

# UV-B Exposure Increases the Activity of Indoleamine 2, 3-Dioxygenase (Ido) and Alters the Levels of Tryptophan Metabolites in Indian Ground Squirrel (*Funambulus Palmarum*) Lens

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## Abstract

The ocular lens of the ground squirrel is an excellent model for studies of eye light interactions that may apply to the human system. UV-B radiation induced damage to the eye lens is believed to be mediated through ROS. Antioxidant systems, both enzymatic and non-enzymatic aid in preventing the damage to the lens. The activity of indoleamine 2,3-dioxygenase (IDO), a first rate limiting enzyme of tryptophan catabolism, has been studied in the lenses of Indian ground squirrel exposed to UV-B radiation. Further, antioxidants and oxidative stress markers have also been investigated to assess the oxidative status. Our investigation revealed an increased indoleamine 2,3-dioxygenase activity and tryptophan metabolites suggesting their protective role in UV stress. The study indicates that UV-B irradiation for 12 h and 24 h (300 nm; 100  $\mu\text{W}/\text{cm}^2$ ) of lens samples led to a marginal decrease in GSH and increase in carbonyls and MDA levels reflecting the role of indoleamine 2,3-dioxygenase in protecting the lens tissue.

**Keywords:** Indoleamine 2, 3-dioxygenase; UV-B radiation; kynurenine; 3-hydroxy kynurenine; Reactive oxygen species; Glutathione; Superoxide dismutase; Malondialdehyde

## Introduction

UV-B radiation (280–320 nm) is absorbed predominantly by the outer structures of the eye, particularly the cornea, with relatively little radiation reaching the lens and retina [1,2]. Under natural conditions these rays are filtered partially by the ozone layer, but some still reach the earth's surface. Near-ultraviolet (UV) radiation represents around 25% of the solar spectrum and approximately 10 mW/cm<sup>2</sup> (300–400 nm) can impinge upon the eye from direct sunlight. Light in the near-UV range at this irradiance level is capable of photochemically damaging mammalian cells [3]. The eye thus does receive UV-B radiation, which is greatly attenuated by the time it gets past the cornea and aqueous humor before reaching the lens [4]. Yet even this amount of UV-B, incident for extended periods of time, can cause chemical and biochemical damage to the constituents of the lens causing cataract [5–7]. Ultraviolet-B (UV-B) and ultraviolet-C (UV-C) irradiation induce the oxidation of tryptophan [8]. The oxidation of tryptophan catalyzed by indoleamine 2, 3-dioxygenase (E.C 1.13.11.52) results in oxidative end products within the kynurenine pathway [9]. They are also produced, albeit, to a lesser extent, by enzyme-independent UV mediated oxidation of tryptophan [10]. Earlier observations from our laboratory with WNIN rats showed changes in indoleamine 2, 3-dioxygenase expression during cataractogenesis [11]. Tryptophan oxidation is enhanced when IDO is activated by interferon- $\gamma$  or by superoxide anion. A series of enzymatic reactions convert kynurenine to anthranilic acid (AA), 3-hydroxykynurenine (3HK) and 3-hydroxyanthranilic acid (3HAA) [12,13]. Subsequently, 3HK is glycosylated to form 3-hydroxykynurenine O-glucoside (3HKG). Van Heyningen [12] first reported the identification of the lens pigment in the grey squirrel (*Sciurus carolinensis leucotis*) as N-acetyl-3-hydroxykynurenine and this was confirmed at a later date using more advanced techniques [3]. Major UV filters (N-acetyl 3-hydroxykynurenine and N-acetyl-kynurenine) in the lens of the thirteen lined ground squirrel (*S. tridecemlineatus*) have been identified and quantified [7]. The lenses of both the grey squirrel and ground squirrel contain N-acetyl-3-hydroxykynurenine as the major

UV filter; however, N-acetyl-kynurenine appears to be unique to the ground squirrel. For the present investigation we have chosen Indian 3-lined ground squirrel which is found naturally in southern India. These kynurenine metabolites may also act as UV filters and reduce chromatic aberration to sharpen the retinal image. Further, they may also minimize or reduce photo-oxidative damage that may result from prolonged exposure of the lens and retina to the high energy wavelengths of light.

