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Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.ror.isrj.net

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ORIGINAL ARTICLE

THE CATARACTOGENIC EFFECT OF NICOTINE AND THE POSSIBLE PROTECTIVE ROLE OF CURCUMIN

[1]Sawsan H. Karam, [1]Laila K. Hanafy, [1]Sahar M. Mansour, [2]Olfat A. Hassanin, [3]Amany Abdel Ghaffar and [3]Atef M. Mahmoud

[1]Histology,
[2]Ophthalmology and
[3]Biochemstry Departments, Research Institute of Ophthalmology, Giza, Egypt.

Abstract:

changes in Slit lamp examination revealed The biochemical results showed the oxidative stress of nicotine by decreased activities of antioxidant enzymes like reduced glutathione and increased malondialdehyde. The total soluble lens proteins lowered in this group. There were changes in UV spectra of lens soluble proteins which suggest a conformational at the tertiary structural levels. On the other hand, curcumin markedly improved the previous changes. In conclusion, consumption of curcumin to cigarette smokers can protect against cataractous changes of nicotine.

KEY WORDS:

Nicotine, Curcumin, Cataract.

INTRODUCTION

Cataract development affects over million people worldwide leading to a decrease in visual function and a reduction in overall quality of life. Risk factors for cataract development include diabetes, smoking, ocular inflammation and excessive sunlight exposure (Tirgan *et al*., 2012). Clinical studies have also established a positive correlation between smoking and an increased risk of cataract formation (Delcourt *et al.*, 2000). Nicotine is the main toxic component of tobacco smoking. Although the mechanism by which nicotine promotes cataract development in not known, nicotine causes oxidative stress and generates reactive oxygen species (ROSs) (Newman *et al*., 2002). Curcumin has been shown to have a wide range of biological activities in various systems including its antioxidant action (Kunchandy and Rao, 1990). Curcumin not only exhibits antioxidative and free radical scavenging properties, but also enhances the activities of antioxidant enzymes. So, curcumin could be effective in delaying or preventing the formation of cataract (Ozgen *et al*., 2012).

The current study appears to be the first in the literature studying the antioxidant effect of curcumin on nicotine-induced cataract.

MATERIALS AND METHODS

Twenty five albino rats with an average of 150-200 gm were randomly divided into 3 groups. The rats were housed in stainless-steal cages and fed with standard rat chow and tap water ad libitum.

Group 1: Consisted of 5 rats and served as control.
Group 2: Consisted of 10 rats and injected with nicotine (2.5mg/kg body weight) intraperitoneal for 8 weeks.
Group 3: Consisted of 10 rats and administrated with curcumine (80mglkg body weight) by stomach tube simultaneous with nicotine injection.

OPHTHALMOLOGIC METHODS:

Rats' eyes were examined clinically using photo slit lamp biomicroscope (Huvitz, HS500, Cornea). A 1% tropicamide eye drops was used as a mydriatic. One drop was applied in each eye followed by another drop after 5 minutes and then the eyes were examined after 30 minutes. Lens images were captured. Slit beam and retroillumination photography were taken.
classing of Surtanarayana *et al.* (2003); (I) lens

HISTOLOGICAL METHODS:

After ophthalmological investigation, the rats of all groups were sacrificed after 8 weeks. The eyes were dissected at the corneo-scleral junction and the lenses were fixed in gluteraldehyde 4% for 6 hours .They were cut into two halves at the equator and left overnight in phosphate buffer solution. Finally they were soaked in Mollifex solution (BHD Company) for 3 weeks in order to decalcify the lenses .The specimens were postfixed in osmium tetroxide and dehydrated in ascending grades of ethanol. The specimens were embedded in araldite CY502 and semi-thin 1um thick sections were cut for light microscopy and stained with toluidine blue (Glauret, 1965).

BIOCHEMICAL METHODS:

Preparation of lens homogenate for biochemical analysis:

The lenses from each group of rats were homogenized in ten times their mass of 50 mM phosphate buffer (pH 7.2) and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant obtained was stored at -70°C in aliquots until used for the analysis.

Estimation of total antioxidant capacity reduced glutathione and malondialdehyde (MDA) content:

The GSH content was estimated by the method of Ellman (1959) as modified by Xu *et al.* (1992). The extent of lipid peroxidation (MDA) was determined by the method of Ohkawa *et al*. (1979).

