

# Effect of Nicotine on Retinal Degeneration in Laboratory Mice – A Route-dependent Study

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**Abstract** Nicotine, the major addictive and psychoactive alkaloid present in tobacco products, has been used by a large population. Although the role of tobacco smoke on various disorders in different organs, such as lungs, liver, kidney, gonads, and eyes, has been studied, scanty reports elucidate the action of nicotine on ocular health disorders. The current investigation aimed to evaluate the deteriorative effect of nicotine via intraperitoneal and oral routes on retina of Swiss albino mice. Nicotine ditartrate salt at a dose of 1mg/kg b.wt. was injected intraperitoneally and fed by oral gavage to mice for 30 consecutive days. Ocular tissues were dissected out and subjected to histological observation and oxidative stress (SOD, CAT enzyme activities, MDA and GSH levels) analysis. Histological studies showed that nicotine administration caused loss and displacement of cellular components from the RPE, PRC, INL and GCL layers that led to alterations in the thickness of these layers. Nicotine administration caused an elevation of lipid peroxidation and suppression of SOD and catalase enzyme activities and reduced glutathione levels. Intraperitoneal administration of nicotine caused stronger suppression of antioxidative defense and strongly elevated lipid peroxidation than orally treated experimental mice. Specific grading to determine retinal degeneration suggested that mice receiving nicotine intraperitoneally had marked retinal degeneration and mice receiving nicotine orally had mild retinal degeneration. The data showed stronger deterioration of retinal tissues in intraperitoneally administered nicotine-treated mice than that in orally treated mice. Thus, the present study may

suggest a route-dependent effect of nicotine on retinal degeneration in the studied mice.

**Keywords** Nicotine, Retina, Oxidative Stress, Retinal Degeneration

## 1. Introduction

Nicotine is the major addictive and psychoactive alkaloid [1] in tobacco products, and it is widely consumed by people by smoking cigarettes or chewing tobacco leaves. It is responsible for a multitude of disorders of major vital organ systems of the body that include lungs, heart, GI tract, ovaries and testes, etc. Nicotine's effect on various pathologies of the ocular tissues has also been studied, including Grave's ophthalmology, macular degeneration, cataracts, glaucoma, and retinopathies due to hyperglycemia [2]. Ill effects of nicotine on these pathologies have been associated with hypoxia and elevated oxidative stress-mediated injury to the ocular tissues [3].

The eye, a sensory organ is responsible for converting visual images from the surroundings to information that can be analyzed by the brain. Retina is situated at the posterior-most part of the ocular tissue and it converts light into electrical signals [4]. The retina is made up of seven layers. The innermost Ganglionic cell layer (GCL) is made of non-image-forming photoreceptors called retinal

ganglion cells [5], followed by the adjacent IPL i.e., Inner Plexiform Layer. The INL or Inner Nuclear Layer consists of synaptic connections between Amacrine cells and Horizontal cells in huge amounts and lower densities of cones and bipolar cell connections [6]. This layer is followed by the adjacent supporting Outer Plexiform Layer i.e., OPL. The ONL or Outer Nuclear Layer lies adjacent to the OPL which consists of an irregular distribution of rod and cone granules but with uniform thickness. The photoreceptor cell layer (PRC) is made of rods and cones, which are responsible for scotopic and photopic visions, respectively [7,8]. The supporting Retinal Pigment Epithelium or RPE, functioning as a blood-retina barrier [9], thus managing the transport of nutrients, ions, water, and gases into and out of the retina [4]. Its close association with the adjacent PRC layer supports the photoreceptor outer segments to maintain adequate visual function [9]. The retina is composed of cells that have a very high oxygen demand and has been categorized as “the most metabolically expensive” tissue [4]. The presence of polyunsaturated lipids and the high metabolic rate of retinal cells make them prone to oxidative damage [10]. The major antioxidant defenses in the eye include superoxide dismutase, catalase, and reduced glutathione, which protect the retina against oxidative damage that occurs due to photo-oxidation and toxic chemical exposure [10]. Cigarette smoking caused oxidative damage to the retinal epithelium and associated vasculature and increased the incidences of developing early macular degeneration in mice [11]. Chronic tobacco smoke exposure also caused degeneration and apoptosis of cells in the RPE because of increased oxidative stress in mice [12]. This layer is particularly prone to damage by toxic compound administration because of its close association with the retinal vasculature. Reports suggest that retinal pigment epithelial cells undergo hypertrophy, detachment from the neural retina, and anomalies in the distribution of pigment granules after toxic chemical exposure [13]. Abnormality in retinal and choroid layer thicknesses relates to the incidences of various pathological conditions of the eye like uveitis, scleritis, and retinopathy [14]. Loss of rods and cones, and retinal epithelial cells is a characteristic of retinal degeneration which initiates loss of visual acuity [15].

