# Effect Of Heparin Sodium In Protection Of The Lens Against Cataract Induced With Intravitreal Injection Of Sodium Selenite- In Rabbits

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**Abstract:** Objective: To evaluate the possible protective role of heparin sodium eye drops against sodium selenite – induced cataract in rabbits.Materials and Methods: A group of 18 adult rabbits (Oryctologus cuniculus) were divided into 3 groups each one of 6 rabbits normal group (without treatment and induction), control group (received DW pre and post induction of cataract), and heparin sodium group (received heparin sodium eye drops pre and post induction of cataract). The cataract had been induced by intravitreal injection of 0.1ml sodium selenite (0.01% w/v) in the right eye. Results: Heparin sodium was effective in prevention of cataract and the mean score of opacity was (0.17±0.01) at the end of trial period in stead of the expected score (4 ±0.00) which observed in DW group, and there was non significant difference comparing to pre induction ($p>0.05$). Conclusions: Heparin sodium eye drops exerted a detectable preventive effect against sodium selenite - induced cataract in rabbits, also it was found to be apparently safe and tolerable along the trial period.

**Keywords**; cataract,intravitreal sodium selenite, oxidation, lens proteins, lens opacity, heparin sodium.

## 1 INTRODUCTION

Cataract is a disease that affects the eye, it causes opacity of the lens which progressively impair the light transmission to the retina and finally prevents the vision 1,2. Oxidation of lens proteins SH- groups induce protein conformational changes leading to protein aggregation and opacification of the lens resulting in block of light transmission to the retina and then blindness 3. **CATARACT IS CURRENTLY TREATED BY SURGERY, BUT IN PRESENT STUDY DISCOVERED THAT THIS DISEASE CAN BE PREVENTED BY HEPARIN SODIUM, THE BENEFIT OF THESE IDEA IT AVOID THE COMPLICATIONS OF SURGERY.**

## 2 Materials and methods

### 2.1 Animal and Housing

A group of 18 adult rabbits (Oryctologus cuniculus) aged about one year with a range of body weight of 1.5-2 kg were obtained from the animal house stock of the Department of Pharmacology, College of medicine, AL-Nahrain University, Baghdad-Iraq. Animals were kept on fresh trefoil diet, water at libitum, suitable temperature and normal light. The included rabbits were allocated into 3 groups, normal group (without treatment and without induction of cataract) (n=6) , control (distilled water) (DW) group (n=6) and treatment (heparin sodium) group (n=6). Right eye of each included rabbit of DW and heparin sodium groups was instilled with 2 drops- 3 times / day of either distilled water (control group) or heparin sodium (treatment group); such administration started five days prior to induction of cataract (i.e. prophylactic use) and continued thereafter for further 21 days after the cataract being induced (i.e. therapeutic use).

### 2.2Preparation of sodium selenite solution

Amount of 10 mg of sodium selenite powder was dissolved in 100 ml distilled water to prepare the 0.01% w/v of selenite solution. A fresh solution was prepared for each use [4].

### 2.3 Induction of cataract

The rabbits were anesthetized by intramuscular injection of 0.5 ml of (50 mg/ml) ketamin, lidocaine (2%) solution was applied on the eye to obtain additional anesthetization locally, the induction of disease was done by inserting needle of (gauge 30,12.7 mm) (4 mm behind the limbus in sclera measured by caliper) to intravitreal injection of 0.1 ml from 0.01% w/v of sodium selenite solution in right eye, it was single injection [5]. After injection ,the rabbits were monitored for caractogenesis which begun after one hour and when opacity progression observed 6(Samuel et al, 2003) ,the rabbits were sent to slit-lamp examination (also to detect of cataract type) after instillation of tropicamide 0.5% and phenylephrine 10% to obtain maximum pupillary dilatation [2,7]. The type of cataract was Posterior sub capsular according opacity classification system [8,9]. In control right eyes the complete opacity (mature cataract) was observed after 48 to 72 hours of induction of disease.