Protein carbonyl measurement:

$E3_{65\ nm}$=21.0 mMcm/mg of protein according to Uchida *et al*. (1998).

Protein measurement:

Lens soluble protein was assayed using the method described by Lowry *et al*. (1951) by using bovine serum albumin as a standard.

Structural alterations:

To understand the mechanism for the structural and conformational changes of lens soluble proteins, the secondary and tertiary structural states of crystalline by UV spectra were monitored.

UV spectroscopy is used to quantify protein and DNA concentrations as well as the ratio of portion to DNA concentration in a solution. A sample of 0.5 ml of the soluble fraction of lens protein was aspired using an aspiration syringe, and diluted to 1 ml with phosphate buffer solution (PH:8.2) in a quartz cuvet to study the UV absorbance of the lens soluble protein. Measurements were taken by using an Uvikon 930 spectrophotometer, KONTRON INSTRUMENTS, Milan, Italy, found at the Research Institute of Ophthalmology, Cairo, Egypt.

Statistical analysis:

The values are expressed as the mean ±SE. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for Windows (version 10.0). A value corresponding to $p<0.05$ was deemed to be statistically significant.

RESULTS

Ophalmological investigation:

Slit lamp examination of the control animals showed clear with light reflection (Fig. 1). Retroillumination photography showed normal red reflex and smooth lens surface. In rats received nicotine, slit lamp examination revealed
Suryanarayana et al. (2003) (Fig. 2). Retroillumination photography showed dim red reflex with the presence of vacuoles (Fig. 3). Administration of curcumin with nicotine greatly ameliorated the observed changes in nicotine group. Slit lamp examination showed clear lens (Fig. 4) and retroillumination photography revealed more or less smooth surface lens.

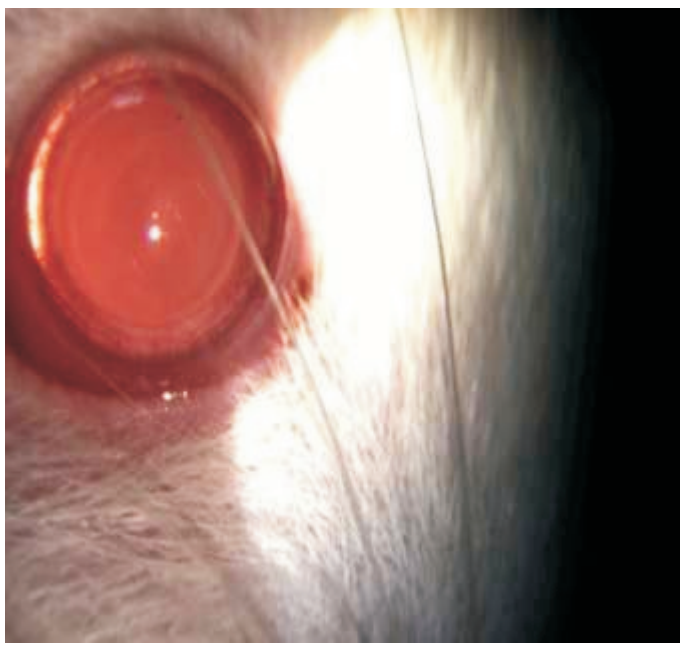

Fig. 1: Slit lamp photography showing clear crystalline lens with normal light reflection in and normal red reflex.

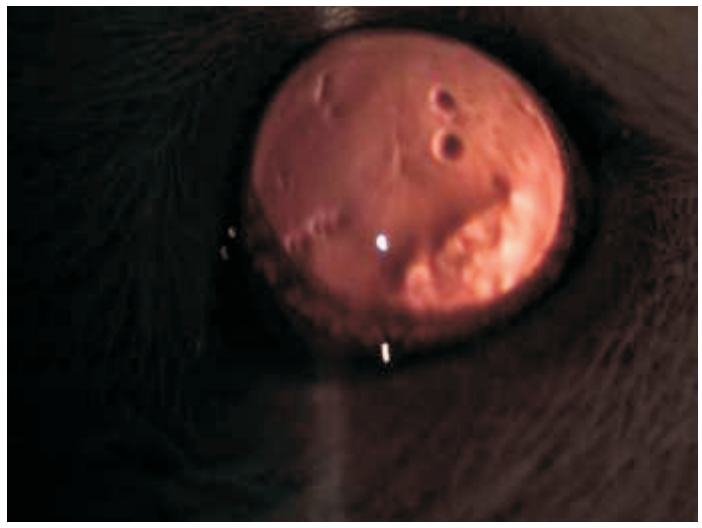

Fig. 2: Slit lamp photography showing: Equatorial vacuoles and granular deposits on the anterior surface of the lens.