Acute and chronic intraperitoneal injection of various doses of nicotine (0.5 mg kg<sup>-1</sup> body weight. - 2 mg kg<sup>-1</sup> body weight) has shown various histopathologic changes in the retina of rats [16]. Nicotine administration to juvenile male Wistar rats via oral gavage has caused a decline in retinal thickness and altered expression of markers of pathologies related to retinal degeneration [17]. Reports suggested variations in deteriorative effects caused by different doses of nicotine administration through oral or intraperitoneal routes, but scanty reports suggest route-dependent effectiveness of nicotine administration. Thus, the current study was directed towards the investigation of

route-dependent efficacy of nicotine administration on retinal degeneration in laboratory mice.

## 2. Materials and Methods

### 2.1. Experimental Design

An institutional guideline on animal experiments was followed for the current study design (Ethical Clearance No. TU/IAEC/2023/1/2-4). Male healthy Swiss albino mice were maintained under appropriate 12 hr light and dark conditions, with temperature maintained at 25±2 °C and humidity at 55±5%. Polycarbonate cages of dimensions 43 centimeters, 27 centimeters, and 14 centimeters were used for housing the mice and they were supplied with feed and water as required. The mice were randomly segregated into three groups. Each group had 5 mice.

Experimental groups

1. Group 1: CON (Control)
2. Group 2: IP (Nicotine i.p. treatment)
3. Group 3: ORAL (Nicotine oral treatment)

The CON group of mice received 0.9% normal saline. IP group of mice were intraperitoneally injected with nicotine {(-)-Nicotine Ditartrate salt Sigma-Aldrich Chemicals} (1mg/kg B.wt.) dissolved in 0.9% normal saline (200 µL/day). ORAL group of mice were fed with nicotine (1mg/kg B.wt.) dissolved in 0.9% normal saline (200 µL/day) using a 22-gauge oral gavage needle. Nicotine treatment was done for 30 consecutive days.

### 2.2. Parameters Studied

#### 2.2.1. Histological Observation

- a. Bouin’s fixative was used to fix the eyes dissected from the experimental mice, which were then processed by routine Hematoxylin-Eosin staining. Slides of ocular tissues were observed under Olympus microscope BX41 and microphotographs were taken at 40X objective.
- b. Thickness Measurement of Retinal Layers

The retinal layers were subjected to thickness measurements from the histological sections of all the experimental groups of mice. Thickness measurements of all the layers were done by Dewinter DIGICAM (version x86, 3.7.7855) from the central and peripheral parts of the retina.

- c. Specific Grading to Determine Retinal Degeneration

The specific histopathologic grading scheme as elucidated by Schaefer et al. [18] has been adopted to determine retinal degeneration from histological sections of ocular tissues of experimental mice as per the following table:

**Table 1.** Specific Grading Criteria for Retinal Degeneration Determination

Level of Retinal Degeneration	Properties
Minimal retinal degeneration	The retina has a loss of or ragged appearance of the PRC layer
Mild retinal degeneration	Loss of the PRC layer, thinning of ONL, occasional outer layer nuclei displacement into the PRC layer
Moderate retinal degeneration	ONL is notably thinned with accompanying thinning of the INL
Marked retinal degeneration	Thinning and decreased cellularity of all layers of the retina

### 2.2.2. Determination of Oxidative Stress in Ocular Tissue of Nicotine-Stressed Mice

#### a. Lipid Peroxidation Level Determination

Malondialdehyde is a lipid peroxidation product which was quantified using the protocol by Ohkawa et al. [19], which measures the interaction with thiobarbituric acid (TBA). TBA reagent (3.3ml) was mixed with 0.1ml of 10% ocular tissue homogenate made in phosphate buffer. After boiling the reaction mixture, the supernatant was obtained after centrifugation. At 532 nm, the supernatant was subjected to optical density measurement.

#### b. Superoxide Dismutase (SOD) Activity Measurement

SOD activity measurement in the ocular tissue was done by the protocol of Das et al. [20] and modified by Singh et al. [21]. Ocular tissue was homogenized in phosphate buffer (pH = 7.4) to obtain a 10% ocular tissue homogenate. A 1.4 ml reaction mixture was prepared from phosphate buffer, L-methionine, Triton-X-100, hydroxylamine hydrochloride, and EDTA. 0.1ml of the tissue homogenate was combined with this reaction mixture. Riboflavin and Griess reagents were added, and optical density measurement was done at 543nm.