The lens has an efficient UV protection system that acts to ameliorate the effects of photo-oxidation. In the present study, we have measured the activity of indoleamine 2,3-dioxygenase and quantified the tryptophan metabolites in Indian ground squirrel (*Funambulus palmarum*) lens samples which were subjected to UV-B irradiation. Our experimental investigation indicated that exposure to UV-B light leads to an increase in indoleamine 2,3-dioxygenase activity, resulting in an increase in tryptophan metabolites (kynurenine and 3-hydroxykynurenine), which act as UV filters. Further, irradiation led to an increased lipid peroxidation and protein carbonyls and a decrease in GSH suggesting an oxidative insult.

## Materials and Methods

### Materials

Acetonitrile, methanol, trifluoroacetic acid (TFA), ammonium

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acetate, catalase, kynurenine, 3-hydroxykynurenine, L-tryptophan, 2-thiobarbituric acid (TBA), 1,1,3,3-tetraethoxy propane (TEP), ortho-phthalaldehyde (OPT), reduced glutathione (GSH), 2,4-dinitrophenylhydrazine, diethylenetriaminepentaacetic acid (DETAPA), pyrogallol and bovine serum albumin (BSA) were obtained from Sigma Chem.co. All other chemicals and reagents used were of analytical grade.

### Animals

Adult three lined ground squirrels (*Funambulus palmarum*), weighing approximately 150 g, were obtained and animal care protocols were in accordance with and approved by Institutional Animal Ethics Committee (383/01/CPCSEA). Animals were sacrificed by Co<sub>2</sub> asphyxiation and lenses were dissected by posterior approach.

### Irradiation

Intact lenses were suspended in RPMI- 1640 medium, containing 10 mM HEPES buffer pH 7.2 and 0.01% sodium azide (1 ml/lens) in the quartz cuvette by employing a perplex sheet. Lenses were irradiated at 300 nm at room temperature (22°C) for different time periods (up to 24 h), placed in the cuvette in such a way that the anterior portion faces the light source. Lenses kept in the dark under similar conditions served as control. The source of UV light was a 150 W xenon high pressure lamp attached to a double monochromator [14]. The distance between the sample holder (cuvette) and exit slit of the monochromator was 6 cm. The intensity of the incident light falling on the sample was found to be 100  $\mu$ W cm, as measured with an ultraviolet meter (Blak-Ray, UVP Inc, Model J-225). At the end of the time period, the lenses of 12 h and 24 h corresponding to control and irradiated samples were taken, homogenized and centrifuged at 14,000 $\times$ g for 20 min at 4°C for indoleamine 2,3-dioxygenase, SOD and protein carbonyls. Total lens homogenate (10%) was used to determine the levels of MDA, GSH and tryptophan metabolites.

### Preparation of the lens homogenate

Four to six lenses have been pooled, weighed and 10% (w/v) homogenate was prepared in 50 mM sodium phosphate buffer pH 7.4 for all assays. Lenses used for the study are categorized as follows: C12 h (Control 12 hours), C24 h (Control 24 hours), E12 h (Experimental 12 hours), E24 h (Experimental 24 hours).

### Protein estimation

Protein (total, soluble and insoluble) was estimated according to Lowry [14] using BSA as reference standard. For total protein estimation the lens homogenate (10%) was prepared in 50 mM sodium phosphate buffer pH 7.4. Total homogenate was centrifuged at 14,000 $\times$ g for 20 min at 4°C. The buffer soluble supernatant was used for estimation of soluble protein and the residue was dissolved in TNEN buffer pH 8.0 [Tris buffer 25 mM containing NaCl (0.1 M), EDTA (0.5 mM), and sodium azide (0.01%)] and used for the estimation of insoluble protein.