### 2.4 Eye drop preparation [4,10]

Heparin sodium (5000IU/ml) eye drop preparation; Mixing 5 ml of heparin sodium (5000 IU / ml) with 1mg of benzalkonium chloride. (Each 1 drop contains 250 IU of heparin sodium).

### 2.5 The treatment design

Heparin sodium (5000IU/ml) eye drops were administered as 2 drops topically 3 times/day to the right eye for five days prior to induction of cataract (i.e. prophylactic use) and continued there after for further 21 days after the cataract being induced (i.e. therapeutic use). where as left eyes were received the distilled water. While the control group was received distilled water. Each group included 6 rabbits. The groups of this part of study were:

1. Negative control (distilled water) group. Distilled water was administered to both eyes of rabbits.
2. heparin sodium (5000IU/ml) group

### 2.6 The parameters

which detected and followed up for both eyes of each included rabbit in this study, which measured at morning and repeated every day;

**1- Maturity of cataract**

The score of lens opacity (by using ophthalmoscope grading criteria) was determined according to the cataract classification [8,11,12].

**2-Pupillary response to light [12,13].**

**3- Signs of hemorrhage**
Daily monitoring of the eyes during the experiment for any bleeding (the inner side of eyelid, conjunctiva, sclera, and cornea) [14,15]. If there was hemorrhagic spots in the vitreous and aqueous humor they can be distinguished during the eye motion, they will be observed move in the media 16, during ophthalmoscopic and slit-lamp examination [12,15,17,18].

**2.7Partial thromboplastine time PTT;**
The heparin treated rabbit groups (prophylaxis and treatment) and control groups were prepared to draw 5 ml of blood was aspirated from heart of each rabbit that received heparin in the present study in order to determine PTT from heart and put 4.5 ml of blood in tubes contain sodium citrate 0.5 ml then sent the samples (prophylactic groups; 6 tubes from control group, 6 tubes of heparin group) and (treatment groups; 6 tubes of control, 6 tubes of topical heparin group, and 6 tubes of injected heparin group) to the laboratory for the test [19].

**2.8Ophthalmoscopic examination and opacity grading**
The eye examinations were daily carried out in a dark room with a direct ophthalmoscopes and instillation of tropicamide (0.5%) and phenylephrine (10%) eye drops to obtain maximum pupil dilatation 9. Opacities that obscured the red reflex were scrutinized from several angles of view to determine their location in relation to the lens. The grading of opacity included assessment of the area of clear red reflex from area without red reflex of retina 9. By using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined according to the classification of Mehra and Minassian and Chylack [8,17].The ophthalmoscopic examination was done in dark room after measuring the pupillary response to light to avoid the influences of mydriatic drops. slit-lamp (Topcon com.\japan) was also used to evaluate the lens opacity [8,13,14]. At the end of experiment, the rabbits were killed and the lenses were extracted by posterior approach [20]. Then the lenses were sent directly to biochemistry lab., to measure each of reduced glutathione (GSH) [17] level, and malondialdehyde (MDA) level [18]., and a small parts of the lenses were fixed in Gluteraldehyde (3%) to prepare semithin sections for electron microscope (EM) study 21,22.

**2.9Statistical analysis:**
All data were expressed as mean (±SD). Paired and unpaired t-tests were used accordingly for assessing the effectiveness of employed therapy for the right eyes of rabbits in a given group, to compare between the results of right and left eyes in the same rabbit, and the right eyes of rabbits of two groups. Chi-test was used whenever it was applicable (i.e. for independent qualitative data). $P<0.05$ was considered significant [25,26].

