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The effect of N-acetylcysteine on the sensory retina of male albino rats exposed prenatally to cypermethrin

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Background: *Cypermethrin (CYP), a pyrethroid that is globally used in the field and house to fight the pests. CYP can induce cellular toxicity and cross the placental barrier. N-acetylcysteine (NAC) can fight the prenatal exposure to the inflammation. This work aimed to study, for the first time, the effects of NAC on the sensory retina of male albino rats exposed prenatally to cypermethrin.*

Materials and methods: *Twenty-four sexually mature female albino rats and 12 male albino rats were allowed for mating and divided equally into the following groups: group I (control group): kept without treatment; group II (NAC group): received 1 g/kg/day NAC diluted in distilled water orally by gastric tube from the 7th day of gestation till delivery; group III (CYP group): received 12 mg/kg/day of cypermethrin orally by gastric tube from the 7th day of gestation till delivery; group IV (CYP and NAC group): received 12 mg/kg/day of cypermethrin and 1 g/kg/day of NAC. The ten male offspring of each group were divided into subgroups a and b that were sacrificed at the age of 7th and 14th days postnatal, respectively. At the end of the experiment, the eye samples were subjected to histological, immunohistochemical and morphometric studies.*

Results: *Concerning the different previous studies, the sensory retina of CYP subgroups showed vacuolation of the inner and outer plexiform layers, dilated congested blood vessels, hyalinisation and disorganisation of the photoreceptor layer. Also, the expression of collagen IV and caspase 3 (a marker of apoptosis) was up-regulated in the CYP subgroups.*

Conclusions: *N-acetylcysteine significantly protected the sensory retina from the damaging effects of CYP. NAC could be considered as a good protective agent against the damaging effect of CYP on the sensory retina.* (Folia Morphol 2021; 80, 1: 140–148)

Key words: cypermethrin N-acetylcysteine rat sensory retina

INTRODUCTION

Pyrethrins are organic compounds, naturally derived from *Chrysanthemum cinerariifolium* flowers [18]. They have a potent insecticidal activity as their synthetic analogues which are called pyrethroids [19]. Cypermethrin (CYP), a pyrethroid that is globally used in the field and house to fight pests [13]. It is capable to disturb the cellular structure and function of pests [1]. CYP induced cellular toxicity may be due to reactive oxygen species (ROS) that cause destruction

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of the cellular proteins, lipids and DNA [8]. CYP can cross the placental barrier causing toxic effects on the development of albino rats causing several congenital malformations and foetal death [10]. Cysteine is the basic amino acid for the synthesis of glutathione which combats the oxidative stress and the resultant free radicles by its antioxidant activity [11]. This activity is classified as enzymatic one through the promotion of glutathione peroxidase and reductase enzymes [14]. Besides this, N-acetylcysteine (NAC) has anti-inflammatory properties through inhibition of the cyclooxygenase enzyme together with its products as prostaglandins [6]. And so, NAC can fight the prenatal exposure to the inflammation preventing the occurrence of behavioural and memory alterations on the offspring [16]. This allows us to study the role of NAC on the retina of prenatally exposed rats to CYP.

MATERIALS AND METHODS

Materials

Cypermethrin. A trade name to an oily solution containing 250 g/L of the active ingredient CYP, it was diluted with distilled water to concentration levels convenient to be used throughout the whole experiment, in which 1 mL of diluted working solution contained 12 mg of CYP.

N-acetylcysteine. A pharmacological preparation (Acetylcistein 600 mg, Effervescent Instant Granules, SEDICO Pharmaceutical Co., 6 October City-Egypt) was diluted with tap water and given to the rats orally.

Animals

Twenty-four sexually mature female albino rats and 12 male albino rats, weighing between 200 and 250 g were allowed for laboratory rat chow diet and water ad-libitum.

Every 2 female rats were housed overnight with a sexually mature male albino rat for mating; every morning, vaginal smears were taken and microscopically examined for the presence of sperms. The detection of sperms in the smears was considered as the 1st day of gestation. All experimental procedures were performed with the approval of the Research Ethics Committee, Faculty of Medicine, Menoufia University, Egypt.