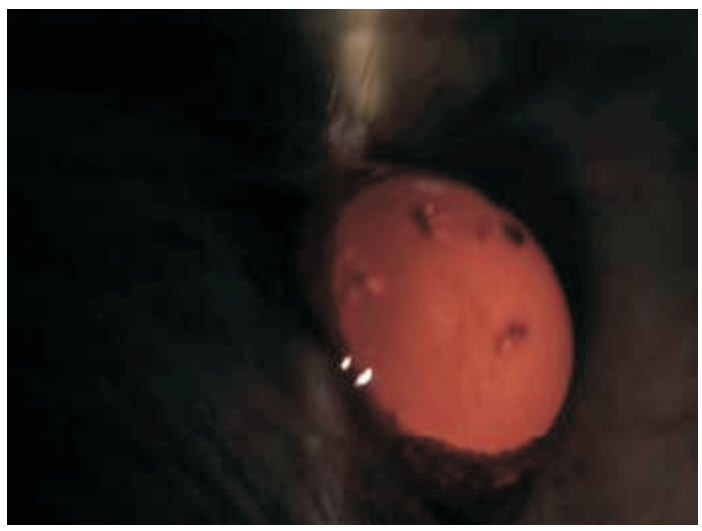

Fig. 3: retroillumination photography with dim red reflex.

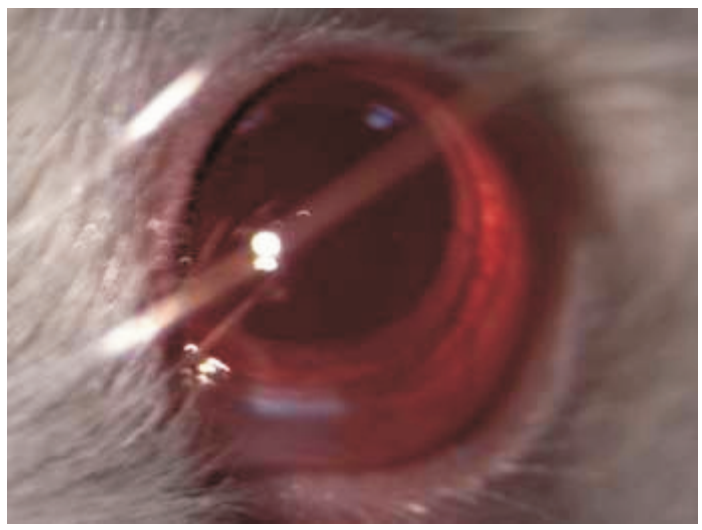

Fig. 4: Slit lamp photography showing clear crystalline lens in.

HISTOLOGICAL RESULTS:

Light microscopic examination of control showed normal crystalline lens with regularly shaped single of anterior cubodial epithelial cells lying under intact lens capsule. Clear and regularly arranged cortical and nuclear lens fibers were noticed in all specimens. Behind the equator of the lens, epithelial cells hand a fusiform and scanty cytoplasm with an oval –shaped nuclei (Fig. 5).

Group 2: This group represented the lens after nicotine injection illustrating thickened and flattening of the epithelial cells and some of these cells appeared necrotic. In addition, the cortical fibers were vacuolated at the anterior region and separated from each other (Fig. 6).

Group 3: The previous changes which observed in group 2 were improved by curcumin administration where light microscopic examination showed lens capsule, epithelium and cortical fibers more or less normal (Fig. 7).