## 3 Results
The type of cataract that could be obtained in the present study was found to be Posterior sub capsular (PSC) according opacity classification system. (Chylack L. et al 1993 and Datiles MB. et al 2003)[8,9] as shown in [Figure 1]. In control right eyes the complete opacity (mature cataract) was observed after 48 to 72 hours after induction of the disease. According to opacity classification system [8,9], the type of induced cataract in the present study was found to be posterior subcapsular one (PSC) [Figure -1]. Complete opacity (mature cataract) could be achieved in lenses of control group after 48-72 hours of intravitreal injection of sodium selenite. [Figure 2] demonstrated the difference between two lenses: cataractous (after cataract being induced) one which appeared opaque and normal one which appeared transparent. As shown in [Figure 3], cataract in the control group could advance with time to reach hypermature stage.

**3.1The EM study of normal and control lenses**
Normal eye lens; shown a homogenous, featureless cytoplasm, and it homogenous stained and has dens appearance. [Figure 4]. Control eye (of DW group); there was enlarged irregularly shaped fibers. Cytoplasm was lost its featureless, homogenous and dens appearance. There is thick darkly stained aggregations inside the fiber and extended along the lens fiber, and make a connected network across fibers, these aggregations represent the insoluble proteins that accumulate and aggregate in the lens fiber (cause of the lenticular opacity) which resulted from the oxidative and sclerotic effect of selenite on the lens proteins, and these aggregations are surrounded by clear or lighter areas, and these areas resulted from losing the cytoplasm its homogenous appearance. [Figure 5].

**3.2 Distilled water (control) group**
Control (DW) group: Prior the cataract induction, lenses of right eyes of the included rabbits were intact, transparent, and had intact response to light; instillation of DW eye drops for 5 days did not affect them and the mean score of opacity remained (0±0.0). After cataract being induced and instillation of DW eye drops was continued, the mean score of opacity increased to be (4± 0.00); comparing to pre induction value, such increment was highly significant. These eyes lacked their response to light and persisted so along the trial period. [Figure 6].

**3.3 Heparin sodium (5000IU/ml) group**
Prior the induction of cataract the lenses of 6 included rabbits right eye were intact and transparent pre and post instillation of heparin sodium eye drops and the mean score of opacity (mean ± SEM) was (0±0.00) for 5 days . After cataract being induced and instillation of heparin sodium was continued the mean score was (0.7 ± 0.21) at the 7th day, and in comparing with right eyes pre induction there was a significant ($0.01 <P< 0.05$) difference, at the 14th day also there was a significant ($P<0.01$) difference , the mean score was (1.0 ± 0.00), at the 21st there was non significant difference ($P > 0.05$) and the mean score was (0.1± 0.01).Figure (7) The heparin sodium (5000 IU/ml) eye drop was more efficient in cataract prevention effect than distilled water during trial period. [Table 1]. All included rabbits right eye had intact light reflex after instillation of heparin sodium for 5 days pre induction of cataract. Post induction of cataract also all right eyes had light reflex along the trial period post induction. Regarding light reflex, there was no significant difference ($P > 0.05$) at any time during the trial period comparing to pre induction in heparin sodium group. However there was significant ($p < 0.01$) difference regarding comparison results of light reflex, in heparin sodium group with those of distilled water post induction.

**Partial thromboplastine time (PTT)**
The PTT is not significantly ($P>0.05$) differed in heparin

sodium treated rabbits with those of control group and the mean of PTT in the control group was (32.3 ± 0.21) sec., and in the heparin sodium group was (32.5 ± 0.22) sec. Also there was no sings of hemorrhage when the eyes were examined.

### 3.4The EM study of heparin sodium treated lenses

Heparin sodium prevented the aggregations of proteins and the cytoplasm seemed homogenous. And there was no clear areas which results from shrinking and accumulation of cytoplasmic material from peripheral to the center of the lens fiber. [Figure 8].

### 3.5The GSH and MDA levels

The GSH and MDA levels that measured at the end of prophylaxis, are shown in [Table 2]. The GSH level in heparin sodium group was higher than that in DW group, and the MDA level heparin sodium group was less than that in DW group. While these levels in heparin sodium group not differed from normal. The comparison in GSH, MDA levels between groups is shown in the table (3), and [Table 4].

