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Full Length Research Paper

Preventive effects of silymarin in retinal intoxication with methanol in rat: Transmission electron microscope study

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The aim of this study was to investigate the ultra-structure of the photoreceptor layer of male rats under the effect of methanol intoxication and protective effect of silymarin against the methanol toxicity. Fifteen adult male rats were divided into three groups: Control group, Experimental group I (received 4g/kg methanol by intraperitoneal injection for five days), Experimental group II (received 4 g/kg methanol by intraperitoneal injection for five days and received 250 mg/kg silymarin orally for three months). At the end of the experiment, the eyes were removed; retina was separated near the optic disc and studied by transmission electron microscope. Results showed that the retina in the experimental group I exhibited loss of outer segments and disorganization in inner segment. Increased extra cellular space, disappearance of outer limiting membrane and pyknotic nuclei were seen in this group. But normal outer segment, organized inner segment and normal outer limiting membrane were obvious after treatment with silymarin in experimental group II. These findings show that methanol causes damage in the photoreceptor layer of the rat retina and silymarin can protect the damage to retina against the methanol intoxication.

Key words: Ultra-structure, photoreceptor layer, methanol intoxication, silymarin, rat.

INTRODUCTION

Silymarin is an antioxidant flavonoid complex derived from milk thistle (*Silybummarianum*) (Mosallanejad et al., 2012).These flavonoids are potential antioxidants for different toxicities and induced oxidative stress. There are many toxicological studies which show the protective activities of silymarin on different organs (Jain et al., 2011; Burczynski et al., 2012; Das and Mukherjee 2012; Muley et al., 2012).

The vertebrate retina is a light-sensitive tissue lining the inner surface of the eye. Methanol intoxication produces toxic injury to the retina and optic nerve, resulting in blindness (Seme et al., 1999; Eells et al., 2003). Formic acid, produced in methanol intoxication, is the toxic metabolite responsible for the retinal and optic nerve toxicity. Previous studies have documented formate induced retinal dysfunction in a rodent model of methanol intoxication (Seme et al., 1999). Also, an ultra-structural examination showed swelling and disruption of the mitochondria in photoreceptor inner segments, optic nerve and the retinal pigment epithelium in methanol-intoxicated rats (Murray et al., 1991). Some methanol intoxication findings support the hypothesis that formate inhibits retinal mitochondrial function and increases oxidative stress (Seme et al., 2001).

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The establishment of rodent model of methanol intoxication will facilitate research into the mechanistic aspects of methanol toxicity and the development and testing of treatments for human methanol poisoning (Eells et al., 2000). In continuation of previous studies on silymarin and methanol intoxication, we investigate preventive effects of silymarin in methanol intoxication on the retina of rats by transmission electron microscope.

MATERIALS AND METHODS

Fifteen mature male Wistar rats aged three months were used in this study. Initially, the animals were randomly divided into three groups of five rats each: Control group, Experimental group I (received 4g/kg methanol by intraperitoneal injection for five days), Experimental group II (received 4g/kg methanol by intraperitoneal injection for five days and received 250mg/kg silymarin orally for three months before methanol injection). At the end of experiment, animals were anesthetized with ketamine and euthanized using compressed CO_2 gas using a Plexiglas chamber. Gas delivered in a predictable and controllable fashion, at a low-flow rate. Started with room air then slowly filled the chamber with CO_2 for 10 min. Euthanasia was carried out in a laboratory away from the animal facility. CO_2 first renders the animal anesthetized and then with adequate exposure time will result in death by CO_2 narcosis. Confirmatory methods to be performed after CO_2 overdose include 50% additional time in the euthanasia chamber filled with 100% CO_2. The retinal sample preparations were obtained as described in our previous study (Esfandiari et al., 2012). Briefly, the eyes were enucleated, cornea and vitreous body were removed and the eyes were placed in 4% gluteraldehyde fixative for 4 h. The retina was separated near the optic disc and processed for transmission electron microscope. Ultrathin section of retina evaluated on a Philips CM-10 (Philips Eind hoven, Netherlands). The photoreceptor layer of retina was compared between the control and each experimental groups. The morphometrical parameters were analyzed using the Statistical Package for the Social Sciences (SPSS) version 16 and analyzed with one-way ANOVA and post hoc Tukey test. The significance level was set at $P \leq 0.05$. Ethical approval was granted by the ethical Committee (Iranian Society for the Prevention of Cruelty to Animal, and Iranian Veterinary Organization) and all studies were performed in accordance with the Guide to the Care and Use of Laboratory Animals (Olfert et al., 1993).

RESULTS AND DISCUSSION

In the control group, the retinal outer segment exhibited bimembranous discs. The inner segment contains round to oval mitochondria present near the outer segment. The inner segment appeared normal without increased extracellular space. The outer limiting membrane appeared normal and contained zonula adherents junction in this group. The outer nuclear layer consisted of round to oval nuclei with normal heterochromatin in the center of the nuclei (Figure 1).

