Lens Protein Changes Associated With Cigarette Smoking

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Abstract: Purpose: Smoking is an independent risk factor that has dose-response effect. The goal of the present work is to study the biophysical and biological effects of smoking on the crystalline lens of the rabbits. **Materials and methods**: Twenty New Zealand albino rabbits used in this study were classified into five groups in which group I (n=4) served as control. The other groups were exposed to different durations of cigarette smoke (five cigarettes per day). Animals were decapitated after 2, 4, 6 and 8 weeks and soluble lens proteins were separated and the following measurements were carried out: estimation of total soluble protein, refractive index measurement, sodium dodocyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and determination of sodium, calcium and potassium concentrations. **Results**: The results showed that, exposure of the animals to cigarette smoke resulted in decrease of the protein concentration and potassium content that was accompanied by an increase in the refractive index of the soluble lens proteins and an increase in sodium and calcium content. In addition, there were changes in the molecular structure of soluble lens proteins demonstrated by SDS-PAGE. **Conclusion**: smoking causes morphological and functional changes to the lens that may lead to cataract.
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1. Introduction

Cigarette or tobacco smoking is a well-recognized major risk factor for a wide range of diseases, such as cardiovascular, respiratory, and malignant diseases[(1)]. Human[(2,3,4)] and animal studies[(5,6,7)] have demonstrated that maternal smoking has several detrimental and teratogenic impacts on pregnancy and its outcomes such as placental insufficiency, foetal growth retardation, low birth weight, foetal brain damage, and sudden infant death syndrome. Smoking also has adverse ocular effects. It has been shown to be a risk factor for many common and severe eye diseases, such as Graves' ophthalmopathy[(8)], age related macular degeneration [(9)], glaucoma [(10)], and cataract [(11,12,13)]. Many of these diseases lead to irreversible blindness. The epidemiological relationship between smoking and cataracts has been well studied by case-controlled [(14,15)], cross-sectional [(16,18)], and prospective studies [(19-20)]. There is a dose-response relationship between the cumulative amount of smoking and the risk of nuclear cataract developing [(21)]. A major teratogenic component of tobacco smoke responsible for adverse effects is nicotine. A typical smoker using 20 cigarettes a day will absorb about 0.3 mg/kg nicotine daily, resulting in peak plasma nicotine concentrations in the range of 10 to 50 ng/ml[(22,23,24)]. The present work deals with the biophysical and biological effects of smoking on the crystalline lens of the rabbits after different periods of exposure namely 2, 4, 6 and 8 weeks to provide support for the hypothesis that cigarette smoking increases the risk of cataract formations.

2. Materials and methods

Twenty New Zealand albino rabbits with an average body weight of 2.5±0.5 Kg were selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The research protocol was approved by the local ethical committee that applies the ARVO (THE ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY) statements for using animals in ophthalmic and vision research. The animals were classified into five groups in which group I (n=4) served as control. The other groups were exposed to cigarette smoke (five cigarettes per day) [(25)] by using eye speculum to insure that their eyes were exposed to smoke. Groups II, III, IV and V were exposed to smoke for 2, 4, 6 and 8 weeks, respectively. Animals were decapitated after different periods of exposure and eyes were enucleated. Then, the lenses were freed from the eye. The lenses without their capsules were weighed, homogenized separately in de-ionized water and centrifuged at 16,000 rpm to extract soluble lens proteins then stored at -20^{o}C for the following measurements.

Estimation of total soluble lens proteins:

Total proteins in the soluble part of the crystalline lens were determined by the method of Lowry et al.[(26)].

Refractive index:

The refractive index of native soluble lens protein was measured using Abb's-refractometer attached with temperature control unit type W Lauda (Germany).

SDS polyacrylamide gel electrophoresis:

Soluble lens proteins were separated according to their molecular weights by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli[27] using 5% stacking gel and 12% separating gel. The data represented graphically with an automatic scanner (model R-112, manufactured by Beckman).