### Kynurenine and 3-hydroxykynurenine standard curve by HPLC

The standard medium (200  $\mu$ l) contained 0.5 M potassium phosphate buffer (20  $\mu$ l, pH 6.5) at a final concentration of 50 mM, 0.2 M ascorbic acid (20  $\mu$ l, neutralized with 1M NaOH solution) at a final concentration of 20 mM, 0.5 mM methylene blue (4  $\mu$ l) at a final concentration of 10  $\mu$ M, 5 mg/ml catalase (4  $\mu$ l) at a final concentration of 100  $\mu$ g/ml, MilliQ water (132  $\mu$ l) and various concentrations of kynurenine and 3-hydroxy-kynurenine (20  $\mu$ l). After addition of 40  $\mu$ l

of 30% (v/v) TCA, the medium was centrifuged (11,500 rpm, 4°C, 15 min). The amount of kynurenine and 3-hydroxykynurenine present in the supernatant was then measured by reversed phase HPLC using the Phenomenex column (C18, 250 $\times$ 4.60 mm, 4 micron), LC-20AT pump (Shimadzu), gradient controller (Shimadzu Chromatography Corp., Kyoto, Japan) and a Rheodyne injection valve with a 20  $\mu$ l fixed sample loop (Model 7725i, Rheodyne, Cotati, CA, USA). A binary solvent system was used as the mobile phase with a flow rate of 0.8ml/min. Ammonium acetate (10 mM) as solvent A and 10% methanol in 10 mM ammonium acetate pH 6.7 as solvent B. The percentage of solvent B in the gradient was 0% (10 min), 0-100% (10 min) and 100-0% (15 min) and the eluant was monitored at 360 nm (UV-VIS detector).

### Assay of Indoleamine 2, 3 -dioxygenase activity

Lens homogenate was centrifuged at 14,000 $\times$ g, 4°C, for 15 min and supernatant was used for the indoleamine 2,3-dioxygenase assay. The assay was carried out according to the method of Mailankot with minor modifications. The standard assay medium (200  $\mu$ l) contained 0.5 M potassium phosphate buffer (20  $\mu$ l, pH 6.5) at a final concentration of 50 mM, 0.2 M ascorbic acid (20  $\mu$ l neutralized with 1M NaOH solution) at a final concentration of 20 mM, 0.5 mM methylene blue (4  $\mu$ l) at a final concentration of 10  $\mu$ M, 5 mg/ml catalase (4  $\mu$ l) at a final concentration of 100 g/ml, 2 mM L-tryptophan (20  $\mu$ l) at a final concentration of 200 mM, MilliQ water (112  $\mu$ l) and enzyme source (total soluble protein 1 mg equivalent protein). The reaction mixture was incubated at 37°C for 1 h and 40  $\mu$ l of 30% (v/v) TCA was added to the assay mixture. The mixture was incubated at 60°C for 15 min to hydrolyze N-formyl kynurenine to kynurenine. It was then centrifuged at 14,000 $\times$ g, 4°C, 15 min and the amount of kynurenine and 3-hydroxykynurenine formed were quantified by method outlined above. Indoleamine 2,3-dioxygenase activity was expressed as nanomoles of both kynurenine and 3-hydroxykynurenine formed per milligram protein per minute.

### Quantification of tryptophan metabolites by HPLC

To 100  $\mu$ l of lens homogenate (control and UV irradiated samples) 0.3 ml of 100% (v/v) ethanol was added and placed at room temperature for 1 h and then centrifuged (14,000 $\times$ g, 15 min). The supernatant was removed, kept at -20°C, whereas the pellet was re extracted twice with 0.3 ml of 80% ethanol. The supernatant was observed to be pale yellow in colour. The supernatants were pooled and dried in a speed-vac concentrator (Eppendorf AG · 22331 Hamburg, Germany). Kynurenines were quantified by the method described by Hains et al. [6]. Metabolites have been estimated by reverse phase HPLC. The column was equilibrated in 0.1% (v/v) TFA, at a flow rate of 0.7 ml/min. Samples were resuspended in 100  $\mu$ l of 0.1% (v/v) TFA, centrifuged for 10 min at 12,000 $\times$ g and loaded on to the column. UV filters (N-acetyl-3-hydroxykynurenine, N-acetyl-kynurenine, kynurenine and 3-hydroxykynurenine) were eluted with the following gradient; 0-5 min 0% buffer B (0.08% (v/v) TFA, 80% (v/v) acetonitrile, water), 5-50 min 0-50% B, 50-60 min 50-0% B. The eluent was monitored using UV detector at 360 nm. In a similar way, reference kynurenine and 3-hydroxykynurenine were used to construct standard curves.