Experimental design

The pregnant mothers were divided equally into the following groups:

— Group I (control group): the pregnant rats were fed ad libitum and allowed free water supply from the 7th day of gestation. Ten male offspring were divided into subgroup Ia and Ib that were sacrificed at the age of 7th and 14th days postnatal, respectively;
— Group II (NAC group): the pregnant rats received 1 g/kg/day of NAC [3] diluted in distilled water orally by gastric tube from the 7th day of gestation till delivery. Ten male offspring were divided into subgroup IIa and IIb that were sacrificed at the age of 7th and 14th days postnatal, respectively;
— Group III (CYP group): the pregnant rats received 12 mg/kg/day of CYP [7] orally by gastric tube from the 7th day of gestation till delivery. Ten male offspring were divided into subgroup IIIa, and IIIb that were sacrificed at the age of 7th and 14th days postnatal, respectively;
— Group IV (CYP and NAC group): the pregnant rats received 12 mg/kg/day of CYP and 1 g/kg/day of NAC diluted in distilled water orally by gastric tube from the 7th day of gestation till delivery. Ten male offspring were divided into subgroup IVa and IVb that were sacrificed at the age of 7th and 14th days postnatal, respectively.

At the end of each detected period, the eye samples were removed from each animal. The eye samples were fixed in 10% formol saline and processed to prepare 5 μm-thick paraffin sections for use in the following histological techniques:

Histological study

Haematoxylin and eosin stain and toluidine blue stain.

Immunohistochemical study

For detection of the immunoreactivity of collagen IV and caspase 3, the sections were deparaffinised, rehydrated, and after antigen retrieval with 10 mmol/L citrate acid solution (pH 6), the specimens were preincubated with goat serum for 5 min and were then incubated overnight at 4°C with anti-collagen IV (Abcam, 6586) and anti caspase 3 (Abcam, 2302) (Working dilution 1:500). The binding was detected using biotinylated secondary antibody (goat anti-mouse IgG; Sigma Aldrich) for 10 min. The specimens were then incubated with streptavidin-peroxidase complex for 5 min, followed by incubation with 3,3-diaminobenzidinetetrahydrochloride (DAB; Sigma Aldrich) for 3 min. Slides were counterstained with haematoxylin and mounted.

Morphometric study

Data were obtained from five different sections from each rat of all subgroups and examined using image J analyser software, version 1.47 v to determine:

— the colour intensity in toluidine blue-stained sections;
— area per cent in collagen IV immune-stained sections;
— the number of positive cells in caspase 3 immune-stained sections.

Statistical analysis

Data obtained from the morphometric study was subjected to statistical analysis using SPSS software version 20 (SPSS, Inc., Chicago, IL, USA). Data were presented as mean ± standard deviation. Differences among the study groups were detected by using Mann-Whitney U test. The results were considered statistically significant with p value < 0.05 [15].

RESULTS

Histological study

Haematoxylin and eosin (H and E) stain

Haematoxylin and eosin-stained retinal sections of the control and NAC groups revealed the same histological features of the sensory retina.

The sensory retina of postnatal rats aged 7 days from control mothers (control group) consisted of the photoreceptor layer, 2 nuclear (outer and inner) layers that were separated from each other by the outer plexiform layer. Moreover, the inner nuclear layer was followed internally by the inner plexiform layer then the ganglion cell layer. The nerve fibre layer was followed by the inner limiting membrane with the developing blood vessels.

However, the sensory retina of 7 days old postnatal rats from mothers treated with cypermethrin (CYP group) showed vacuolations of inner and outer plexiform layers. The nuclear layers showed pyknosis of their nuclei, also the blood vessels showed vasodilatations and congestion. On the treatment of the mothers with cypermethrin and N-acetylcysteine (CYP and NAC group), the sensory retina of their 7 days old postnatal offspring showed preservation of normal development and differentiation, so the retina appeared more or less normal. The sensory retinal layers of postnatal rats aged 14 days from control mothers (control group) appeared more differentiated than that of the age of 7 days (Fig. 1).

On the treatment of the mothers with cypermethrin (CYP group), the sensory retina of their 14 days postnatal offspring showed hyalinisation and disorganisation of the photoreceptor layer. Also, the vacuolations of the plexiform layers and nuclear pyknosis of the nuclear layers appeared. On the treatment of the mothers with cypermethrin and N-acetylcysteine (CYP and NAC group), the sensory retina of their 14 days old postnatal offspring showed preservation of normal development and differentiation, so the sensory retina appeared more or less normal (Fig. 2).

Toluidine blue stain

The sensory retina of postnatal rats aged (7 and 14) days from control mothers (control group) showed dark blue staining of its neural cells indicating the presence of dense Nissl's bodies in their cytoplasm. The CYP sensory retinae showed a significant decrease in the colour intensity as compared to that of the control group, while the NAC treated sensory retinae showed a significant increase in the colour intensity of the toluidine blue stain as compared to that of CYP group (Figs. 3, 4).

