

Research Article



The Possible Protective Effect of Bupropion (0.5%) Eye Drops Against Selenite Induced Cataract in Rabbits

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ABSTRACT

Cataract is the opacity of the crystalline lens that leads to visual impairment or blindness. The study aimed to investigate the possible protective role of bupropion (0.5%) eye drops against selenite induced cataract in rabbits. Adult rabbits with body weight in a range of (1.5-2 kg) with no signs of ocular inflammation were used in the present study. The induction of cataract was done by intravitreal injection of 0.1 ml (0.01% w/v) of sodium selenite solution in the right eyes. Lens opacity, pupil diameter, intraocular pressure (IOP), pupillary response to light, corneal sensation, conjunctival redness, level of malondialdehyde (MDA) and reduced glutathione (GSH) in aqueous humor of rabbit eyes and histopathological study of lens were done. Bupropion (0.5%) eye drops resulted in prevention of cataract development and there was no change in pupil diameter, positive pupillary response to light, no corneal sensation, no conjunctival redness, decreased level of MDA and increased level of GSH. Histopathological study of lens showed that bupropion (0.5%) eye drops resulted in prevention of the aggregations of proteins and the cytoplasm appeared as homogenous and there was no dark and light areas in cytoplasm when compared to cataract lens.

Keywords: cataract, selenite, bupropion, lens opacity

INTRODUCTION

Cataract is the opacity of the crystalline lens that lead to visual impairment or blindness¹. There are several risk factors may involved in cataract formation, including effect of aging, genetic, diabetes, exposure to sunlight (ultraviolet B radiation) for long-term, heavy smoking², and several drugs like corticosteroids tranquilizers, radiomimetic drugs³, quinoline, methotrexate, oral contraceptives, ergot, streptozotocin, methoxsalen, thiazide also may cause cataract⁴. Oxidative stress clearly known to induced lipid peroxidation of membranes of lens fibers⁵. Nitric oxide (NO) has an important role in the development of selenite-induced cataracts⁶. Bupropion is a pharmacologically unique antidepressant, as it is a weak inhibitor of neuronal reuptake of both dopamine and norepinephrine. Bupropion, in general is well tolerated and it does not block muscarinic, histaminergic, or adrenergic receptors⁷. Oxidative-antioxidant-related study effects of bupropion on animal model showed that bupropion had positive antioxidant effects⁸.

The aim of the study is to investigate the possible protective effects of bupropion (0.5%) eye drops against selenite induced cataract in rabbits.

MATERIALS AND METHODS

Experimental animals

Adult rabbits (*Oryctolagus cuniculus*), age about one year with body weight in a range of (1.5-2 kg) with no signs of ocular inflammation were used in this study. The rabbits were housed in animal house of The High Institute for

Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University. All rabbits were maintained during the study with appropriate temperature and good ventilation.

Animals were kept on fresh diet and allowed for free access to water. The experiments were approved by Animal Ethical Committee, College of Medicine/Al-Nahrain University, Baghdad, Iraq.

Rabbits were divided into three groups: normal, cataract and bupropion (0.5%) eye drops.

Induction of cataract

The rabbits were anesthetized by an intramuscular injection of 0.5 ml of Ketamin (50 mg/ml). In addition Lidocaine solution in concentration of (2%) was applied locally to the eyes to obtain additional anesthesia. The induction of cataract in the right eyes was done by single intravitreal injection of 0.1ml from of sodium selenite solution (0.01% w/v)⁹. After injection, the rabbits were monitored every day for cataractogenesis¹⁰.

Bupropion (0.5% w/v) eye drop preparation

The tested drug used was powder of high purity (Bupropion reference standard from USP Rockville, MD LOT). A 500mg bupropion powder and 1% (w/v) of benzalkonium chloride were dissolved in 100 ml of isotonic buffer solution. All substances used are of high purity and eye drops are prepared freshly.

The selection of the concentration and frequency of drug administration was based on a pilot study which was done preceding the study, and the concentration was



found with beneficial effects and the formulation found to be effective without side effects on rabbits eyes (there was no irritation, no conjunctival redness).

Lens opacity

The score of lens opacity (by the use of ophthalmoscope grading criteria) was done in accordance with the classification of Kador¹¹ and Chylack¹².

Pupil diameter

By using the pupil gauge, measuring of pupil diameter was done and the results would be presented in millimeter units¹³.

Pupillary response to light

The light reflex was examined by swinging flashlight to investigate a relative afferent pupillary defect. The obtained results would be expressed as either it was intact or absent^{14,15}.

Corneal sensation

Corneal sensation could be examined with wisp of cotton wool which applied and moved from side to side. The results was presented as either the corneal sensation was intact or absent¹³.

Conjunctival redness

It could be detected by examination of conjunctiva of both eyes and the results would be either present or not¹⁵.