#### c. Catalase (CAT) Activity Measurement

The catalase activity in ocular tissues was measured following the method proposed by Sinha [22] and modified by Hadwan [23]. Ocular tissue was homogenized in phosphate buffer (pH = 7.4) to obtain a 10% ocular tissue homogenate. Hydrogen peroxide and potassium dichromate were mixed to form a reaction mixture. The 10% homogenate thus obtained was allowed to mix with the reaction mixture and put in a water bath to boil. After centrifugation the supernatant collected was subjected to optical density measurement at 570nm to estimate H<sub>2</sub>O<sub>2</sub> content deterioration per minute.

#### d. Reduced Glutathione (GSH) Activity Measurement

Reduced glutathione is a non-enzymatic antioxidant marker, measured following the protocol elucidated by Ellman [24] and adjusted by Gupta et al. [25]. Ocular tissue was homogenized in phosphate buffer (pH = 7.4) to obtain a 10% ocular tissue homogenate. Trichloroacetic acid was mixed with the homogenate at a ratio of 1:1 and centrifuged.

The supernatant thus collected was subjected to a reaction with Ellman's reagent (1% sodium citrate, 0.04% DTNB in 0.1M phosphate buffer). At 412 nm, optical density measurement was taken, and the level of GSH in terms of mg/g of tissue was observed.

### 2.3. Analysis of Statistical Data

One-way ANOVA and Tukey's multiple-range test were done for experimental data interpretation. The significance of data was considered at  $p < .05$ . Statistical Package for the Social Sciences was used for data analysis and histograms were prepared with the help of Microsoft Excel program.

## 3. Results

### 3.1. Histological Observations

The retinal sections of the control group (Figure 1A, 1D) showed normal histoarchitecture of the retina with intact layers. The retinal section of the nicotine-treated mice showed visible loss of epithelial cells from the RPE layer of the retina in both intraperitoneal (Figure 1B) and oral (Figure 1C) groups. The intraperitoneally treated mice showed regular loss of cells, whereas the orally treated mice showed irregular loss of cells in the RPE layer. The PRC layer in the intraperitoneally treated mice showed a ragged appearance, whereas it remained unchanged in the orally treated mice (Figure 1B, 1C). Displacement of ONL and INL nuclei into the outer plexiform layer was observed in both intraperitoneally (Figure 1E) and orally (Figure 1F) treated mice, but greater displacement of nuclei was noted in intraperitoneally treated mice.

#### 3.1.1. Central Retinal Layers Thickness Measurement

RPE, PRC, INL, and GCL are four retinal layers showing pathophysiological changes. The intraperitoneal treatment in mice revealed a significant ( $p < .01$ ) decline in the thickness of these layers in the central retinal regions as compared to control. Significant ( $p < .05$ ) and ( $p < .01$ ) decrease in thicknesses of INL and GCL layers were noted, respectively, in mice that were orally-treated compared to control. INL and GCL layers underwent a significant ( $p < .01$ ) decline of thickness in the intraperitoneally

treated group as compared to orally treated mice (Table 2).

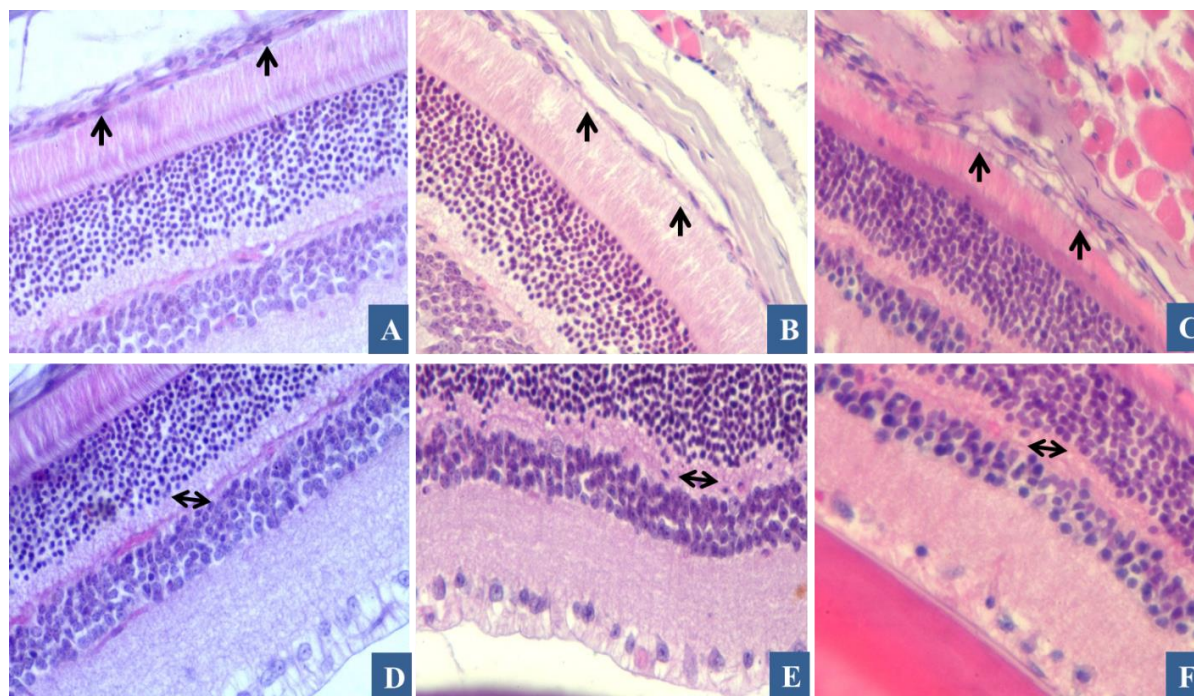
### 3.1.2. Peripheral Retinal Layers Thickness Measurement