Sodium (Na^+), Potassium (K^+) and calcium (Ca^{++}) content:

Sodium, potassium and calcium content in lens homogenate were recorded using atomic absorption spectroscopy in order to study the membrane permeability of the lens.

Statistical analysis:

Data were expressed as the mean ± SD. Comparison between groups was performed using analysis of variance (ANOVA), commercially available statistical software package (SPSS-11, for windows) was used where the significance level was set at $p<0.05$.

3. Results:

Fig (1) shows the total soluble lens proteins of control and exposed rabbits to cigarette smoking after 2, 4, 6 and 8 weeks. The protein concentration in control (group I) was 290.7±3.5 mg/g lens tissue wet weight. After 2 week of exposure to smoke the protein content was 288.1±3.9 that indicate no change in group II. But the rest of exposed animals' groups show a significant decrease in the soluble lens protein content to 280±4 (group III), 260.4±5.2 (group IV) and 255±4.3 (group V) after 4, 6 and 8 weeks, respectively.

Fig (2) shows the refractive index of soluble lens protein for control and after exposure to smoke for all periods. It is clear from the figure that the groups exposed to smoke were characterized by an increase in the refractive index relative to control, except group II.

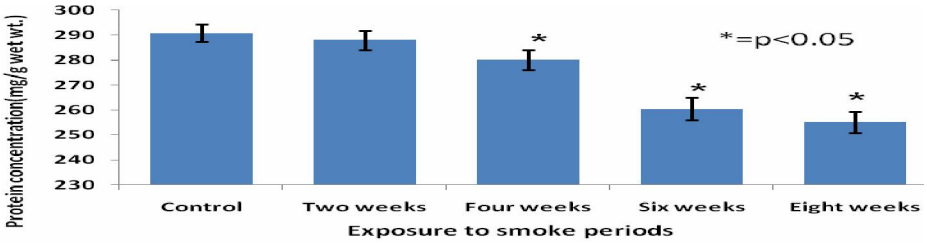


Fig 1. Protein concentration of rabbit lens for control and after exposure to smoking for different periods.