## 4 Discussions

Cataract is any opacity in the lens. Cataract is progressively impairs the light transmission to the retina and finally prevents the vision [27]. heparin sodium (5000 IU/ml) 3 times/day used prophylactically; had no effect on the transparent lens in normal eyes after 5 days of its instillation. Furthermore, it was able to prevent sodium selenite from raising the mean score of opacity to its expected value (4± 0.00) (that had been detected in distilled water group) after the injection of selenite. After 21 days of selenite injection, and with continuation of heparin sodium instillation, the right eyes were not significantly ($P > 0.05$) differed comparing to preinduction and the mean score of opacity was (0.17± 0.01). The EM study showed the role of heparin sodium to protect the lens proteins from opacification, because heparin prevented the aggregations of proteins, and the cytoplasm seemed homogenous. These result revealed that heparin sodium caused excellent prevention of cataractogenesis. The effect of heparin clearly was better than DW. These results pointed out to the beneficial prophylactic and therapeutic anticataract effect of heparin (5000IU/ml). Prophylactic study in the rabbit eyes which treated by heparin sodium eye drop they stay in their suppressed state without any progress of opacity for many months after induction, while in control eyes the opacity completed within 48-72 hours and persist along this period. In addition to that the response to light in heparin sodium group was present and intact, and this referring to that the opacity cannot reached and spreads to whole lens due to the lens protected with heparin. The results of PTT, approved the safety of heparin applied to the eye in tested doses, and had no systemic adverse effect.

### 4.1Possible mechanism of action

The antioxidant character of heparin. [28]. Heparin boosts the antioxidant effect of superoxide dismutase by releasing it near the endothelial cells of the vessels. On the other hand, heparin, as a sink of free radicals of oxygen [25,29.30]. Gilbert et al., (1997) found, the potential pathophysiological importance of the ability of heparin to alter NO production was relevant in the endothelial cells [31]. Whereas Karlsson et al., (1993); Zehnder, (2007) recorded that heparin has the ability to bind with enzymes and increase their activity, and among these enzymes is superoxide dismutase [19, 32]. In addition to that; NO reacts with thiols (compounds containing the SH groups) to form nitrosothiols . The proteins containing this groups will accumulated and their activity inhibit by this nitrosothiols [33], on the other hand heparin has suppressing effect on NO [31]. The heparin-binding affinity of the tetrameric extracellular superoxide dismutase (EC-SOD) is a result of the cooperative effect of the heparin-binding domains of the subunits, located in the hydrophilic, strongly positively charged C-terminal ends [32]. Liu et al., (2009) found the effects of heparin-superoxide dismutase conjugate (heparin-SOD) on CCl4-induced acute liver failure that altered the redox state with a decreased hepatic GSH and increased formation of lipid peroxidative products, which were partially normalized by treatment with heparin-SOD [34]. These results are agreed with the GSH and MDA determination results in the present study, in lenses that received heparin sodium. The results expressed that heparin has a considerable antioxidant activity, which take place in the protection the lenses from cataractogenesis, and this antioxidant action was noticeable in prophylaxis when heparin kept the level of GSH near to the normal level and there was no significant difference ($p > 0.05$), also heparin prevented the MDA level from elevation and there was no significant difference ($p > 0.05$) comparing with normal value. So, such antioxidant activity would be attributed to the obtain anticataract activity of heparin in present study, and this mechanism which expected in prophylaxis.