### Synthesis of $\alpha$ - N-acetyl-kynurenine and $\alpha$ -N-acetyl-3-hydroxykynurenine

Kynurenine (2 mM in 800 mM sodium phosphate buffer pH 7.5) was taken in a reaction chamber at 37°C and to this 8 mM acetic anhydride was added drop wise. The reaction was allowed for 1 hr with continuous and gentle magnetic stirring under subdued light

conditions. The final reaction mixture contained 1 mM kynurenine in 400 mM sodium phosphate buffer pH 7.5 with 4 mM acetic anhydride. The products were separated by HPLC as described above and the major components were analyzed by mass spectrometry.

### Liquid chromatography mass spectrometry

Peaks were analyzed in positive ion mode using Agilent LC-MSD-Trap- SL with ESI. MS settings were as follows; capillary Exit 111.5 Volt, skimmer 40.0 Volt, Dry Temp (set) 355°C, Nebulizer (set) 40.00 psi, Dry Gas (set) 8.00 l/min, HV Capillary 3500 V, HV End Plate Offset -500 V and mass range 50-500 m/z.

### Oxidative stress markers and antioxidants

Protein carbonyl content of soluble protein was measured spectrophotometrically using the 2,4-dinitrophenyl-hydrazine [15,16]. Malondialdehyde (MDA) production was determined by thiobarbituric acid reactive substances (TBARS) [17]. Reduced glutathione was estimated by the spectrofluorimetric method reported by [18]. Total superoxide dismutase (SOD, E.C. 1.15.1.2) activity was assayed by [19] monitoring the rate of inhibition of pyrogallol reduction. One unit of SOD represents the amount of enzyme required for 50% inhibition of pyrogallol reduction/min.

### Statistical Analysis

The differences between the control and UV irradiated samples were analyzed using simple t- test and the differences were considered significant if  $p < 0.05$ . All data is expressed as mean  $\pm$  SD.

### Results

The present study is an attempt to understand the role of indoleamine 2,3-dioxygenase and antioxidants under UV-B stress. Indoleamine 2,3-dioxygenase enzyme was assayed by the method described [9] with minor changes. Enzyme activity was expressed as the quantity of both kynurenine and 3-hydroxykynurenine (Table 1) formed and standard graphs were plotted. The retention times for 3-hydroxykynurenine and kynurenine are 11 and 21 min respectively. Significant ( $p < 0.05$ ) increase in 3-hydroxykynurenine and marginal increase in kynurenine (Figure 1) was observed when lens homogenate was exposed to UV-B irradiation for 12 h in relation to control and 24 h UV exposure (Figure 2) led to a significant ( $p < 0.05$ ) increase in both kynurenine and 3-hydroxykynurenine.

### Content of kynurenes in control and UV-B irradiated groups

A typical HPLC profile of the UV filters in control and experimental (12 h and 24 h) of the ground squirrel lens homogenate is depicted in (Figure 3). All the four metabolites viz., Kynurenine, 3-hydroxykynurenine, N-acetyl-3-hydroxykynurenine and N-acetyl-kynurenine in squirrel lens were quantified (Table 2) by HPLC. Mass spectrometry was carried out to confirm their conversion and mass values were in agreement with those reported in literature [7] several major peaks have been observed that absorbed at 360 nm. Our data confirms the conversion of 3-hydroxykynurenine to N-acetyl-3-hydroxykynurenine and a clear cut wavelength shift was also observed. As a result of conversion, the maximum absorption of N-acetyl-3-hydroxykynurenine was recorded at 300 nm. A marginal increase in both 3-hydroxykynurenine and kynurenine for 12 h exposure and significant ( $p < 0.05$ ) increase in kynurenine for 24 h exposure was observed. However, the levels of N-acetyl-3-hydroxykynurenine

and N-acetyl-kynurenine were found to be unaltered in both 12 h and 24 h exposures. The  $\alpha$ -amino groups of both Kynurenine and 3-hydroxykynurenine can act as acceptors for the acetyl group suggesting an efficient lenticular mechanism for acetylation of the  $\alpha$ -amino group of these metabolites. Acetylation of the  $\alpha$ -amino group will prevent the deamination of the tryptophan derived UV filters and this perhaps represents an efficient mechanism for stabilizing UV filters in the squirrel lens (Figure 4).