In the peripheral region of the retina, the RPE, INL and GCL layers underwent a significant ( $p < .01$ ) decline of thickness in intraperitoneally treated mice compared to control. A significant ( $p < .01$ ) decline of thickness of PRC layer of intraperitoneally treated mice was also noted as compared to control mice. Orally-treated mice showed a significant ( $p < .01$ ) decline of thickness in the INL layer

compared to control. Further, a significant ( $p < .01$ ) decline in thickness was observed in INL in intraperitoneally treated group compared to orally treated mice (Table 3).

### 3.1.3. Specific Grading to Determine Retinal Degeneration

According to the Specific Grading of Retinal Degeneration criteria described by Schaefer [18], the orally treated group of mice showed Mild retinal degeneration, and the intraperitoneally treated group of mice showed marked retinal degeneration (Table 4).



**Figure 1.** Histology of retinal sections showing effects of nicotine treatment. Control (A, D), IP (B, E) and ORAL (C, F). The upward black arrow shows loss of cells from RPE (Retinal Pigment Epithelium) layer of the retina. The double-headed black arrow shows displacement of INL and ONL nuclei into outer-plexiform layer. The microphotographs were taken at 40X objective

**Table 2.** Central retinal thickness (in  $\mu\text{m}$ ) of the 7 layers of retina of the experimental mice groups: RPE, PRC, ONL, OPL, INL, IPL, GCL, TRT (Total Retinal Thickness)

GROUP	RPE ( $\mu\text{m}$ )	PRC ( $\mu\text{m}$ )	ONL ( $\mu\text{m}$ )	OPL ( $\mu\text{m}$ )	INL ( $\mu\text{m}$ )	IPL ( $\mu\text{m}$ )	GCL ( $\mu\text{m}$ )	TRT ( $\mu\text{m}$ )
CON	9.08 $\pm$ 1.33	31.34 $\pm$ 2.81	29.46 $\pm$ 3.37	15.24 $\pm$ 2.17	27.23 $\pm$ 3.18	44.69 $\pm$ 13.19	15.59 $\pm$ 1.86	160.61 $\pm$ 5.86
IP	4.57 $\pm$ 0.10 <sup>a</sup>	25.85 $\pm$ 4.98 <sup>a</sup>	31.19 $\pm$ 4.53	6.75 $\pm$ 0.37	12.30 $\pm$ 0.46 <sup>a,c</sup>	45.83 $\pm$ 8.08	6.59 $\pm$ 1.20 <sup>a,c</sup>	133.09 $\pm$ 19.16
ORAL	8.51 $\pm$ 0.32	28.03 $\pm$ 4.53	32.39 $\pm$ 3.75	12.28 $\pm$ 1.50	22.71 $\pm$ 1.45 <sup>b</sup>	39.55 $\pm$ 1.11	11.64 $\pm$ 1.86 <sup>a</sup>	158.43 $\pm$ 2.72

Data were represented as Mean  $\pm$  SEM, n = 5. a,  $p < .01$ , Con vs IP, Con vs ORAL; b,  $p < .05$ , Con vs IP, Con vs ORAL; c,  $p < .01$ , IP vs ORAL

**Table 3.** Peripheral retinal thickness (in  $\mu\text{m}$ ) of the 7 layers of retina of the experimental mice groups: RPE, PRC, ONL, OPL, INL, IPL, GCL, TRT (Total Retinal Thickness)