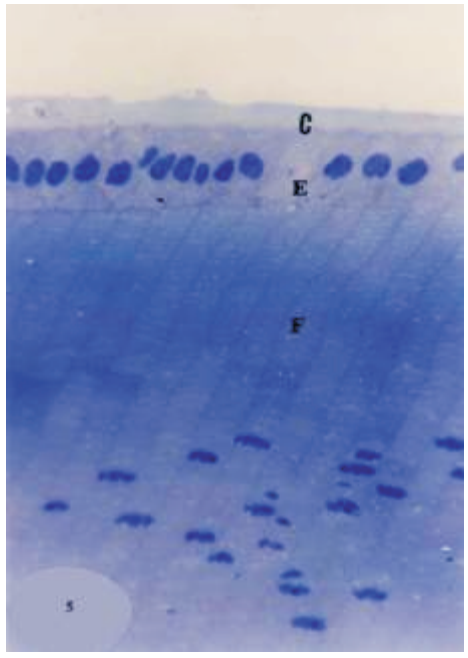

Fig. 5: Light micrograph of control lens showing a lens capsule, lens epithelium (E) and regular cortical fibers (F) (Toluidine blue X=500).

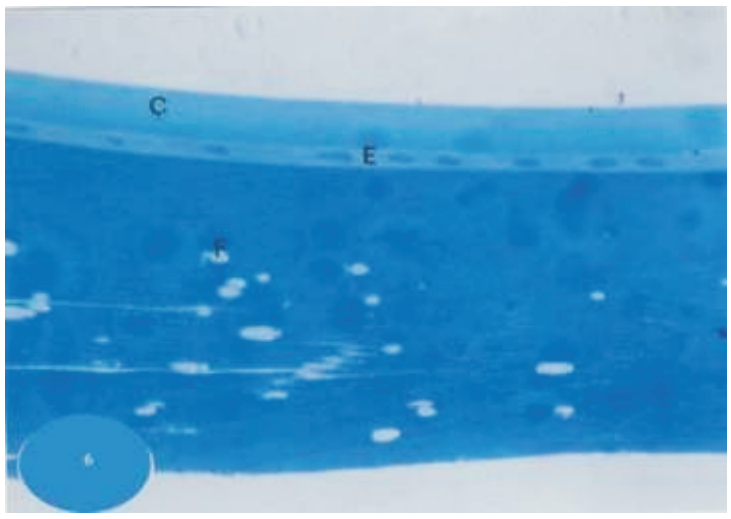

Fig. 6: Light micrograph of rats lens of nicotine group showing thickening of the lens capsule (c)., flattening of the epithelial cells (E) vacuolar changes and fissuring of the fibers(F) (Toluidine blue X=500).

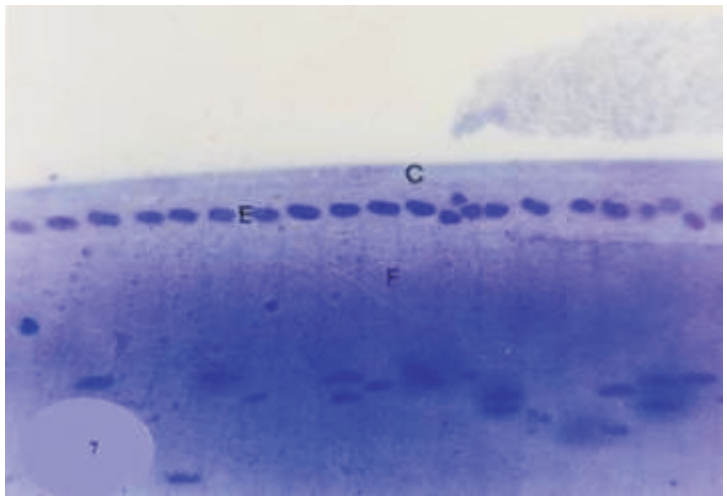

Fig. 7: Light micrograph of rats lens of nicotine group treated with curcumin in showing intact lens capsule(c), epithelial cells(E)and cortical fibers (F) (Toluidine blue X=500).

BIOCHEMICAL RESULTS:

The Status of the total antioxidants and reduced glutathione in the present study were significantly decreased in lenses of rats in nicotine- treated group as compared to that in the controls. A statistically significant increase in the level of lipid peroxide and carbonyl group were observed, in the lenses of rats in nicotine- treated group as compared to those of the control group. Curcumin treatment showed a significant increase in the levels of TAC and GSH and reduction in the levels of MDA and carbonyl group in lenses of Group 3 compared to nicotine -Group (Table 1).

Table 1: Levels of Total antioxidant capacity, reduced glutathione, malondialdehyde and carbonyl group in lenses of rats in different groups.