The ultra-structure and morphometry of photoreceptor layer in the experimental groups were evaluated after methanol intoxication and with protection effect of silymarin. The experimental group I had major signs of pathology. The loss of the outer segment was seen and vacuole formation in bimembranous discs of outer segment was obvious (Figure 2). Scattered and disorganized inner segments were seen in group I (Figure 2). Increased extracellular space was apparent in inner segments (Figure 2). However, the inner segment region looked abnormal and diffused. Some pyknotic nuclei in the outer nuclear layer were obvious. The outer limiting membrane disappeared in this group (Figure 2). The minor signs of histopathology were seen in experimental group II. The outer segment appeared normal and organized inner segments were seen but some vacuole in inner segments was obvious (Figure 3). The outer limiting membrane appeared normal but pyknotic nuclei in outer nuclear layer were seen (Figure 3). The mean thickness of the photoreceptor layer in the control group was 85.82±0.99 µm, whereas it was 61.92±2.75 µm and 85.44±1.61 µm in the experimental group I and II, respectively.

The quantitative study showed that the reduction in the thickness of outer segment, inner segment and outer nuclear layer in experimental group I were due to outer segment loss, scattering of inner segment and karyolysis in outer nuclear layer. Measurements of thickness of outer segment, inner segment and outer nuclear layer were significantly lower in experimental group I than in the control and experimental II groups.

The present study was undertaken to test the hypothesis that silymarin, as an antioxidant, would protect the retina against the methanol toxicity. Methanol can severely damage many systems, especially visual function (Osborne, 1977; Patra et al., 2006). As in the case of many chronic degenerative diseases, increased productions of reactive oxygen species (ROS) have even been considered to play an important role in the pathogenesis of methanol toxicity (Pawlosky et al., 1997; Paula et al., 2003). Also, Alaa El-Din et al. (2011) indicated that methanol treated rabbits show a significant decrease in the level of endogenous antioxidant. The data obtained in the present study shows that silymarin protected the different retinal parts which may relate to its antioxidant effects in prevention of oxidative stress. The outer segment loss was obvious in experimental group I. Probably, oxidative stress of methanol intoxication in the outer segment is associated with double bond breaking of 11-cis-retinal during cis-trans isomerization. This photochemical reaction has been reported to cause the formation of oxygen free radicals. Oxygen free radicals can also form protein peroxides (Williams, 2008). The changes of the primary structure of protein may induce loss of outer segment layer. The scattered and disorganized inner segment occurred in experimental group I. Therefore, these changes give rise to mitochondrial damage and reduce oxidative metabolism which may also lead to photo oxidative damage by ROS formation. The pyknotic nuclei was observed in experimental I and II groups. These changes are responses to injury or damage cells. The histological change decreased in experimental group II compare with experimental group I. Results of the present investigation show that the silymarin treatment is useful in functional recovery of the

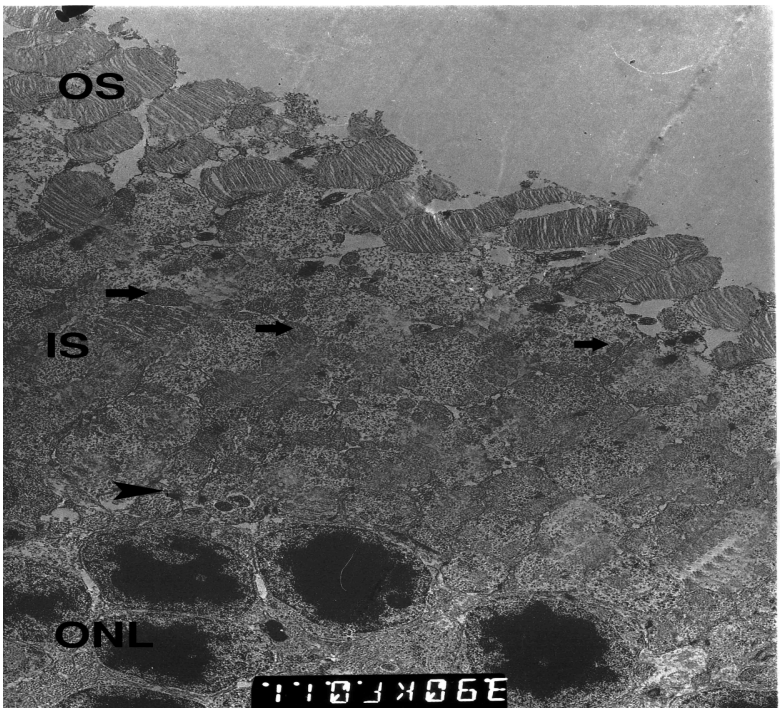

Figure 1. Electro micrograph of the photoreceptor layer from the control group. OS, outer segment; IS, inner segment; outer limiting membrane (arrowhead); ONL, outer nuclear layer; mitochondria (tick arrows). (× 3900).