Immunohistochemical study

The sensory retina of postnatal rats aged (7 and 14) days from CYP mothers (CYP group) showed significant increase in the percentage of positively reacting area for collagen IV as compared to that of control group, while the NAC treated sensory retinae showed significant decrease in the percentage of positively reacting area for collagen IV as compared to that of CYP group (Figs. 5, 6).

The sensory retina of postnatal rats aged (7 and 14) days from control mothers (control group) showed minimal expression of caspase 3. The CYP sensory retinae (CYP group) showed significant increase in the number of positively immunostaining cells for caspase 3 as compared to that of control group, while the NAC treated sensory retinae (CYP and NAC group) showed significant decrease in the number of positively immunostaining cells for caspase 3 as compared to that of CYP group (Figs. 7, 8).

DISCUSSION

The postnatal exposure to CYP leads to neurodegenerative effects on human and rat [22], so the aim of this work was to study for the first time, the effect of prenatal exposure to CYP on the development of the sensory retina and the possible role of

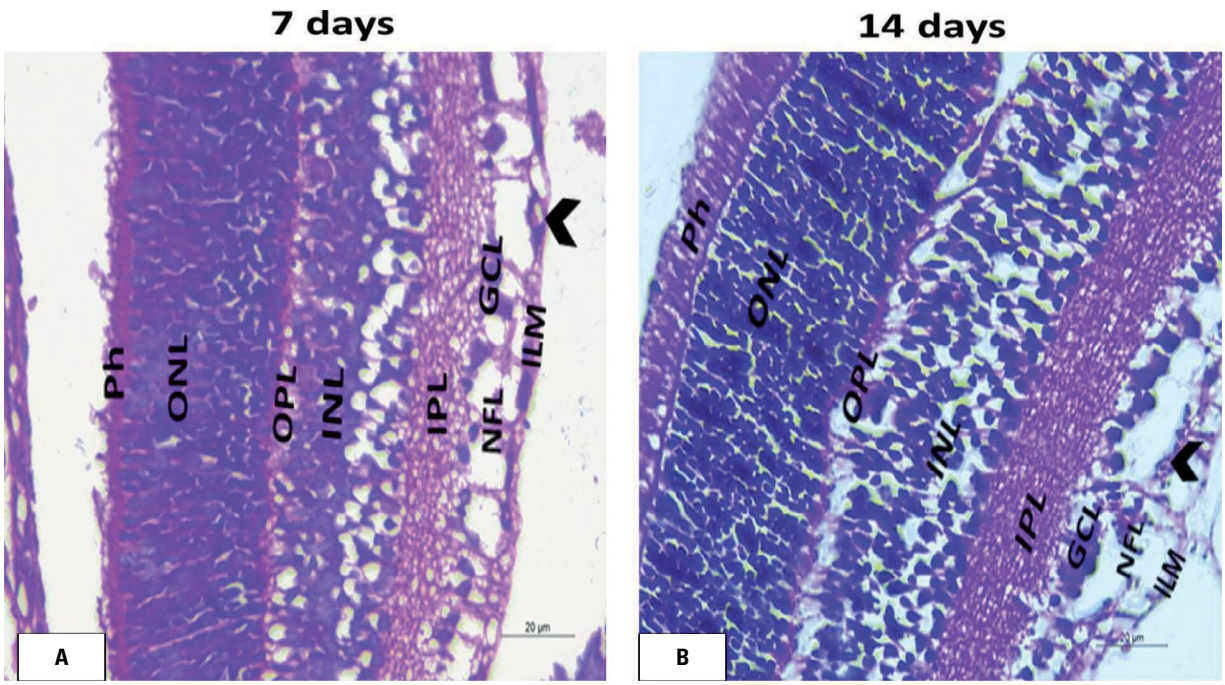

Figure 1. Haematoxylin and eosin stained sensory retinal sections (×400) of 7 and 14 days postnatal offspring of control group: the sensory retina of 7 (**A**) and 14 (**B**) days postnatal offspring of (control) group showing photoreceptor layer (ph), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear (INL), inner plexiform layer (IPL), ganglionic cell layer (GCL), nerve fibre layer (NFL) and inner limiting membrane (ILM). Also, the normal blood vessels (arrowhead) are present on the inner limiting membrane.