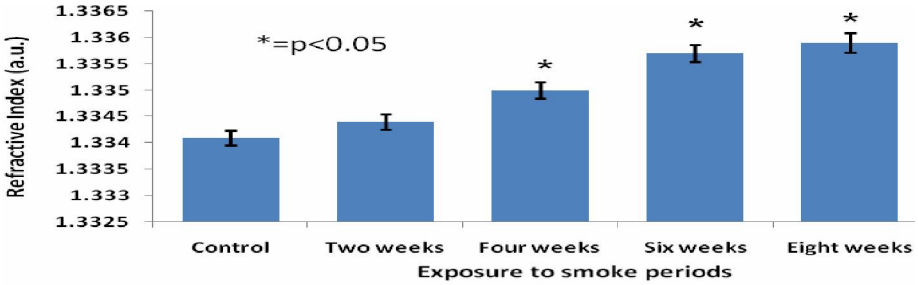
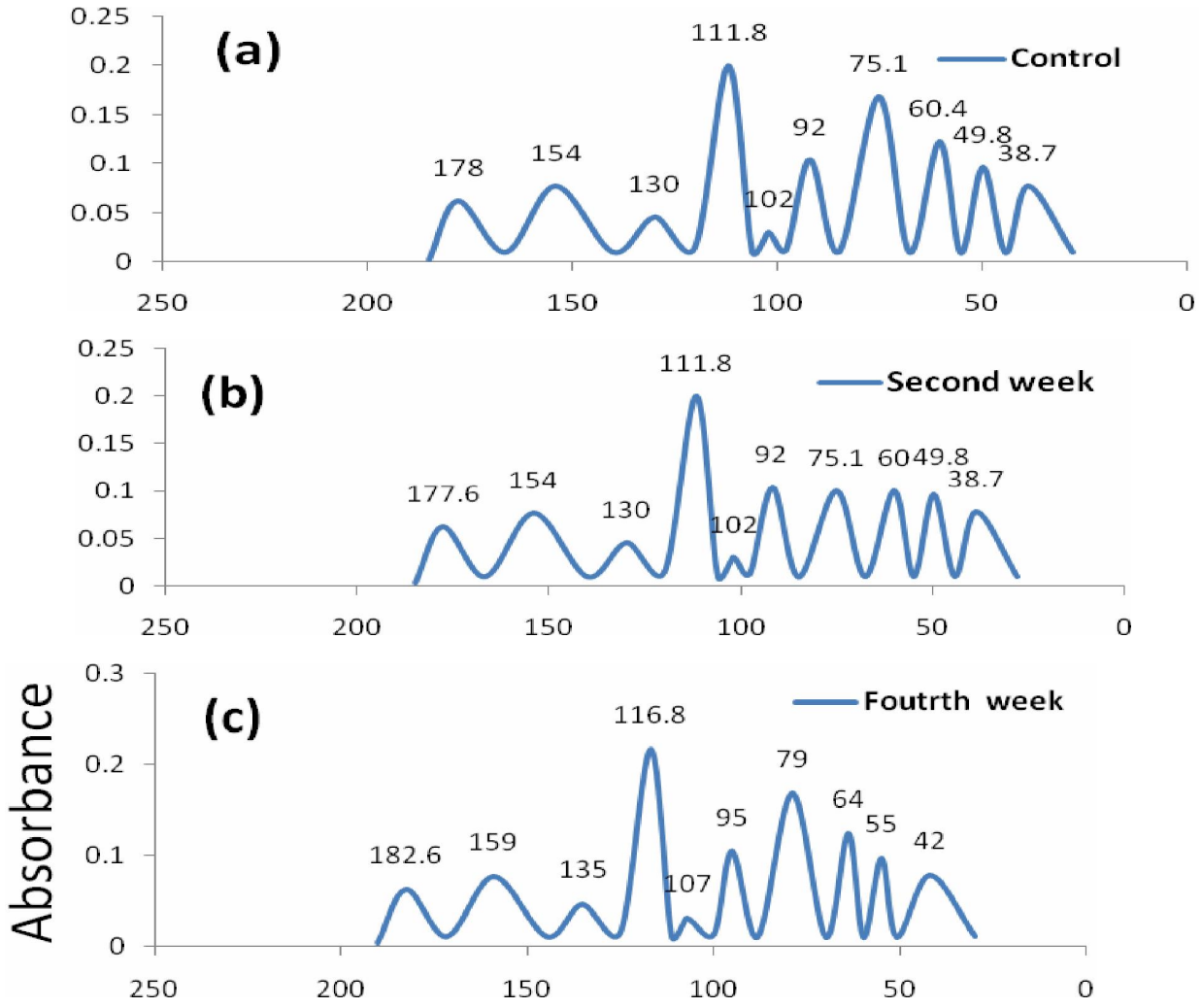


Fig 2. Refractive Index of rabbit lens protein for control and all groups exposed to smoking for different periods.

Panel (a) of Fig (3) shows the electrophoretic patterns of lens proteins for control rabbits, it was characterized by the presence of 10 peaks, which reflect the different soluble protein fractions with specific intensities and broadening that covered the molecular weight range 39 – 178 KDa. In panel (b) of fig (3), the patterns of lens proteins for group II which were exposed to cigarette smoke for 2 weeks revealed no change in the electrophoretic mobility and the intensity of all peaks proteins fractions. After 4 weeks of exposure to cigarette smoke (panel c of fig 3), the pattern revealed some shift to high molecular weight for all fractions and covered the molecular weight range 42-183 KDa. Also a decrease in the intensity of the 75 KDa peak is observed to be 0.1 compared to the control which is 0.17. Panels (d) and(e) of fig (3) shows the electrophoretic pattern of soluble lens proteins for animals exposed to cigarette smoke for 6 and 8 weeks, respectively. The two patterns revealed propagation of the same phenomenon; shift in all fractions to high molecular weight and covered the molecular weight range 40-185 KDa and 65-195 KDa after exposure to smoke for 6 and 8 weeks, respectively. Panel (d) characterized by decrease in the intensity of low fractions mobile groups. Also panel (e) (8 weeks exposure to smoke) is characterized by reduction of fractions to 9 peaks.