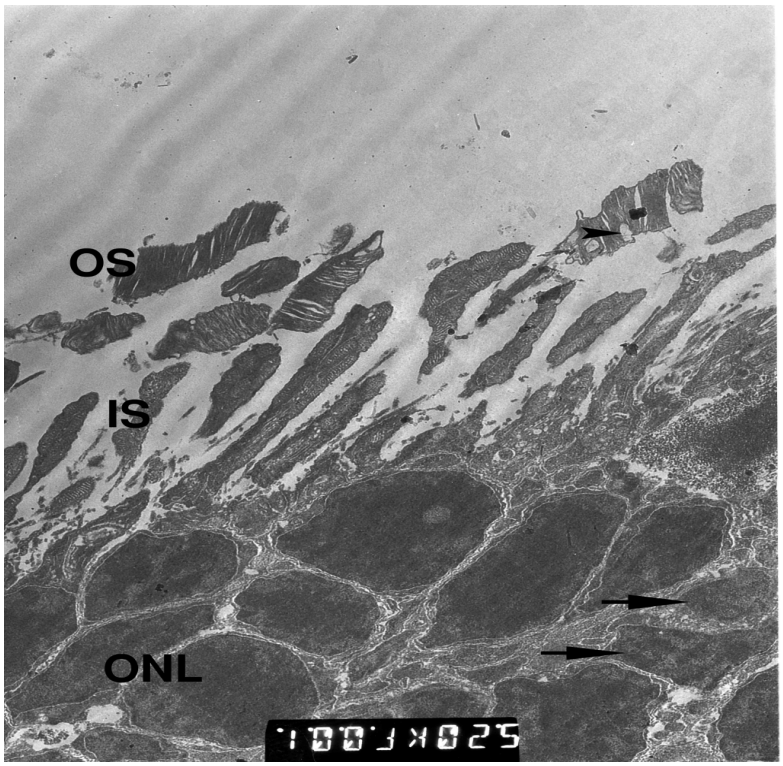

Figure 2. Electro micrograph of the photoreceptor layer from the methanol group. OS, disorganized outer segment; IS, scattered inner segment; pyknotic nuclei (arrows) and vacuole in outer segment (arrow head). (× 5200).

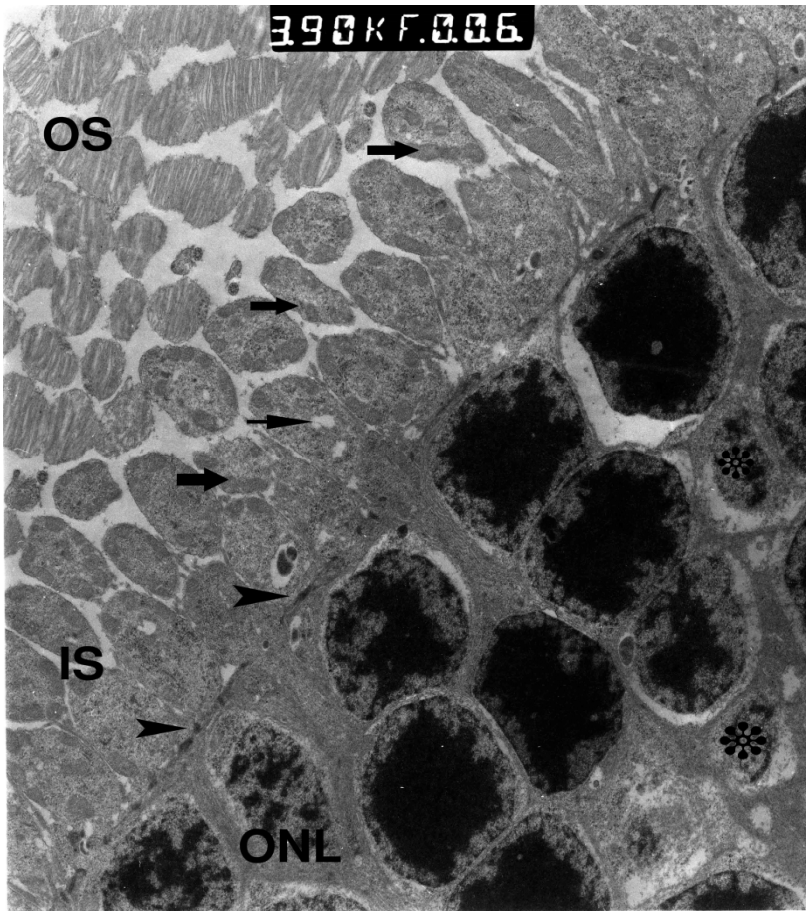

Figure 3. Electro micrograph of the photoreceptor layer inmethanol-silymarin group. OS, Normal outer segment; IS, inner segment; ONL, outer nuclear layer; mitochondria (thick arrows); vacuole in inner segment (arrow); outer limiting membrane (arrow head); pyknotic nuclei (asterisks). (× 3900).

retina after injury by the methanol intoxication. It is concluded that silymarin treatment with 250 mg/kg dosage protects the photoreceptor layer from the histopathology changes of methanol intoxication and advances the recovery of photoreceptor function.

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