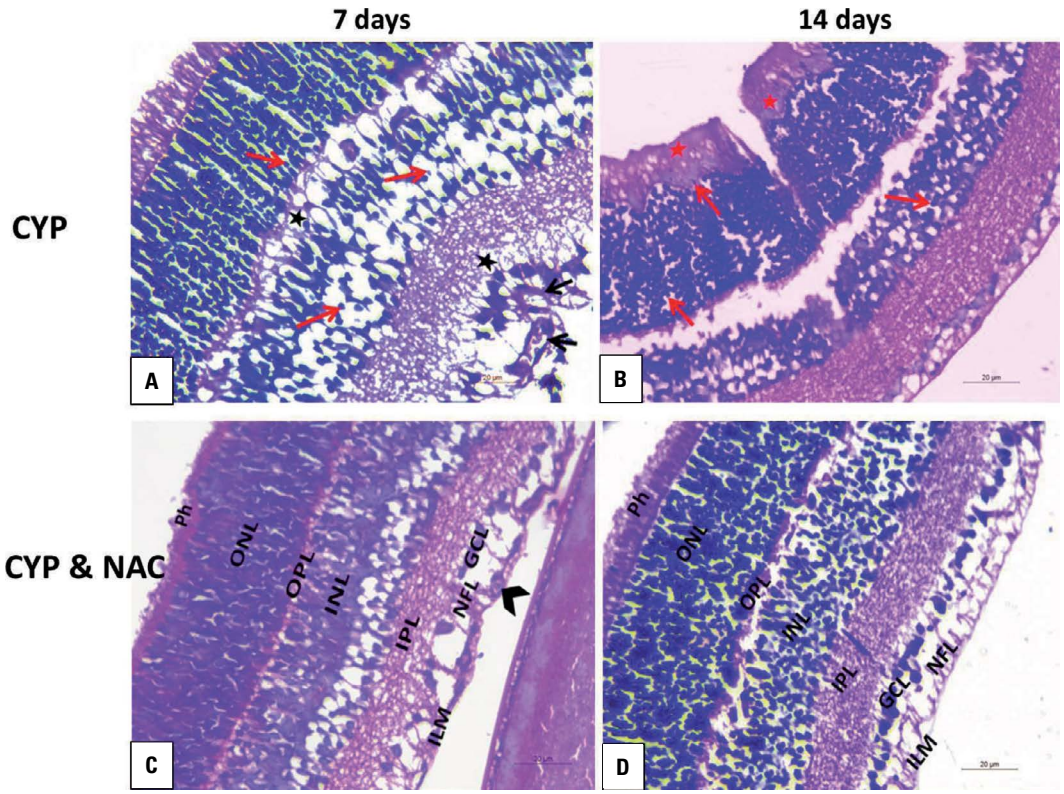

Figure 2. Haematoxylin and eosin stained sensory retinal sections (×400) of 7 and 14 days postnatal offspring of cypermethrin (CYP) and cypermethrin and N-acetylcysteine (CYP and NAC) groups. **A, B.** Sensory retina of 7 and 14 days postnatal offspring of CYP group showing vacuolations (black star) of plexiform layers, pyknosis (red arrow) of nuclear layers, vasodilatation and congestion of the blood vessels (black arrow). Moreover, hyalinization and disorganisation (red star) are present in the photoreceptor layer; **C, D.** Sensory retina of 7 and 14 days postnatal offspring of CYP and NAC group showing preservation of normal development and differentiation.