Groups / Parameters	Control Mean ± SD	Nicotine Mean ± SD	Nicotine + curcumin Mean ± SD
TAC (nmol/g lens)	0.50±0.19	0.25±0.16[a]	0.43±0.09[b]
GSH (Omoles/g lens)	7.2±1.39	5.4±1.64[a]	6.7±1.43[b]
MDA (nmol/g lens)	1.08±0.13	2.14±0.51[a]	1.2±0.09[b]
Carbonyl group (nmol/mg protein)	1.7210.37	3.0 0.67[a]	1.9510.67[b]

[a]: p?0.005 vs. control group, [b]: p?0.005 vs. nicotine group.

Table 2: Levels of total soluble protein in lenses of rat in different groups.

Groups / Parameters	Control Mean ± SD	Nicotine Mean ± SD	Nicotine + curcumin Mean ± SD
Total soluble lens protein (mg/g wet wt).	277±2.95	185±3.2[a]	265±2.95[b]

[a]: p?0.005 vs. control group, [b]: p?0.005 vs. nicotine group

Table (2) shows the level of total soluble protein in lenses of rats in different groups. As shown in

this table, there was a significant drop in total soluble protein in lenses of nicotine group when compared to the control group. There was a significant increase of total soluble protein contents in lenses of curcumin - treated group as compared to nicotine group.

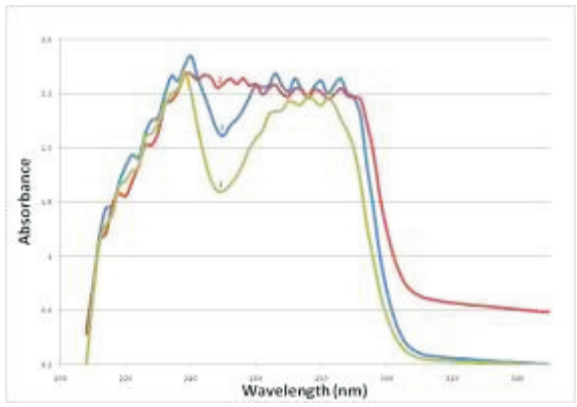

Fig. 8: UV absorption spectrum of lens soluble protein in different groups (Trace 1: control; Trace 2: nicotine –treated group; Trace 3: nicotine + curcumin treated group).

Fig. (8) shows UV absorption spectrum of lens soluble protein in different groups. There were changes in UV spectra of lens soluble proteins in nicotine-treated group when compared to control group. These changes were suggested conformational changes at the tertiary-structural level. These changes were improved in curcumin-treated group as compared to nicotine group.

DISCUSSION

An epidemiological association between cigarette smoking and the development of cataract has been well recognized (Raju *et al.*, 2006). Nicotine had been proposed to be a major environmental risk factor for a variety of human diseases (Kalpana *et al*., 2007). Although the mechanism by which nicotine promotes cataract development is not known, nicotine causes oxidative stress and generates reactive oxygen species (ROSs) including super oxide and hydrogen peroxide (Newman *et al.,* 2002). The biochemical results done in this study, explained the oxidative stress in nicotine injected group by marked lowering activity of antioxidant like reduced glutathione and increased malonaldehyde. Also reduction in level of total soluble protein in lenses of this group and change in UV spectra of soluble lens proteins were noticed.

Nicotine, a potential carcinogen, used in the present study has been reported to be oxidized into its metabolite continine, formaldehyde and (methyllnitros amino)-1-(3pyridyl)-1-butanone and plays a key role in the pathogenesis of tissues (Dicke *et al*., 2005). Mostly, nicotine undergoes hydroxylation induced by CYP2A6 to form continine and related metabolites, including formaldehyde giving rise to (ROS) (Yamazaki *et al*., 1999). Thus excessive generation of (ROS) as a consequence of induction of cytochrome CYP2A6 by nicotine plays a major role in the development of lipid peroxidation (LPO) and formation of lipid peroxidative end products. These findings support the elevation of lipid peroxidation in the lens in nicotine injected rats. Therefore, nicotine induced ROS can interact with lens protein and lipids causing oxidation of lens soluble protein, as indicated by increase the level of lens carbonyl group and decrease the total soluble lens protein leading to protein oxidation and cataract formation. Also, ROS and oxidative stress may inhibit the cellular antioxidant and inhibit the activity of antioxidant enzymes (Dey and Roy, 2010). In the current study, slit lamp examination showed equatorial vacuoles and granular deposits on the anterior surface of the lens. The present ophthalmological findings were also in agreement with previous clinical studies that confirmed the correlation between smoking and an increased risk of cataract formation (Delcourt *et al*., 2000). Accumulation of (ROSs) in the eye lens may contribute to cataractogenesis. This change in lens configuration could be emphasized in the current histological results in nicotine injected group, the changes were in the form of thinning and, flattening of epithelial cells and fissuring and vacuoles of lens fibers. These findings were in agreement with (Avunduk *et al.*, 1999) who found edematous epithelial cells and cortical lens fiber cells swelling and sometimes liquefaction. Recently, Tirgan *et al*. (2012) reported that nicotine induced ROS can interact with the protein and lipids, causing further damage to the already compromised lens fibers, leading to intense cataract. There is an increasing interest in

