1998).

The results indicates constant decrease in the activity of catalase in group III. The decrease is nonsignificant in group I,A and group II,A but in the group I,B and Group II,B it shows rapid decrease.
The observed decrease in the activity of catalase enzyme may increase the accumulation of H$_2$O$_2$ in the LEp. Such an increased level of H$_2$O$_2$ inhibits the enzyme catalase (Pigeolet et al., 1990). Post-translational changes in protein impairs the ability as well as the catalytic capacity of the enzyme which finally lead to the formation of inactive enzyme molecules (Dovrat and Gershon, 1981).

As catalase of the eye affords protection to the lens from H$_2$O$_2$ and it also protects SOD of the lens from inactivation by H$_2$O$_2$. Depletion in the activity of catalase directly inhibits SOD activity. Actually SOD inhibition is potentiated by the inhibition of activity of catalase (Kalaria, 1997). Due to decrease in the activity of both SOD and catalase, tissue is exposed to high levels of $O_2^{-*}$ and H$_2$O$_2$. Decrease in catalase activity and its prevention by a level application of catalase, suggest a key role of oxyradicals in the damage of the eye by UV radiation (Cejkova and Kojda, 1994). The decrease in the activity of catalase causes degeneration of LEp and cytoplasmic vacuolization (Bhuyan and Bhuyan, 1979).

4.2.6 Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) is a selenoenzyme. It requires GSH as a cofactor. Like catalase GPx can also hydrolyse hydroperoxides. GPx has a high affinity for H$_2$O$_2$ and has a lower Km for H$_2$O$_2$ degradation. It might therefore be expected to have a higher rate of H$_2$O$_2$ degradation than catalase in the H$_2$O$_2$ concentration range found in normal lens and aqueous humor (O’Brien and Little, 1969). Further, GPx is more important in removing H$_2$O$_2$ probably because it is located in the same subcellular compartment mostly in cytosol and some in mitochondria (Helliwell, 1991) while catalase is located in microperoxisomes that are distributed randomly throughout the cytoplasm. Hence GPx is main route for the detoxification of H$_2$O$_2$ in the lens and removes at least 80% of the endogeneous
H$_2$O$_2$ in certain types of cataracts (Fecundo and Augusteyn, 1983) and particularly it is important to protect membrane cytoskeleton as catalase may be too isolated (Giblin et al., 1990). Therefore GPx plays an important role in the maintainance of lens clarity.

Results of these investigation suggest constant decrease in the activity of GPx enzyme. The observed decreased GPx activity may be due to posttranslational changes and further inactivation or due to decreased level of GSH. Due to observed decrease in the levels of GPx in the lens epithelia, the levels of H$_2$O$_2$ increases which can cause inhibition of SOD. Particularly lipid peroxidation rate increases because GPx is a principle enzyme which acts rapidly on lipid peroxides of cell membrane (O’Brien and Little, 1969; Bergad et al., 1982). Due to decreased GPx, ROS could be removed at much lowered rate and cause an increased accumulation of oxidative stress in the lens (Bhat et al., 1991).

4.3 EFFECTS OF SELENITES

Selenium compounds have been shown to cause cellular dysfunction or cytotoxicity in a number of tissues (Zia, 1993; Bell et al., 1997; Lin-Shiau et al., 1989; Mani, 1994; Moxon and Rhian, 1943). The cataractogenic potential of selenites was first reported by Algana and D’Aquino (1957).

Selenium reacts with various sulphydryls to form selenotrisulphides (R-S-SeS-R) and oxidized sulphydryls of protein (ASH). Selenium is incorporated in poteins as a selenoamino acids such as selenocystine (Shearer et al., 1992). The identification of selenoamino acids is reported in $\alpha$- and $\beta$-crystallins (Shearer et al., 1984). Selenium can also retard protein synthesis by direct inhibition of elongation factor 2 by selenodiglutathione (Vernie et al., 1975) and by inactivation of the initiator met-t-RNA binding factor (Safer et al., 1980). Leakage of crystallins is also
reported from lens into the aqueous humor and vitreous humor. In rat crystallin concentrations increases upto 10-fold to 20-fold increases in the vitreous humor during mature selenium cataract (Watanable and Shearer, 1989; Watanable et al., 1990). Degradation of noncrystallin cytoskeletal proteins in the early stages of opacification is reported by Matsushima and coworkers (1997).