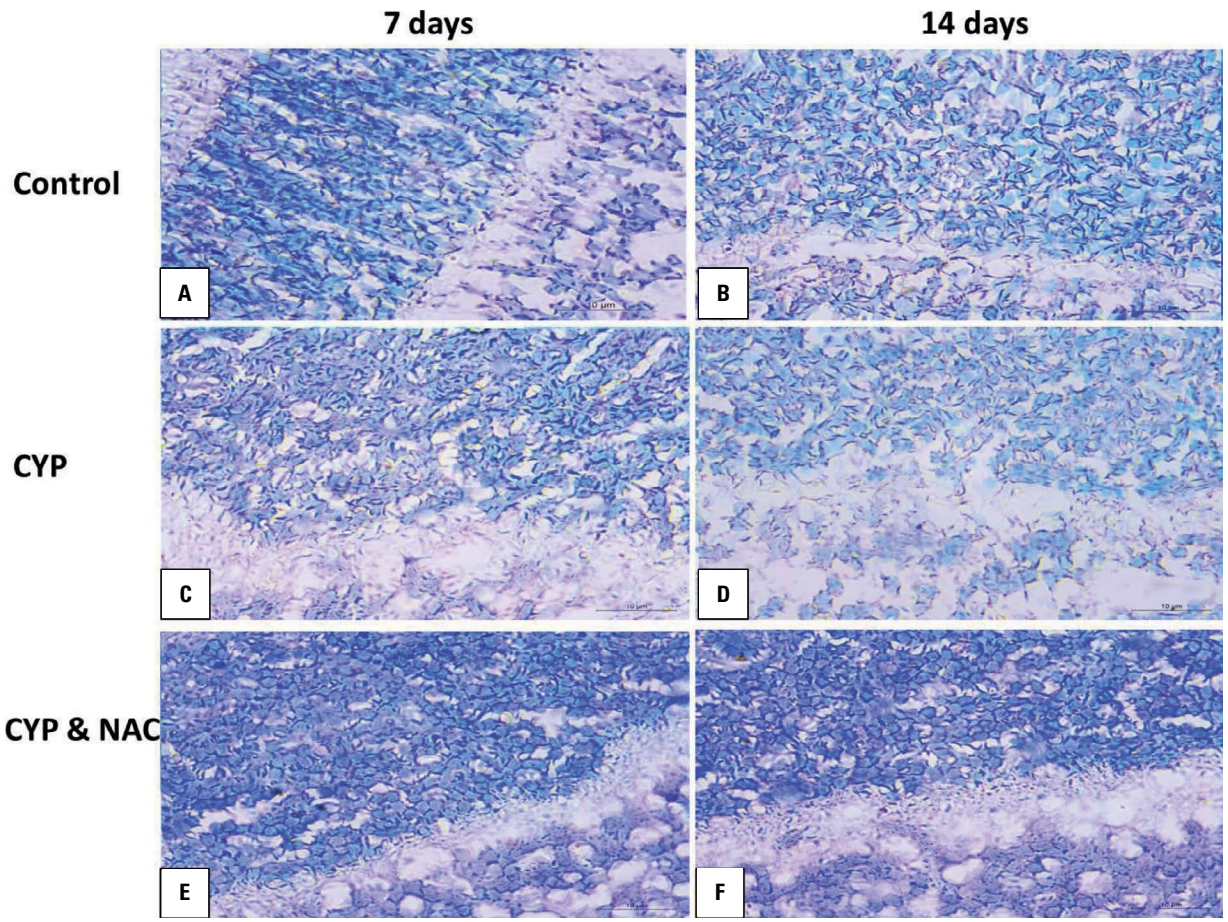

Figure 3. Representative toluidine blue-stained sensory retina (×1000) of all experimental groups; **A, B.** Dark blue staining of Nissl's bodies in retinal neural cells of control group; **C, D.** Significant decrease (p value < 0.05) in colour intensity of the sections of cypermethrin (CYP) group as compared to control group; **E, F.** Significant increase (p value < 0.05) in colour intensity of the sections of CYP and N-acetylcysteine (NAC) group as compared to CYP group.

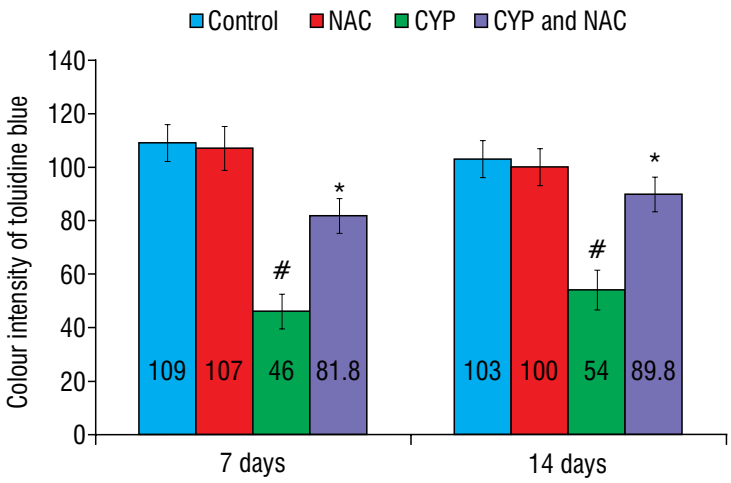

Figure 4. Regarding the colour intensity of toluidine blue staining, the cypermethrin (CYP) group showing a significant decrease ($^{\#}$p value < 0.05) as compared to the control group while the CYP and N-acetylcysteine (NAC) group showing a significant increase (*p value < 0.05) as compared to CYP group.