The cigarette smoke influence on the concentration of cations in the lens protein homogenates was illustrated in Figs 4-6. Sodium, Ca^{++} and K^{+} concentrations did not change after 2 weeks of exposure to cigarette smoke. Four, 6 and 8 weeks of exposure produced a remarkable increase of both Na^{+} and Ca^{++} concentrations in exposed rabbits (Fig 4, 5). On the contrary exposure to cigarette smoke caused a pronounced decrease of K^{+} concentration with respect to control samples (Fig 6).



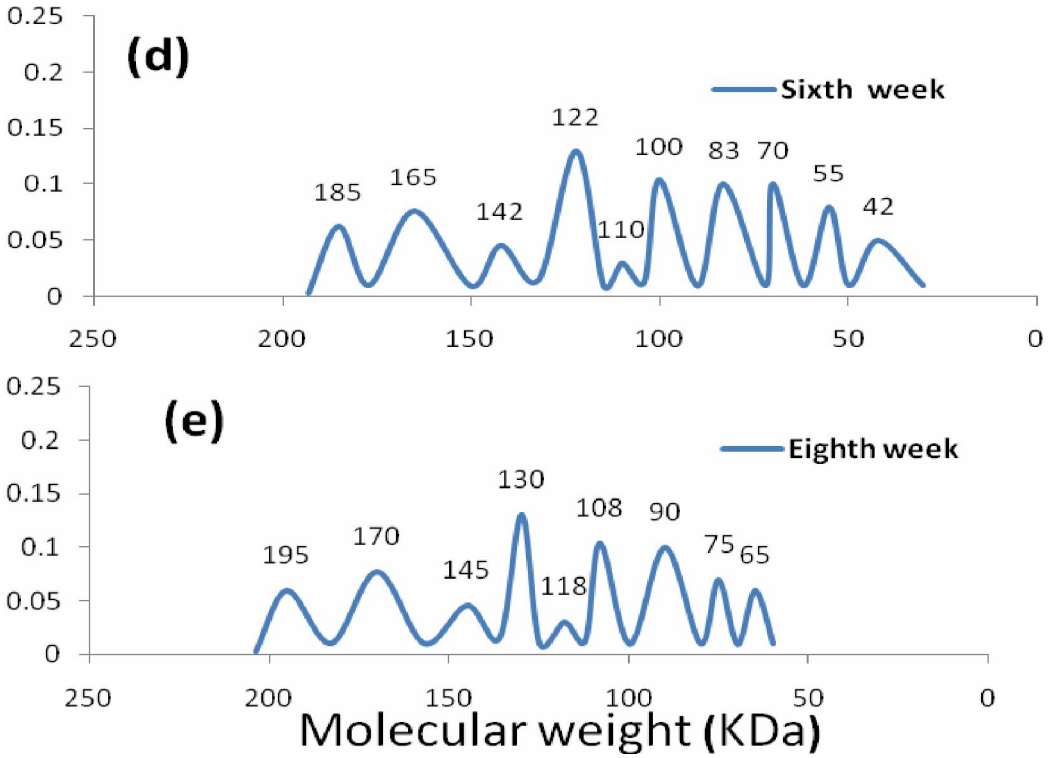


Fig 3. Electrophoretic pattern for (a) control animals, (b), (c),(d) and (e) animals exposed to cigarette smoking for 2,4,6 and 8 weeks, respectively