### 4.2Another possible mechanisms;

could be suggested as heparin aceylate lysine terminal of proteins and prevents conformational changes in proteins [35, 36]. And these conformational changes which cause the opacity of the lens [5]. So heparin prevented the opacity by these mechanism. Also heparin has ability to bind and activate the proteins [19]. So heparin may bind and activate the secondary proteolytic defense mechanism, which enhance to remove the degraded lenticular proteins that cause opacity. Crystallin, a major lens protein in all vertebrates, has been shown to act in a chaperone-like manner to inhibit protein aggregation [38]. Calpain which is a proteolytic enzyme activated by selenite, causes the loss of the C-terminus of alpha-crystallin polypeptides. This is important because the loss of the C-terminal peptide from -crystallin may induce conformational changes and reduce chaperone activity [39,40,41]. Because the formation of large protein aggregates in the lens causes light scatter and leads to cataracts, the net effect of the chaperone activity of -crystallin is the maintenance of lens transparency [40]. Decreased chaperone ability would promote formation of insoluble protein in selenite cataract [42]. heparin-binding domains of the subunits, located in the hydrophilic, strongly positively charged C-terminal and leads to cooperative effect [30]. So heparin expected to prevent the conformational changes and help to maintain the chaperone activity of - crystallin proteins and prevents the proteins precipitation. These mechanism which expected to involved in the treatable action of heparin sodium.

## 5 Conclusions

Heparin sodium eye drops exerted a detectable preventive effect against sodium selenite - induced cataract in rabbits, also it was found to be apparently safe and tolerable along the trial period.

## 6 Acknawlegement

A special thanks for ophthalmology consultant stuff- of Al-Dewanyiah Hospital , for thier coopartion and assistance.

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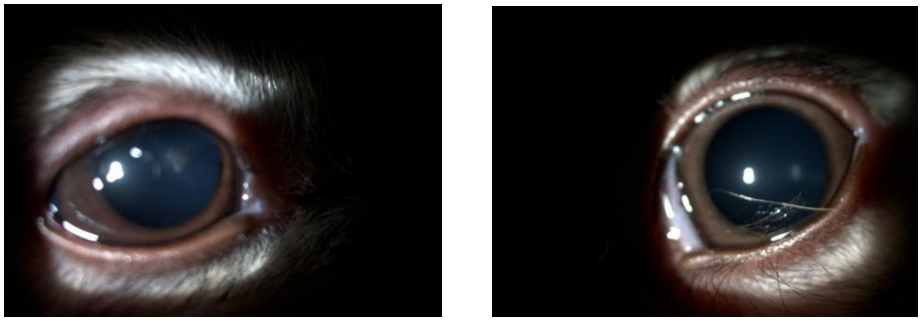


***(A)*** ***(B)***

***Figure (1)*** *Slit- lamp photograph for rabbit eye; A: Left normal eye. B: Right cataractous eye.(posterior subcapsular opacity).*