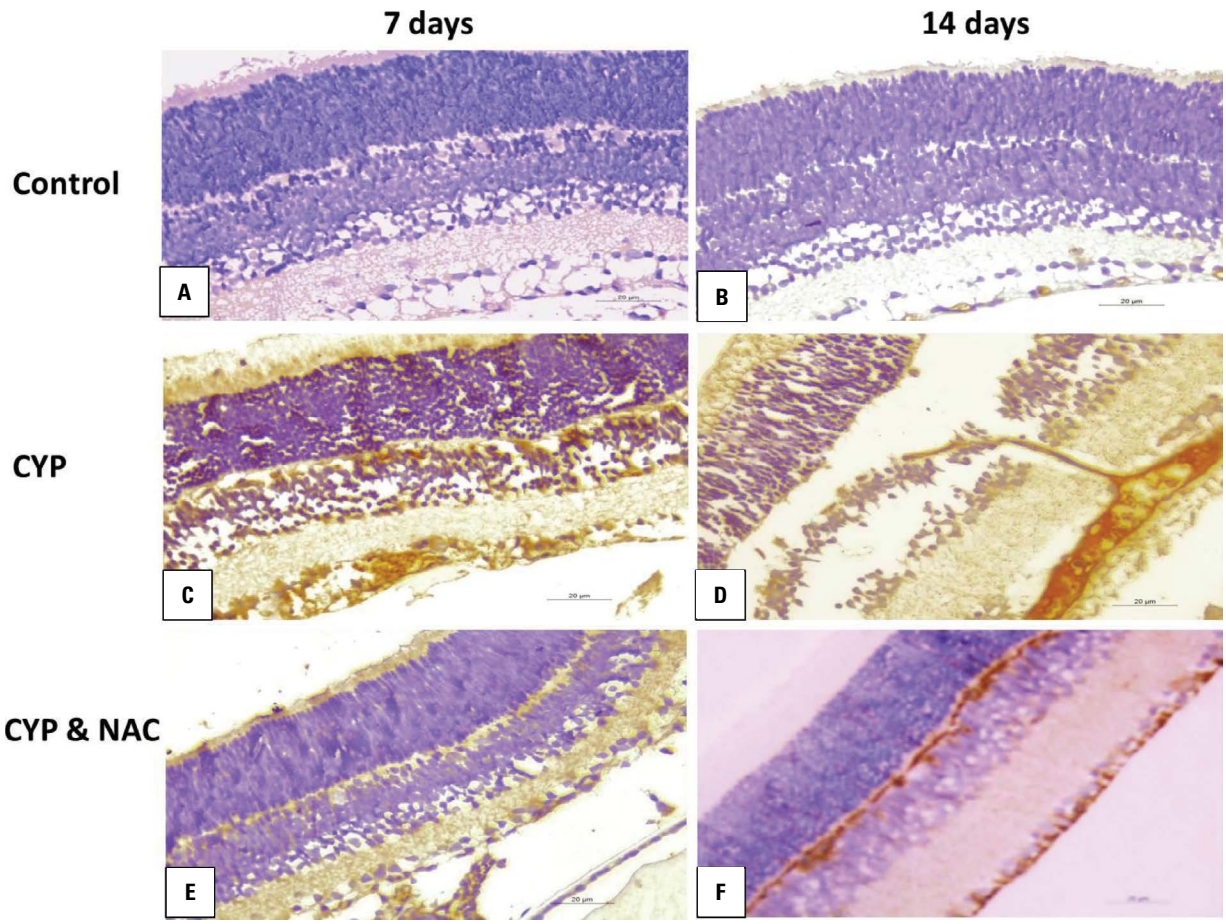

Figure 5. Representative collagen IV immunostained sensory retina (×400) of all experimental groups; **A, B.** Normal expression of collagen IV in retinal blood vessels of (control) group; **C, D.** Significant up-regulation (p value < 0.05) in percentage of collagen IV immunoreacting area in retinae of cypermethrin (CYP) group as compared to those of control group; **E, F.** Significant down-regulation (p value < 0.05) in percentage of collagen IV immunoreacting area in retinae of CYP and N-acetylcysteine (NAC) group as compared to CYP group.

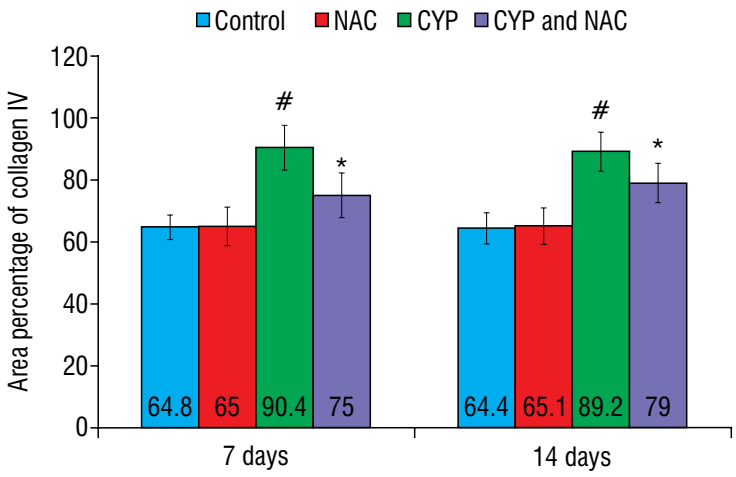

Figure 6. Regarding the expression of collagen IV immunostaining, the cypermethrin (CYP) group showing a significant increase ($^{\#}$p value < 0.05) as compared to the control group while the CYP and N-acetylcysteine (NAC) group showing a significant decrease (*p value < 0.05) as compared to CYP group.