GROUP	RPE ( $\mu\text{m}$ )	PRC ( $\mu\text{m}$ )	ONL ( $\mu\text{m}$ )	OPL ( $\mu\text{m}$ )	INL ( $\mu\text{m}$ )	IPL ( $\mu\text{m}$ )	GCL ( $\mu\text{m}$ )	TRT ( $\mu\text{m}$ )
CON	7.30 $\pm$ 0.64	26.66 $\pm$ 1.19	33.64 $\pm$ 4.17	9.67 $\pm$ 1.66	27.08 $\pm$ 1.71	25.22 $\pm$ 7.72	11.95 $\pm$ 4.6	141.26 $\pm$ 14.31
IP	3.55 $\pm$ 0.71 <sup>a</sup>	23.17 $\pm$ 3.17 <sup>b</sup>	28.38 $\pm$ 8.28	7.80 $\pm$ 1.10	11.56 $\pm$ 3.63 <sup>ac</sup>	27.54 $\pm$ 5.77	7.31 $\pm$ 1.13 <sup>a</sup>	109.33 $\pm$ 18.73
ORAL	6.99 $\pm$ 0.55	24.34 $\pm$ 3.03	28.01 $\pm$ 0.87	10.51 $\pm$ 0.74	17.73 $\pm$ 0.42 <sup>a</sup>	23.17 $\pm$ 2.00	10.21 $\pm$ 1.41	120.99 $\pm$ 4.56

Data were represented as Mean  $\pm$  SEM, n = 5. a, p<.01, Con vs IP, Con vs ORAL; b, p<.05, Con vs IP, Con vs ORAL; c, p<.01, IP vs ORAL

**Table 4.** Specific Grading of Retinal Degeneration ('+' showed presence of property and '-' showed absence of property)

Groups/Properties	CON		IP		ORAL	
	Central	Peripheral	Central	Peripheral	Central	Peripheral
Ragged look of the PRC layer	-	-	+	-	-	-
Loss of PRC layer	-	-	+	+	-	-
Thinning of ONL	-	-	-	+	-	+
Outer Nuclei displaced	-	-	+	+	-	-
Thinning of INL	-	-	+	+	+	+
Decreased Cellularity of all layers of the retina	-	-	+	-	-	-

## 3.2. Observations of Oxidative Stress Parameters

### 3.2.1. Lipid Peroxidation (MDA) Levels

A significantly (p<.01) increased level of malondialdehyde in ocular tissues was noted in the intraperitoneally treated and orally treated mice compared to the control group of mice. Further, the intraperitoneally treated group showed significantly (p<.01) elevated malondialdehyde levels in ocular tissue compared to orally treated mice group (Figure 2).

### 3.2.2. Superoxide Dismutase (SOD) Enzyme Activity

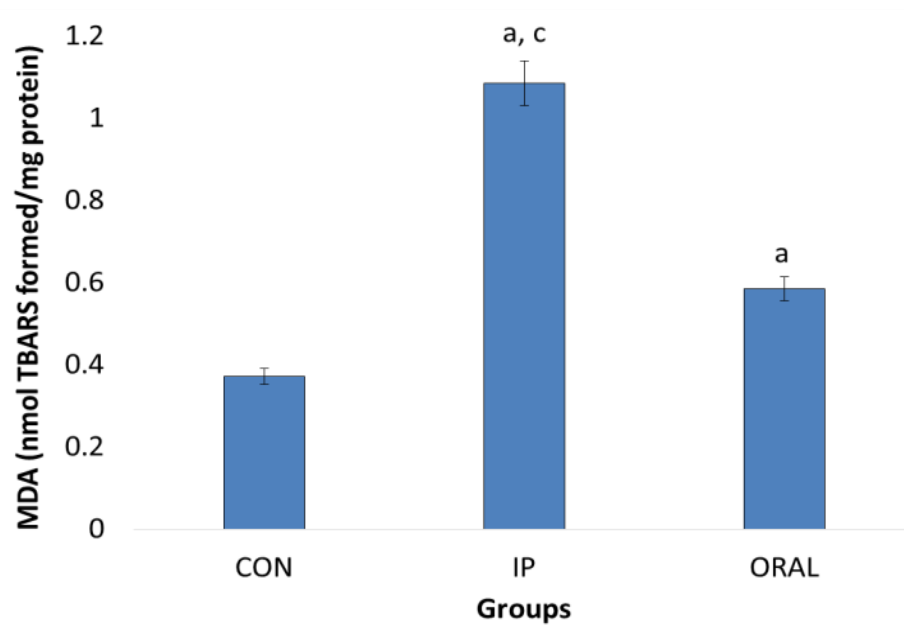
Significant (p<.01) suppression of SOD enzyme activity was noted in the ocular tissues of both intraperitoneal treatment and oral treatment of nicotine in mice groups compared to the ocular tissue of control mice (Figure 3).