### Protein content

There was no net change in the soluble and insoluble protein content (Table 3) in both control and UV-B (12 h and 24 h) exposed samples.

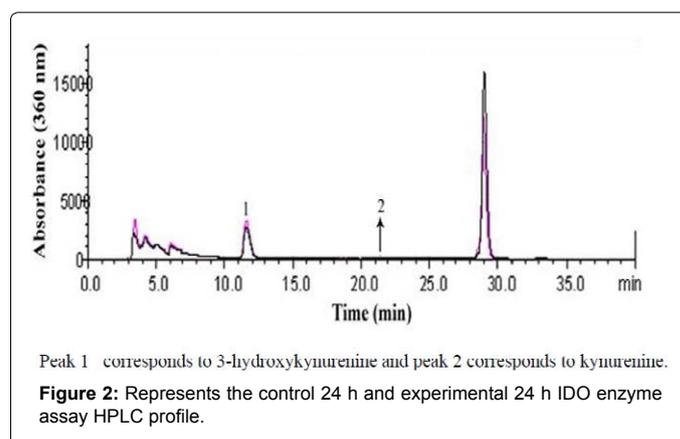
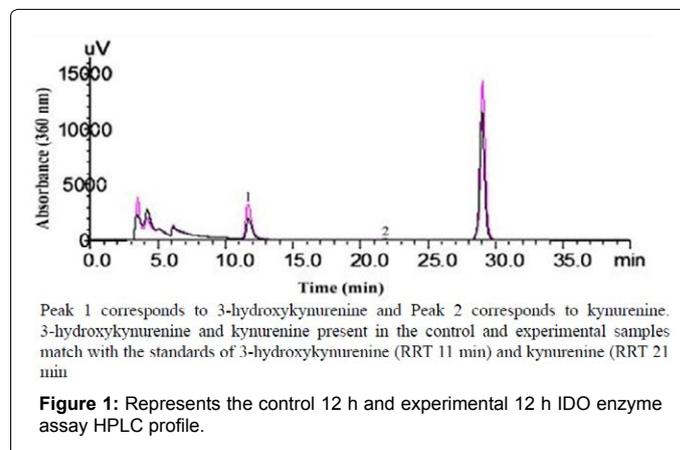
### Antioxidants

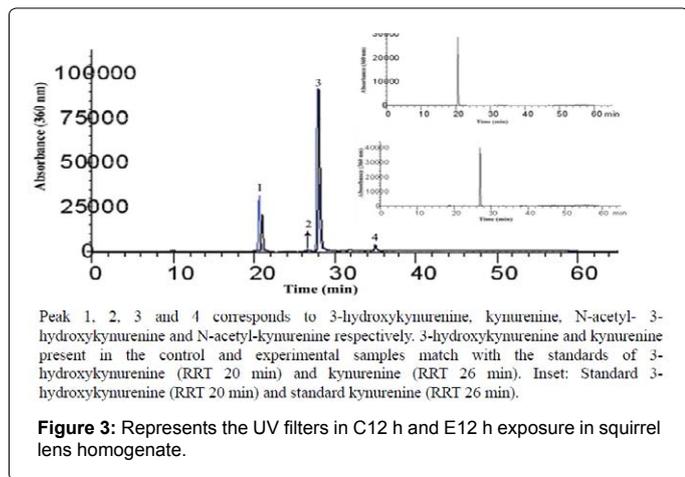
In oxidative stress, the parameters commonly considered are glutathione (GSH), superoxide dismutase (SOD), malondialdehyde

Groups	KYN (1mg protein)	3-OHKYN (1mg protein)
C 12 h	0.05 $\pm$ 0.01	2.24 $\pm$ 0.03
E 12 h	0.09 $\pm$ 0.01	3.94 $\pm$ 0.08*
C 24 h	0.03 $\pm$ 0.00	3.02 $\pm$ 0.04
E 24 h	0.05 $\pm$ 0.00*	3.80 $\pm$ 0.39*

IDO measured using nanomoles of kynurenine and 3-hydroxykynurenine formed per milligram protein per minute. Values are mean  $\pm$  SD (n=3). The \* indicates the significance  $p < 0.05$ . [C (Control); E (Experimental)]