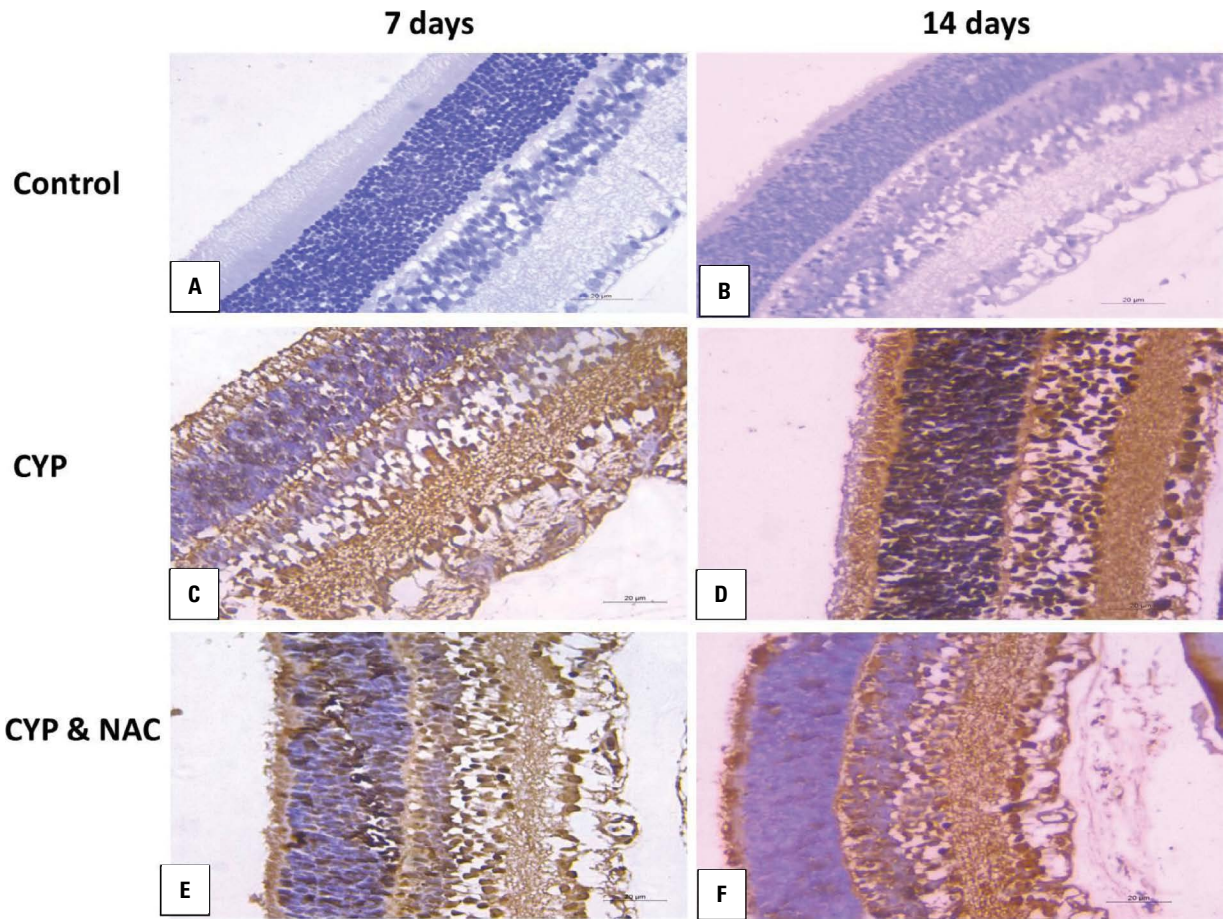

Figure 7. Representative caspase 3 immunostained sensory retina (×400) of all experimental groups. The retinae of control group showing negative (**A**) and minimal (**B**) expression of caspase 3; **C, D.** Significant increase (p value < 0.05) in number of caspase 3 immunopositive cells in retinae of cypermethrin (CYP) group as compared to those of control group; **E, F.** Significant decrease (p value < 0.05) in number of caspase 3 immunopositive cells in retinae of CYP and N-acetylcysteine (NAC) group as compared to those of CYP group.