### 3.2.3. Catalase (CAT) Enzyme Activity

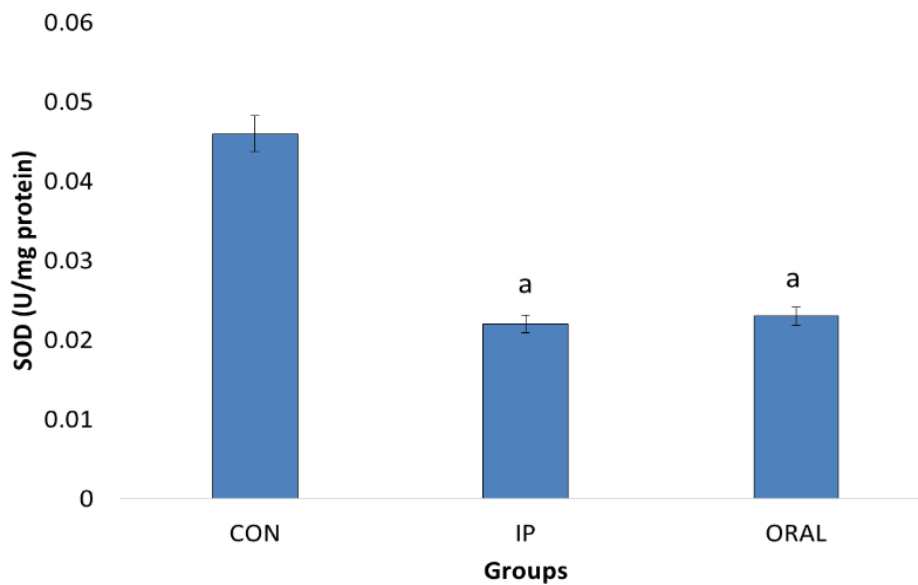
A significant (p<.01) decline in the activity of catalase enzyme was observed in the intraperitoneally treated group in comparison with the control group. A significant (p<.05) decrease in catalase activity was seen in the ocular tissue of orally treated group in comparison with the control group. Further, a significant (p<.01) suppression of catalase activity occurred in the intraperitoneally treated group compared to the orally treated group of mice (Figure 4).

### 3.2.4. Reduced Glutathione (GSH) Level

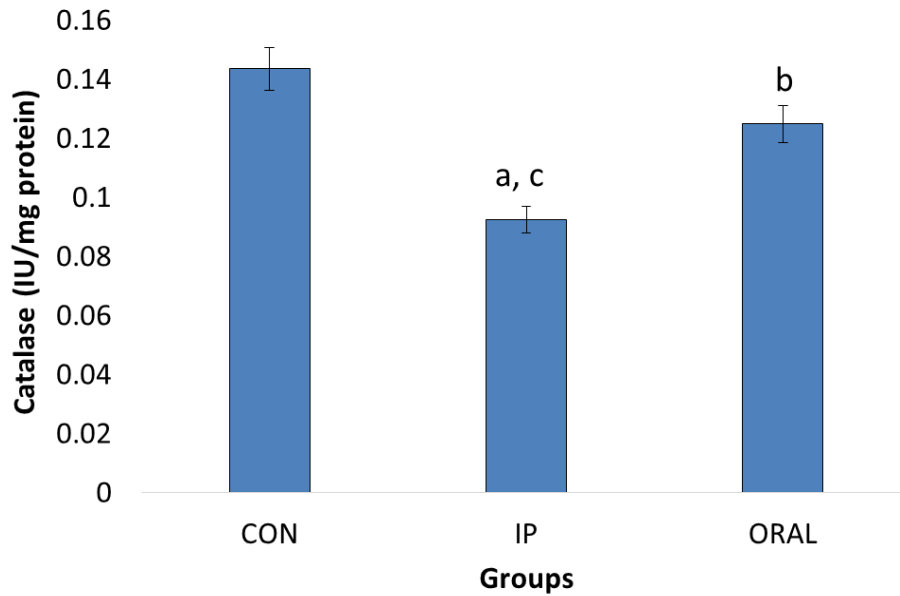
Significantly (p<.01) low levels of reduced glutathione (GSH) were observed in the ocular tissues of the intraperitoneally treated and orally treated groups of mice compared to control mice. Further, a significant (p<.01) decline in the level of GSH occurred in the intraperitoneally treated group compared to the orally treated mice group (Figure 5).