Intraocular pressure (IOP measurement)

IOP measurement were done by anesthetization of the cornea with a local anesthetic (2% lidocaine hydrochloride), and "the foot plate of the tonometer is placed on the cornea (90° on the pupil), a small force (weight) is applied to a central plunger, readings from the tonometer is converted to the corresponding mmHg of tension by referring to a standard chart"¹⁶.

Measurement of glutathione (GSH) and MDA level in aqueous humor of rabbit eyes

Glutathione was measured in accordance with the method of Godin¹⁷ regarding to the reaction of glutathione with 5,5- Dithiobis (2-nitrobenzoic acid) (DTNB) at PH of 8, the result was a colored complex which absorbed light at 412 nm and this was directly proportional to the concentration of GSH.

The technique to find out the MDA level is based on the reality that, in acid medium, MDA reacts with thiobarbituric acid (TBA) to form a pink-colored MDA-TBA complex that exhibits an absorption maximum at 532 nm^{18,19}.

Histopathological study

The rabbit eye lens samples fixed by Gluteraldehyde (3%) solution for 48 hours. Following washing, treatment with osmium tetra oxide (1%) for 20 minute, washing,

dehydration at 4C° and embedding, tissues capsules sectioned at (1micron), these sections stained with solution A (0.4% basic fucshin in 25% methanol) and B (Prepared by mixing the same volumes of (azure II, methylene blue, Na₂ CO₃, absolute methyl alcohol) and examined microscopically²⁰.

RESULTS

Lens opacity

The lenses of eyes of rabbits before cataract induction were intact and transparent, after one week of cataract induction the mean of cataract maturity of right eyes (\pm SEM) was (4.0 \pm 0.0). The right eyes received bupropion (0.5%) eye drops and treatments to this group were twice daily drops for five days before cataract induction and extended for twenty-one days after cataract induction which resulted in prevention of lens opacity, the results were same until the end of this study, figure (1) and associated table.

Pupil diameter

Instillation of bupropion (0.5%) eye drops two times daily could not induce any alteration in pupil diameter and the mean measurement was (9.12 \pm 0.29 mm).

Pupillary response to light

The pupillary response to light of both eyes was positive before and after instillation of bupropion (0.5%) eye drops twice daily and until the end of study.

Corneal sensation

The corneal sensation of both eyes was positive before and after instillation of bupropion (0.5%) eye drops.

Conjunctival redness

There was no conjunctival redness in rabbits eyes before and after instillation of bupropion (0.5%) eye drops.

Intraocular pressure

The mean \pm SEM of intraocular pressure (IOP) of rabbits eyes was represented in figure (2) and associated table.

The GSH and MDA levels

The levels of GSH and MDA were measured at the end of the study in the aqueous humor of normal, cataract and bupropion (0.5%) eye drops groups, figure (3) and figure (4) and associated tables.

Histopathological study of rabbit lens

The cytoplasm of normal eye was uniform, featureless, and it was stained homogenously as shown in figure (5A). In the lens of cataract group, there was thick darkly stained collectives inside the fiber which extended along the lens fiber, these aggregations characterize the insoluble proteins that build up and aggregate in the lens fiber which caused by the oxidative and sclerotic outcome of selenite on the lens proteins. These aggregations are surrounded by plain or lighter areas produced as a result

of losing the cytoplasm its homogenous form as shown by figure (5B). As shown in figure (5C), bupropion (0.5%) eye drops administered twice daily prevented the aggregations of proteins and the cytoplasm appeared homogenous and there was no dark and light areas in cytoplasm when compared to cataract lens.