developing suitable antioxidant nutrient, both synthetic and plant origin, that could be effective in delaying or preventing the formation of cataract (Ozgen *et al*., 2012). Several studies have suggested that intake of antioxidant –rich food may slow the progression of cataract (Kocer and Taysi, 2007; Ertekin *et al.*, 2004 and Hegde *et al.*, 2011). Previous studies reported that dietary intake of antioxidant such as riboflavin, vitamins C, E and carotene has an inhibitory effect on cataract formation (Tirgan *et al.*, 2012). Curcumin, the yellow phenolic pigment, antioxidant properties (Kunchandy and Rao, 1990).

Curcumin had been reported to be a stronger antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group, as it has polyphinolic structure and B. diketone functional groups (Kunchandy and Rao, 1990). There was decrease in total soluble protein content in lenses of second group, in this study, compared to control group (1st gr). This could be explained by oxidation of proteins and in solubilization. Also, the changes in near UV spectra of lens crystalline at the second group could be suggested conformational changes at the tertiary structural level due to nicotine injection. On the other hand, curcumin administration not only prevented the decrease in total soluble proteins but also prevented cross – linking/aggregation and distribution of soluble proteins. Again it improved changes in UV spectra of lens soluble proteins. In the present study, it was noticed that, the antioxidant effect of curcumin was attributed to the delayed progression of nicotine-induced cataract in rats, this in agreement with Manikandan *et al.* (2011) who studied the effect of curcumin on cataract –induced by selenium. Thus curcumin exerts its protective effect against nicotine induced toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant to defense system (Kalpana *et al.*, 2004). At histological and opthlomolgical level, administration of curcumin in the third group greatly ameliorated the changes in the lens (that noticed in nicotine induced group (where the lens appeared more or less normal). Some experimental studies done by (Jain *et al*., 2006) suggested that curcumin can suppress cataract development promote wound healing and lower blood lipids and glucose levels. This was in accordance with studies done by (Manikandan *et al*., 2010) who supported the possibility that natural consumption of curcumin in food can help prevention of onset of selenite cataract. Awashi *et al* (1996) showed that curcumin protects against 4-hydroy-2-trans-nonenal-induced cataract may be an effective protective agent against cataractogenesis induced by lipid peroxidation, and this protective effect may be mediated through the induction of glutathione s-transferase isozyme. They demonstrated that curcumin induced the glutathione linked detoxification pathway activates a protective mechanism associated with GSH and provides it as a free radical scavenger. Suranayans *et al.* (2003) investigated the effects of curcumin in the galactose-induced cataract model with two levels of curcumin, 0.002 and 0.01% in the diet. Although curcumin delayed the onset of cataract at both levels, maturation was delayed by 0.002 curcumin but not by 0.01%. The maturation was faster with 0.01% curcumin. Biochemical analyses demonstrated that at the 0.002% level appeared top Later,Suryanayana et al. (2005)its source turmericIn this study, both curcumin, and turmeric did not prevent strptozotocin-induced hyperglycemia, but delayed the progression and maturation of cataract. To our knowledge, this study may provide pioneer evidence of the role of curcumin in the pathogenesis of nicotine induced cataract model. Its protective activity noticed can possibly be mediated through its antioxidant potential.

CONCLUSION

The curcumin can be considered as an effective cytoprotective compound against oxidative stress-induced cataract.

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