**Table 1:** Effect of UV-B irradiation on IDO activity in squirrel lens homogenate.

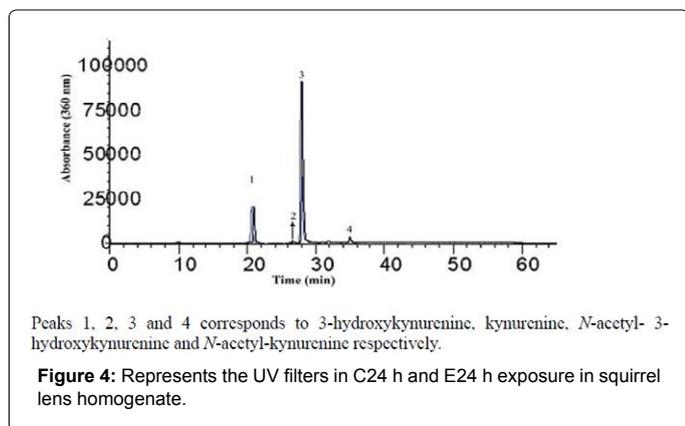




Groups	3-OHKYN(nM)	KYN(μM)	NAK(nM)	NA3OHK(nM)
C 12 h	7.44 ± 0.68	0.14 ± 0.28	1.55 ± 0.37	1.04 ± 0.08
E 12 h	12.96 ± 1.80	0.26 ± 0.04	1.30 ± 0.11	0.90 ± 0.06
C 24 h	7.08 ± 1.84	0.05 ± 0.00	1.13 ± 0.17	0.73 ± 0.10
E 24 h	0.33 ± 0.03*	0.33 ± 0.03*	0.79 ± 0.02	0.56 ± 0.01

Showing the quantitative HPLC profile of 12 h and 24 h UV-B exposure in squirrel lens homogenate. Values are mean ±SD (n=3). The \* indicates the significance p<0.05. [C (Control); E (Experimental)].

Table 2: Quantitative profile of tryptophan metabolites.



Groups	Soluble protein (mg/100 mg lens weight)	Insoluble protein (mg/100 mg lens weight)
C 12 h	31.85 ± 2.38	3.66 ± 1.74
E 12 h	37.83 ± 0.84	4.28 ± 0.48
C 24 h	31.72 ± 8.65	2.94 ± 0.05
E 24 h	36.38 ± 14.08	3.84 ± 0.08

Represents the soluble and insoluble protein content of squirrel lens homogenate. Mean ± SD (n=3). [C (Control); E (Experimental)].

Table 3: Soluble and insoluble protein content of control and UV-B exposed squirrel lens samples.

(MDA) and protein carbonyls. GSH had marginally decreased in 12 h UV exposure whereas, a significant (p<0.05) decrease in 24 h exposure was noticed (Table 3). SOD activity marginally increased when the lens sample was exposed to UV light for 12 h. However, exposure to UV-B for a longer time (24 h) resulted in marginal inhibition of SOD activity (Table 3) in relation to the 12 h exposure and the activity had reverted to the control level.

## Oxidative stress markers TBARS and protein carbonyls content

A marginal rise in MDA levels was observed upon 12 h exposure and 24 h UV exposure showed significant (p<0.05) increase (Table 4). Protein carbonyls (12 h and 24 h UV exposures) were significantly (p<0.05) higher (Table 4) in irradiated groups than their corresponding control groups.

## Discussion

Risk of cortical cataract mediated by UV light was reported by Heck [24]. Near-UV radiation is known to cause oxidative damage to the lens by a variety of free radical mediated reactions. In the present investigation, characteristic alterations in indoleamine 2,3-dioxygenase activity were observed in 12 h UV-B exposure, which points to the fact that shorter duration of UV-B was found to induce the changes in enzyme activity and other concomitant changes reported herein.