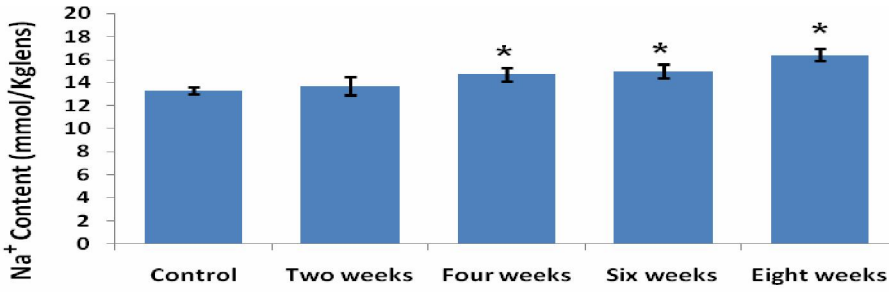


Fig 4. Sodiem concentration in lens homogenate of control and exposed to smoking for different periods.

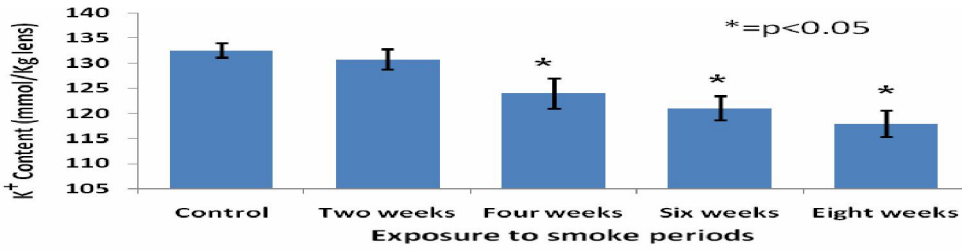


Fig 6. Potassium concentration in lens homogenate of control and groups exposed to smoking for differemt periods.

4. Discussion

Cigarette smoke contains numerous organic and metallic compounds emitted as gases and condensed tar particles, many of them being oxidants and prooxidants, capable of producing reactive oxygen species (ROS). These chemically ROS are known to be present or formed in cigarette smoke which may lead to modification of biological macromolecules [28]. Smoking causes morphological and functional changes to the lens due to its atherosclerotic and thrombotic effects on the ocular capillaries. Smoking also enhances the generation of free radicals and decreases the levels of antioxidants in the blood circulation, aqueous humour, and ocular tissue. Thus, the eyes are more at risk of having free-radical and oxidation attacks in smokers.

Total soluble lens proteins of the rabbits lens and refractive index were quantitatively changed after exposure to cigarette smoke more than 2 weeks. These changes may give an interpretation about the appearance of high molecular weight aggregates which could be attributed to the formation of new protein molecules that differ from the native protein of the control lens. Also these changes are supported by the electrophoretic studies in which shift of all fractions to high molecular weight due to either loss of surface charge or increase in the molecular weight. The present study supports the hypothesis that damage to lens cell membrane affects ion exchange mechanisms with associated formation of cataract [29,30]. It has been demonstrated that in cataract formation there is a significant increase in lens cytosolic calcium and sodium concentrations, together with a decrease in cytosolic potassium levels [31] .The results obtained in our study suggest that increased levels of calcium and sodium and decreased levels of potassium are related to the oxidative damage of cigarette smoking relative to the period of smoking time. Duncan et al.[32] studied the physiological status of human eye lens membranes of different ages in the population. One likely mechanism of cataract formation is oxidation and precipitation of lens proteins. Smoking may increase the oxidative stress in the lens that increases lipid peroxidation and decreases plasma antioxidant levels. The relationship between smoking and cataracts has been well studied [14,16,19]. There is a dose-response relationship between the cumulative amount of smoking and the risk of nuclear cataract developing [21]. Heavy smokers are more at risk than other groups. The cigarette smoking contributes to the formation of cataracts in two ways. First, free radicals present in smoke assault the eye directly, potentially damaging lens proteins and fiber cell membrane in the lens. Second, smoking reduces the body's levels of antioxidants and certain enzymes which may help remove damaged protein from the lens.

In conclusion, Smoking -if continued- may lead to cataract, perpetuate further ocular damage and lead to permanent blindness. Cessation of smoking and avoidance of passive smoking is advised to minimize the harmful effects of smoking on the eyes.

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