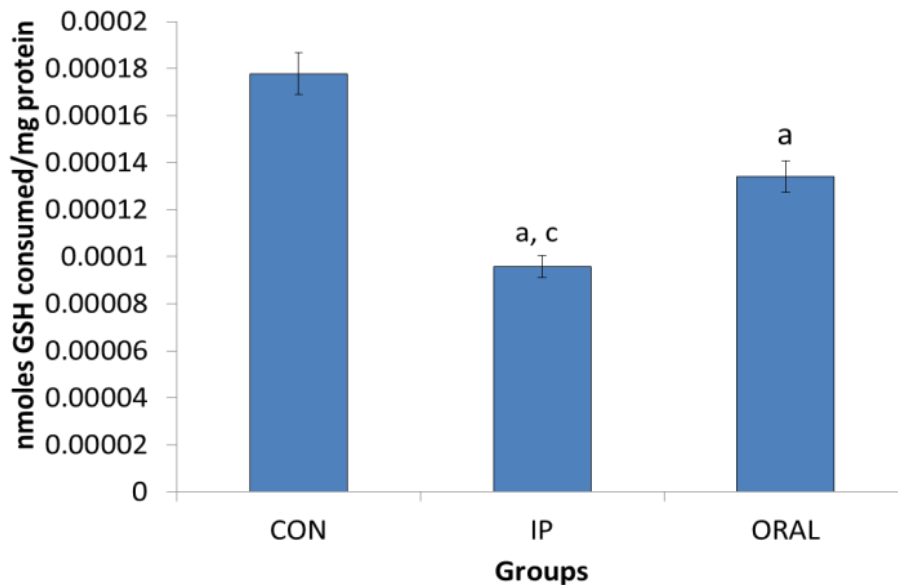
**Figure 2.** Histogram showing effects of nicotine treatment on lipid peroxidation in ocular tissue of experimental mice groups. Histogram represents Mean  $\pm$  SEM. a  $p < .01$ , CON vs IP, CON vs ORAL; c,  $p < .01$ , ORAL vs IP



**Figure 3.** Histogram showing effects of nicotine treatment on the activity of SOD enzyme in ocular tissue of experimental mice groups. Histogram represents Mean  $\pm$  SEM. a,  $p < .01$ , CON vs IP, CON vs ORAL



**Figure 4.** Histogram showing effects of nicotine treatment on the activity of catalase enzyme in the ocular tissue of experimental mice groups. Histogram represents Mean  $\pm$  SEM. a,  $p < .01$ , CON vs IP; b,  $p < .05$ , CON vs ORAL; c,  $p < .01$ , ORAL vs IP



**Figure 5.** Histogram showing effects of nicotine treatment on GSH levels in ocular tissue of experimental mice groups. Histogram represents Mean  $\pm$  SEM. a,  $p < .01$ , CON vs IP, CON vs ORAL; c,  $p < .01$ , ORAL vs IP

## 4. Discussion

The major psychoactive and harmful alkaloid found in tobacco-based products is nicotine, which is consumed by a majority of the population. The harmful impact of nicotine on numerous organs of the body like the lungs, heart, liver, and kidneys, has been elucidated in various reports. The action of tobacco on increased incidences of cataracts and its effect on the lens [26] has been reported earlier. The current investigation evaluated the deteriorative effects of intraperitoneal and oral

administration of nicotine on retinal degeneration in laboratory mice. Histological observation showed changes in the retinal layers in the nicotine-treated group of mice. Retina layer thicknesses vary in the central and peripheral regions of retina in the eye. The macula (retinal region accountable for vision) is thicker in the central retinal region and is thinner towards the periphery, with a depression in the central region, making the thinnest part of the retina, fovea [27]. Hence, thickness measurements were done from the central and peripheral regions and are presented in Table 2 and Table 3 respectively. The four