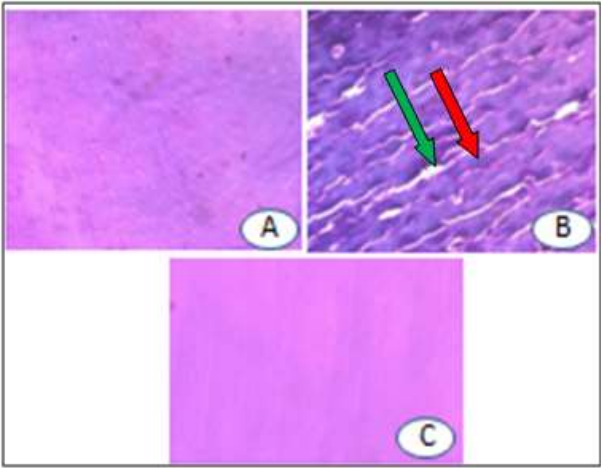
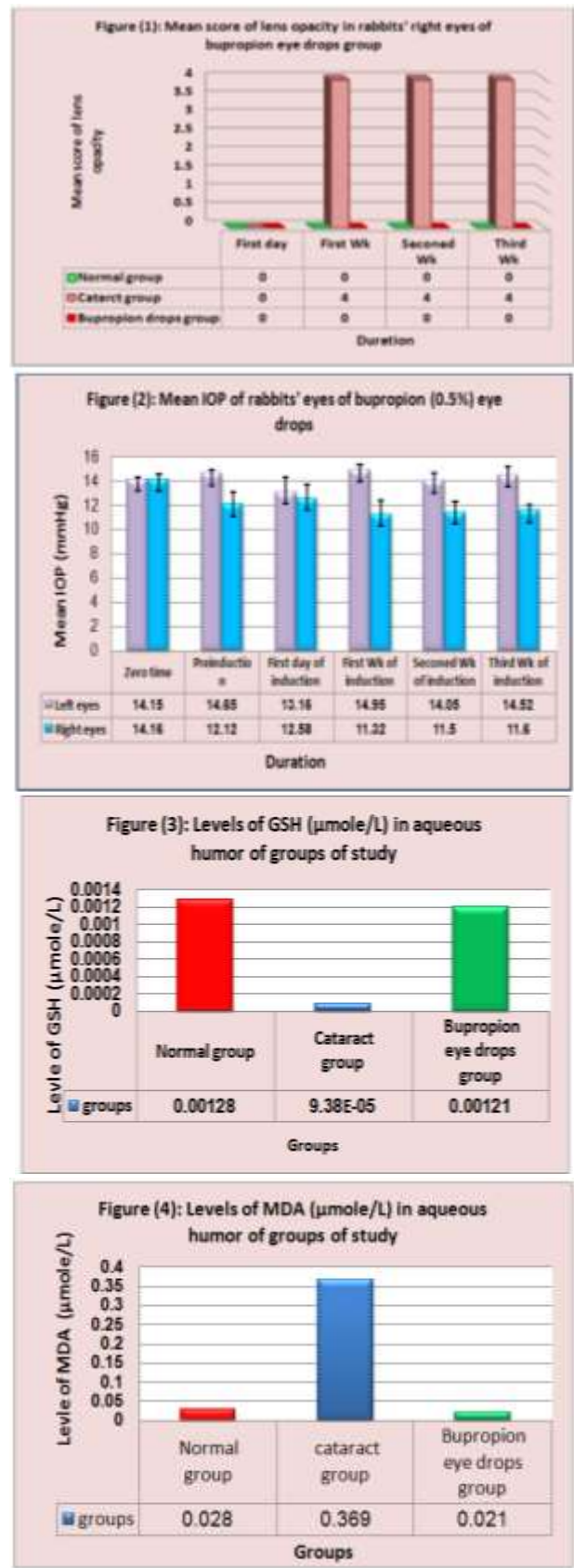


Figure 5: longitudinal section of rabbits' lens stained with solution A and B for semithin section (40X). A: Normal group shown homogenous cytoplasm of the normal lens fibers. B: Cataract group shown darkly stained aggregation (red arrows), alternating with clear areas (green arrows) with loss of the homogenous architecture of the cytoplasm of lens fibers. C: longitudinal section of lens of bupropion eye drops shown normal like homogenous and structureless cytoplasm of lens fibers.

DISCUSSION

The results gave indication to the beneficial prophylactic and anticataract effect of eye drops of bupropion. Some data support that a number of antidepressants including bupropion are capable of "modulate NO synthesis and nitrosative stress-associated signalling cascades". Dhir and Kulkarni²¹ experienced different dosages of bupropion in male rats. The antidepressant effect of bupropion was prohibited by pretreatment with L-arginine which is a substrate of nitric oxide synthase (NOS) and pretreatment with 7-nitroindazole which is a specific neuronal NO synthase inhibitor caused potentiating of bupropion's effects. Also, treatment with methylene blue which is a direct inhibitor of NOS and soluble guanylate cyclase caused potentiating of the bupropion effect in the forced swim test²². In the present study, the IOP of right and left eye were measured to exclude if that bupropion had been caused increase in IOP as one of its side effects, but the results showed that bupropion had decreased the IOP in normotensive rabbit eyes and these results could be a benefit to the use of bupropion.

The norepinephrine-dopamine reuptake inhibitor property of bupropion is thought to also inhibit tumor necrosis factor (TNF) production²³, so the action of bupropion may include targeting TNF production which is secondary to neuroinflammatory processes. Because researches suggest that neuroinflammation have a role in animal models of glaucoma²⁴ and there is increasing evidence that bupropion may help decrease neuroinflammation, in other study, bupropion users had a significantly decreased risk of developing open angle glaucoma when compared to non-users.

TNF is a biomarker connected with retinal ganglion cells death in patients with glaucoma²⁵.

CONCLUSION

Bupropion (0.5%) eye drops twice daily had protective effect against selenite induced cataract in rabbits eyes.

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