Several researchers have reported that initial attack of selenite in both cortical and nuclear cataract is the LEp. Further, if only posterior part of lens is treated with selenite, cataract do not develop but if the anterior part is treated, cataract develops. The effects of selenite on the LEp observed during the treatment and some reported effects are:

1. Being the most important site of protein synthesis, selenite can cause decreased protein synthesis in LEp as it is also reported in the lens. The proteins which are affected due to such effects are defensive proteins like crystallin and important enzymes. Selenites can also cause degradation of proteins by activating calcium dependent proteolytic enzymes or can also cause leakage of water soluble proteins from LEp. All these changes may be the reasons for the observed decrease in the WSP and TP in the LEp.

2. The binding of selenium with GSH can bring about reduction in the reducing environment and a concominent increase in the oxidative stress to the LEp. Such increased oxidative insult may lead to affect cellular components as it is found in the oxidative stress.

3. Selenites can oxidize critical -SH groups leading to the inactivation of Ca\(^{2+}\) ATPase and Na\(^+\) - K\(^+\) - ATPase enzyme and an opening of ion channels in the epithelial membranes. Such an event disturbs ion homeostasis of lens and can further cause liquification of lens. The opening of ion channels can
also increase calcium influx and further degradation of proteins due to increased proteolytic activity. The partial oxidation of such proteins can also cause exposure of their hydrophilic regions and -SH groups. Such exposed -SH groups can also form disulphide links due to effect of ROS and forms insoluble aggregates. Such an event in LEP may be responsible for decrease in WSP and simultaneous increase in USP.

4. Selenite cataract is also reported to be associated with abnormal metabolism and LEP being most important site of metabolism in the lens, selenite may affect the LEP. Such an event leads to decrease in the production of NADPH and decrease in the activity of enzymes like GR. Therefore conversion of GSSG to GSH fails which can also cause decreased oxidative defence mechanism particularly in LEP. So LEP and lens becomes more susceptible.

5. Selenite insult to the LEP is largely confined to germinative epithelial cells. Our observations also suggest extensive effects in the germinative zone cells which are in S or pre S phase of cell cycle. Such an effect of selenium can cause meridional row disorganisation and defective fibre formation.

According to the observation and as it is reported, before any change which takes place in the lens, LEP is affected. So LEP is a first target of selenite which may further cause the cataract formation. However, at low dose treatment (Group I,A) the effects on both histological and biochemical parameters are less and it leads to development of nuclear cataract. Such observations may be due to that first LEP may have been affected and that portion of lens have became opaque but then after LEP have repaired themselves and while the time of opening of eyes and on the day of dissection such a repair have been in much advance state.
4.4 EFFECTS OF UV RADIATION

The UV radiation causes various effects on lens which includes biochemical and histological changes. The production of N-formyl kynurime (NFK) and its association with lens proteins actively generates various ROS which has varied effects on lens proteins (Fecundo and Augusteyn, 1983), DNA and RNA synthesis (Kleiman et al., 1990; Sidjanin et al., 1996), inhibition of cellular division (Sidjanin et al., 1996) and mobility. The generation of diffusive ROS suggests that overall process should have the accelerating kinetics of an autooxidation (Foote, 1976; Creed, 1984; Grossweiner, 1984). If a similar situation exists in vivo, some of the oxidants would be detoxified by antioxidant defence systems of the lens. However, some important enzymes may themselves be susceptible to photooxidative damage (Spector, 1984; Giblin et al., 1987; Jedziniak et al., 1987).

The epithelial cells are the first site for interaction of UV radiation with the lens. The wavelength from 295 nm to 320 nm is believed to act via a mechanism involving the lens epithelium (Bochow et al., 1989). It is further supported by the facts that $O_2$ consumption increases five times upon irradiation and causes accelerated respiratory functions in the LEp cells, which can also suggest that the necessity of the LEp cells may play a role in cataractogenesis (Xu et al., 1993). Both $O_2^-$ and $H_2O_2$ are thought to act by LEp. $H_2O_2$ and $O_2^-$ radicals may react through the Haber-Weiss reaction producing OH*. OH*-radicals are considered to be most damaging ROS and is exceedingly reactive towards most of cellular components, although their in vivo existence has been questioned but it can act at the production site or nearby (Spector, 1995). As most of the activity of lens resides in the epithelium, epithelium seems to be better protected than the cortex and nucleus against the ROS.
According to present observations, UV radiation has following effects on LEp,

1. Decreasing levels of WSP and USP suggests modifications of proteins in the LEp. Such altered proteins can affect the cytoskeleton, crystallins and enzymes.

2. It is reported that $O_2^-$ can cause degradation of collagen, thus it affects the lens capsule. Such an event leads to alteration in the capsule permeability which may allow leaking of WSP into the aqueous or vitreous humor. Such thing is also supported by our observation of decreasing TP. It is also possible that UV may lead to destruction of membranes in LEp and leaking can also take place inside the lens.