The present investigation was an attempt to study direct effects of UV-B radiation in squirrel lenses *in vitro* on indoleamine 2,3-dioxygenase which is involved in many biological processes. Indoleamine 2,3-dioxygenase activity has been expressed by taking into consideration of the levels of both 3-hydroxykynurenine and kynurenine and this is in agreement. In the present study a significant (p<0.05) increase in indoleamine 2,3-dioxygenase activity has been observed in 12 h and 24 h exposure of UV-B radiation. Under normal physiological conditions indoleamine 2,3-dioxygenase activity is reported to be low [20,21]. However, the indoleamine 2,3-dioxygenase activity of the 24h exposure was higher than that of its corresponding control lens sample. Eventhough the 24 h UV exposure control values decreased, the fold difference remains the same for both 12 h and 24 h exposures, indicating an increased indoleamine 2,3-dioxygenase activity under UV-B exposure. The rise in indoleamine 2,3-dioxygenase has been in agreement with the previous studies [22] which suggest its protective influence on corneal endothelial cells mediated by UV by reducing rate of apoptosis and lipid peroxidation. It has been previously reported that indoleamine 2,3-dioxygenase is an antioxidant enzyme since it happens to be a direct scavenger of superoxide radicals [23]. The native state of lens sample continued to be intact and this may be attributed to an increase in indoleamine 2,3-dioxygenase activity and hence not much oxidative damage has been observed during 12 h exposure. The increase in levels of kynurenines may be linked to an increase in indoleamine 2, 3 dioxygenase activity which cleaves the pyrrole ring of tryptophan and forms formyl kynurenine using free oxy radicals. They are also produced, albeit, to a lesser extent, by enzyme independent UV mediated oxidation of tryptophan. These kynurenine metabolites may also act as UV filters and reduce chromatic aberration to sharpen the retinal image. Further, they may also minimize or reduce photo-oxidative damage that may result from prolonged exposure of

Groups	LPO (nmol/g <sup>-1</sup> lens)	GSH (μmol/100mg Lens weight)	SOD (IU)	Protein carbonyl content (nmol/1mg protein)
C 12 h	3.70 ± 0.42	0.17 ± 0.01	62.17 ± 15.73	3.84 ± 0.29
E 12 h	4.50 ± 0.01	0.13 ± 0.02	74.10 ± 12.59	6.14 ± 0.32*
C 24 h	4.25 ± 0.35	0.08 ± 0.01	61.06 ± 14.05	3.77 ± 0.18
E 24 h	9.75 ± 0.03*	0.02 ± 0.01*	50.17 ± 9.27	5.54 ± 0.13*

Effect of UV-B on lipid peroxidation, glutathione concentration, superoxide dismutase and protein carbonyl content in ground squirrel. The data presented here is mean ±SD (n = 3). The \* indicates the significance p<0.05. [C (Control); E (Experimental)].

Table 4: Effect of UV-B irradiation on various biochemical parameters.

the lens and retina to the high energy wavelengths of light.

John had reviewed the activity of IDO in relation to tryptophan metabolism [23]. This review clearly indicates that >95% of tryptophan is channelized through kynurenine pathway. Upon UV exposure tryptophan metabolites such as kynurenine, 3-hydroxy kynurenine are also formed and, it can be speculated that based on the previous studies, under UV exposure it is quite logical to infer that almost all the tryptophan would be oxidized through the kynurenine pathway as well as the UV derived tryptophan derivatives due to enhanced IDO activity whose presence was reported by Heck [24]. This could perhaps be the reason that no detectable tryptophan was recorded in the present investigation after UV exposure.

Our observations suggest that N-acetyl-3-hydroxykynurenine is the major UV filter in the ground squirrel lens and besides this, 3-hydroxykynurenine and kynurenine have also been found in reduced amounts. N-acetyl-kynurenine is unique to ground squirrel but its concentration is far less when compared to N-acetyl-3-hydroxykynurenine. Our observations revealed a significant ( $p < 0.05$ ) elevation in kynurenine for 24 h exposure and other metabolites remain unchanged for both 12 h and 24 h exposures indicating a protective mechanism. Besides this, 3-hydroxykynurenine was also found to show a marginal increase for 12 h exposure (Figure 5).

The role of glutathione (GSH), as an essential and primary lenticular antioxidant is well known. Exposure for 12 h showed a marginal fall in GSH content and exposure for 24 h clearly demonstrated a significant ( $p < 0.05$ ) depletion of GSH [25,26]. This perhaps suggests the generation of oxyradicals in the lens homogenate mediated by UV-B stress and the impaired lens antioxidant status may be correlated to increase in ROS, as evidenced by decrease in GSH content.