retinal layers (RPE, PRC, INL, and GCL), having major pathological importance were considered to evaluate the changes between different experimental groups. The retinal pigment epithelial layer showed a pronounced decline in thickness in central and peripheral regions in the retina of IP compared to ORAL and control mice. Intraperitoneal administration of nicotine also resulted in the loss of cells from the RPE layer, as seen from the photomicrographs; while similar observations were made in the orally treated group as well, the loss was minimal in this group. The RPE layer is responsible for forming the blood-retina barrier and for the transport of gases and nutrients across the retina. Although the RPE layer is not responsible for vision, it is necessary for the viability of the adjacent photoreceptors and is an easy target for systemically administered drugs [13]. Thus, RPE thinning is associated with ill effects on the neural retina. Thinning of the photoreceptor cell layer was also more prominent in IP compared to both ORAL and control. Intraperitoneal treatment with nicotine caused a ragged appearance of this layer as opposed to the comparatively intact PRC layer in the retina of the orally treated mice group. Reports by Piccolino et al. [28] elucidated that damage to the central photoreceptor layer and its detachment from the underlying RPE are associated with loss of visual acuity and in serious cases, lead to the development of chorioretinopathy. The inner nuclear layer thickness was reduced in both IP and ORAL experimental mice; however IP group showed prominently reduced thickness compared with the ORAL mice group. The displacement of nuclei from the outer nuclear layer as well as the inner nuclear layer into the outer plexiform layer is also very evident in the IP group. The displacement of these nuclei affects the relay of information from the photoreceptors to the ganglionic cell layer for further processing. The intact nature of inner nuclear layer, as seen in control mice, is lost in both intraperitoneally and orally nicotine-treated mice groups. The INL consists of nuclei of bipolar cells, Muller, horizontal, and amacrine cells [29]. As one moves from the central to the peripheral part of the retina, the density of all cell populations alters, but the neuronal constitution of the INL stays relatively constant [30]. Retinal signals are processed via the synapses between various neurons, among which the bipolar cells act as excitatory interneurons, amacrine cells help in the interaction between rods and cones, and ganglion cells act as the output neurons [31]. Thus, any displacement of its nuclei, thinning, or loss of the cell constituents of the INL has a deep impact on vision and the overall health of the retina. Finally, a significant thinning of the ganglionic cell layer was observed along with a loss of ganglion cells in IP group. The ganglion cells relay information from retina to brain for further processing and development of an image [32]. A loss of axons and cell bodies from the retinal ganglion cells, with a total loss of thickness of the nerve fibre layer of retina, has been reported in tobacco consumers [33]. Tobacco smoking was reported to influence GCL thickness in human subjects [34]. The

histological observation based on Specific Grading Criteria [18] showed that intraperitoneal nicotine treatment caused Marked Retinal Degeneration since it underwent a loss of cellularity in all the retinal layers mostly in the central region, while the orally treated group had Mild Retinal Degeneration (Table 4).

The current investigation observed that administration of nicotine in Swiss albino mice led to increased lipid peroxidation levels in ocular tissues of the experimental mice. However, intraperitoneal treatment showed higher lipid peroxidation. A high metabolic activity observed in retina, along with high amounts of polyunsaturated fatty acids, makes it an easy target for lipid peroxidation. Nicotine-treated mice showed suppression of SOD and catalase antioxidant enzyme activities in ocular tissues. Intraperitoneal supplementation with nicotine caused greater suppression of catalase activity in ocular tissues. Oyeyipo et al. [35] suggested that nicotine treatment caused decreased serum catalase enzyme activities in rats. Tobacco smoke was reported to negatively impact SOD enzyme activity in individuals with cataracts [26]. SOD enzyme is responsible for the formation of hydrogen peroxide from superoxide anions. The hydrogen peroxide is thus converted to water by catalase enzyme in living organisms. Low catalase enzyme activity in the IP group caused the accumulation of peroxides, which led to increased oxidative stress in ocular tissues. Thus, a suppressed activity of these antioxidant enzymes led to insufficient removal of free radicals and oxidative components from the ocular tissues and caused elevated oxidative stress in the retina of nicotine-administered mice groups. Similarly, a decreased GSH level was observed in both nicotine-treated groups; however, intraperitoneal administration of nicotine more effectively suppressed the GSH level. An increase in stress because of the elevated reactive oxidative radicals is correlated to the advancement of various degenerative diseases of the eye [36]. The decrease in efficiency of the antioxidant system of the ocular tissue due to nicotine exposure has led to an overall imbalance in the removal of the reactive oxygen species. The reports by Saenz-de-Viteri et al. [37] suggested the association of phototoxicity-induced oxidative stress with alterations in the morphology of the neurosensory retina in rabbits. Further, studies by Sasaki et al. [38] suggested the association of oxidative stress with the advancement of neuronal degeneration in retina due to diabetes in mice.

## 5. Conclusions

The increase in oxidative stress in the retinal tissue due to nicotine results in changes in the histoarchitecture of the retinal layers. The present study showed more prominent cellular loss and nuclear displacement with alterations in retinal layer thickness in intraperitoneally nicotine-treated mice, which manifest marked retinal degeneration. Intraperitoneal administration of nicotine caused greater

suppression of the antioxidant defense system in ocular tissues of mice. Therefore, present observations may suggest the effectiveness of the intraperitoneal route of nicotine administration in greater degeneration of the retina.

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