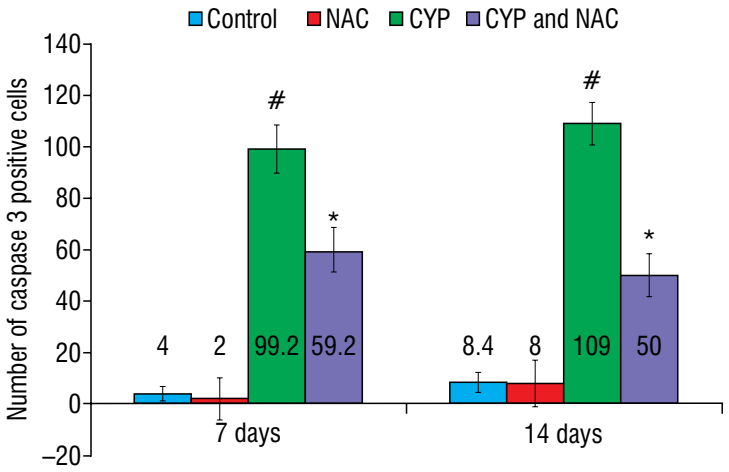

Figure 8. Regarding the expression of caspase 3 immunostaining, the cypermethrin (CYP) group showing a significant increase (#p value < 0.05) as compared to the control group, however, the CYP and N-acetylcysteine (NAC) group showing downregulation of caspase 3 expression (*p value < 0.05) compared to CYP group.

NAC. The sensory retina of 7 days old postnatal rats from mothers treated with cypermethrin (CYP group) showed vacuolations of inner and outer plexiform layers. The nuclear layers showed pyknosis of their nuclei, also the blood vessels showed vasodilatations and congestion. Moreover, the sensory retina of 14 days postnatal rats from mothers treated with CYP showed hyalinisation and disorganisation of the photoreceptor layer. These degenerative changes were explained by the toxic effect on the retinal blood vessels causing retinal hypoxia. Hypoxia-induced reactive changes in glial cells (responsible for retinal vasculature development), which, in turn, result in an imbalance of pro-angiogenic and anti-angiogenic factors leading to retinal degeneration during vascular development and migration of blood vessels in the neural retina [17]. Massengill et al. [12] explained the retinal degeneration by the occurrence of the inflammation. The inflammation was mediated by the accumulation of cyclic guanosine monophosphate causing the opening of calcium channels in the rod cells and subsequent rod cell death which was followed by the degeneration of other retinal layers [9]. Our results also showed a concomitant significant decrease in the intensity of the toluidine blue-stained retinae (CYP group) as compared to the control group. The reduced intensity was explained by the loss of Nissl's bodies (basophilic ribosomal ribonucleic acid substances) as reported by Ajibade et al. [2] which added that neuronal degeneration followed by defective protein synthesis and loss of neuronal function. Our immunohistochemical results showed a significant increase in the expression of collagen IV in the sensory retina of the CYP group as compared to the control group denoting the neovascularisation (proliferation and abnormal organization) of retinal blood vessels, as reported by Dorrell et al. [5]. The retinal neovascularisation was owed to two factors (hypoperfusion and inflammation), but the inflammatory process might precede the ischaemia [21]. The inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) were claimed to cause endothelial proliferation and pathologic angiogenesis [4]. Also, the sensory retina of the CYP group showed a significant increase in the number of caspase 3 immune-positive cells as compared to that of the control group, this explained that the retinal neuro-degeneration occurred through caspase-dependent apoptotic mechanism stimulated by the oxidative stress [23]. NAC allowed significant protection of the sensory retina of the CYP and NAC group by preventing the neural degeneration and pathological apoptosis with the preservation of its Nissl's bodies. Moreover, NAC allowed normal development of retinal blood vessels preventing neovascularisation. The protective role of NAC could be explained by acting as a glutathione precursor (cysteine is required for endogenous antioxidant glutathione production), down regulator of the expression of several inflammatory cytokines genes and microglia proliferation inhibitor [20].

CONCLUSIONS

The prenatal exposure of the rats to CYP led to damage to their sensory retina and the use of NAC as a protective agent resulted in the preservation of the normal structure of the sensory retina.

The experiment was conducted at Human Anatomy and Embryology Department, Faculty of Medicine, Menoufia University.

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