SOD plays an important role as an antioxidant protein by reducing the level of intracellular superoxide radical induced by extracellular stimuli such as UV radiation. In the present study, the change in SOD activity suggests the release of superoxide radicals mediated through UV-B exposure. SOD activity marginally increased when the lens

sample was exposed to UV for 12 h, suggesting an activation of SOD by scavenging superoxide radical to protect the lens from UV stress. It has been reported that an increase in SOD activity is probably a response to increased ROS generation [26]. However, exposure to UV light for longer duration (24 h) resulted in marginal inhibition of SOD activity in comparison with the 12 h exposure, and the activity reverted to the control level. This is in accordance with the earlier findings which suggest that the longer duration of UV exposure suppresses the activity of SOD.

Protein carbonyl content was found to be significantly elevated in lens homogenate exposed to UV-B for 12 h and 24 h exposures in relation to control suggesting UV-B mediated oxidative stress. Protein carbonyls tend to get accumulated on the side chains of proteins as a result of oxidative stress [26]. MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, has been used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress. A marginal rise in lipid peroxidation has been observed in 12 h exposure. This marginal rise of MDA may be due to the enhanced activities of antioxidant enzymes which can scavenge excess lipid peroxidation. And, a significant ( $p < 0.05$ ) increase was noticed upon 24 h exposure in relation to control. These results are in agreement with the observation of [14] and Reddy [13] who reported variations in lipid peroxidation and GSH in rat lens subjected to UV-B radiation.

Our observations have revealed that squirrel lens homogenate exposed to UV-B light has led to an increase in IDO activity and tryptophan metabolites. Among UV radiation blocking compounds, certain aromatic amino acids of proteins, such as tryptophan and its metabolites including kynurenine and 3-hydroxykynurenine play a key role as UV filters in lenticular tissue by absorbing UV radiation between 300 to 400 nm and thus protecting both the lens and the retina from photo-oxidative damage. Keeping all this in view and based on previous findings we hypothesize that indoleamine 2,3-dioxygenase renders distinct a protective influence against UV damage in squirrel lens.

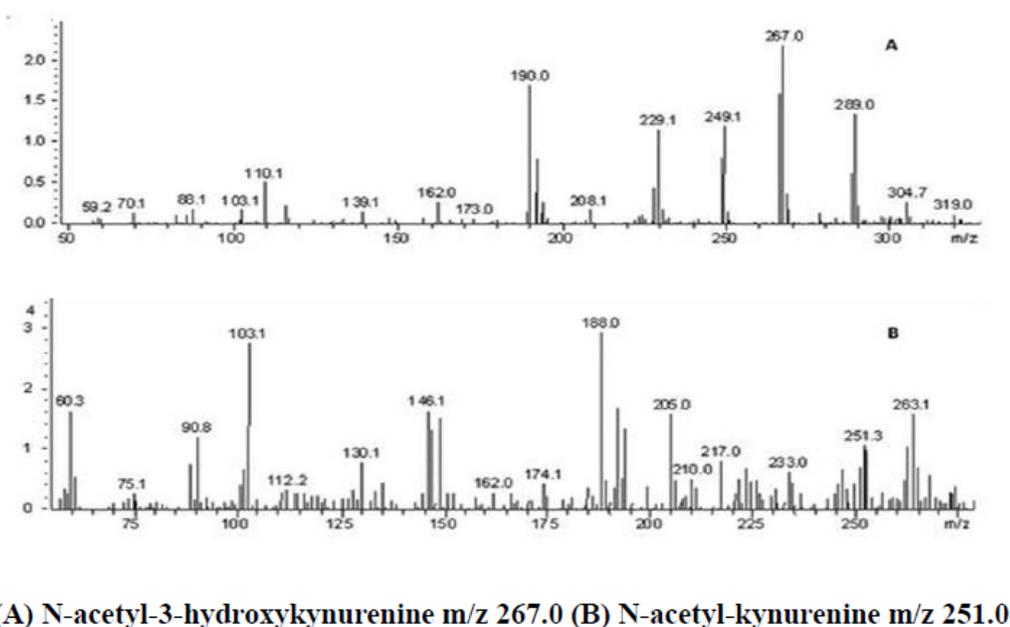


Figure 5: Shows ESI-MS of tryptophan metabolites from the ground squirrel lens.

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