3. During UV irradiation, first cortical cataract develops which may be due to effects on Na⁺ - K⁺ - ATPase and membrane transport which subsequently leads to disturbed ion homeostasis and hydration.

4. UV radiation can also cause destruction of light sensitive molecules.

5. UV radiation can directly affect the DNA (Douki et al., 1999) and can also cause altered DNA repair synthesis. Such things can have varied effects on lens. The occurrence of various nuclear forms suggests the DNA damage.

6. All the above changes can directly cause cell death in the LEp and decrease in the cell density, which is evident by this investigation. The decreasing value of proteins and enzymes may be due to such decrease in the cell density.

Our observations suggest that UV radiation primarily cause two types of effects on the LEp. First is nuclear changes and second is cytoplasmic effects. The
decrease in cell density is found but alterations in the biochemical parameter is less pronounced. After long duration exposure a drastic change was found in both histological and biochemical parameters. So it clearly indicates that when oxidative stress induced by the UV radiation and antioxidant defence becomes imbalanced, it results in observed changes. However, it is likely that the accelerated oxidative damage may be a factor in cataractogenesis.

4.5 EFFECTS OF HYDROGEN PEROXIDE

There are several reports which suggest that major factor involved in the development of cataract is oxidative insult (Spector, 1995; Giblin et al., 1990; Ortwarth, 1998; Spector et al., 1998). Both intra- and extracellular oxidative stress affects the lens (Andley and Clark, 1989). O₂ tension in the vicinity of the lens is low, less than 30 to 60 mm Hg (Kwan et al., 1972). Yet this is sufficient to support some aerobic lens metabolism and is sufficient to act as a source of ROS (Kwan et al., 1972; Hanling and Crabbe, 1984). H₂O₂ is a reactive oxygen species (ROS) produced during the photochemical reaction in the lens along with other ROS. H₂O₂ is not a radical and is less reactive than O₂⁻ or OH⁻. However, its relative stability allows it to move from its origin to other locations passing rapidly through cell membranes (Spector et al., 1993). H₂O₂ is found in aqueous humor and its concentration during cataract increases 2 to 7 fold. Similarly inside the lens its concentration become 30 fold higher (Spector, 1995). H₂O₂ is also important because a complex oxidative stress consisting of H₂O₂, OH⁺ and O₂⁻ was imposed on rat lenses in culture eliminating H₂O₂ prevented the cataract formation (Spector et al., 1993).
The single layer of epithelial cells are the first site of oxidative damage (Spector, 1995). Our results and reports indicates that H$_2$O$_2$ has following effects on LEp which may lead to the cataract development.

1. The results indicate constant decrease in GSH which may be due to its oxidation. Epithelial cells are able to maintain a remarkably high reducing environment, quickly recycling the oxidized glutathione (GSSG) to GSH (Spector et al., 1987). Results also show decreasing activity of GR, so the capacity of LEp to reduce oxidized glutathione also decreases. When constant oxidative stress is given to the lens, the loss of a reducing environment provides the opportunity for oxidative damage to the epithelial cell layer.

2. H$_2$O$_2$ can also cause DNA strand breaks through Fentons reaction producing OH (Zigler et al., 1985). The nuclear modification observed during H$_2$O$_2$ treatment supports such a notion.

3. Due to H$_2$O$_2$ treatment, intracellular free Ca$^{2+}$ increases (Delamere et al., 1983). The increasing Ca$^{2+}$ can activate proteases which can disturb cytoskeleton and can also activate endonucleases that produces DNA strands breaks (Orrenius et al., 1989).

4. H$_2$O$_2$ can also cause metabolic disruptions in the lens and LEp.

5. H$_2$O$_2$ can also lead oxidation and further inactivation of several susceptible enzymes like glyceraldehyde-3-phosphate dehydrogenase (G3PD) (Helliwell, 1987). Oxidative stress leads to inhibition of Na$^+$ - K$^+$ – Pump (Kobayashi et al., 1983), uncoupling of ATP hydrolysis (Garner et al., 1983) and ion
translocation. It may also lead to hydration of lens and subsequent cortical cataract.

7. The level of oxidative stress determines the mode of the cell death. 400 μm of H₂O₂ causes necrosis while 15 μm causes apoptosis (Lennon et al., 1991) 150 μm H₂O₂ causes apoptotic cell death in rat lens epithelia. Histomorphological observations suggest that major nuclear forms occurring due to H₂O₂ insult show condensation (pyknosis), similar to the characteristics of apoptosis.