***Figure (2):*** *Normal (transparent) and Cataractous (opaque) Lenses of Rabbit*

***Figure (3)*** *Hypermature induced cataract*

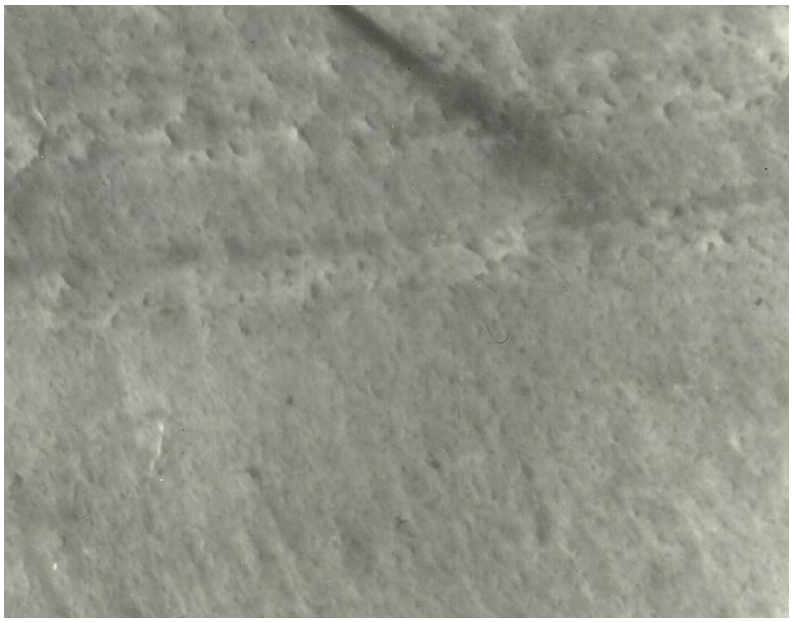


***Figure (4).****Electron micrograph; Longitudinal section of the normal lens shown the homogenous and featureless cytoplasm in the normal fibers. (10500 x).*

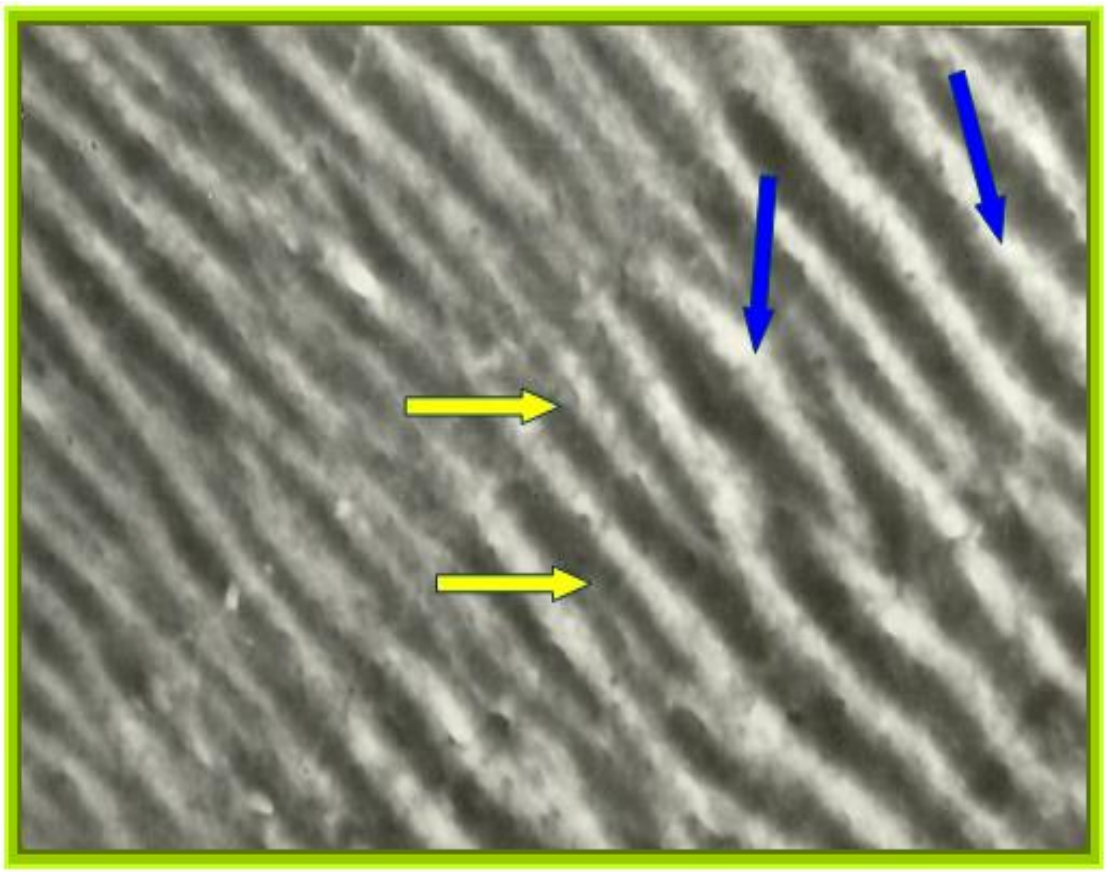
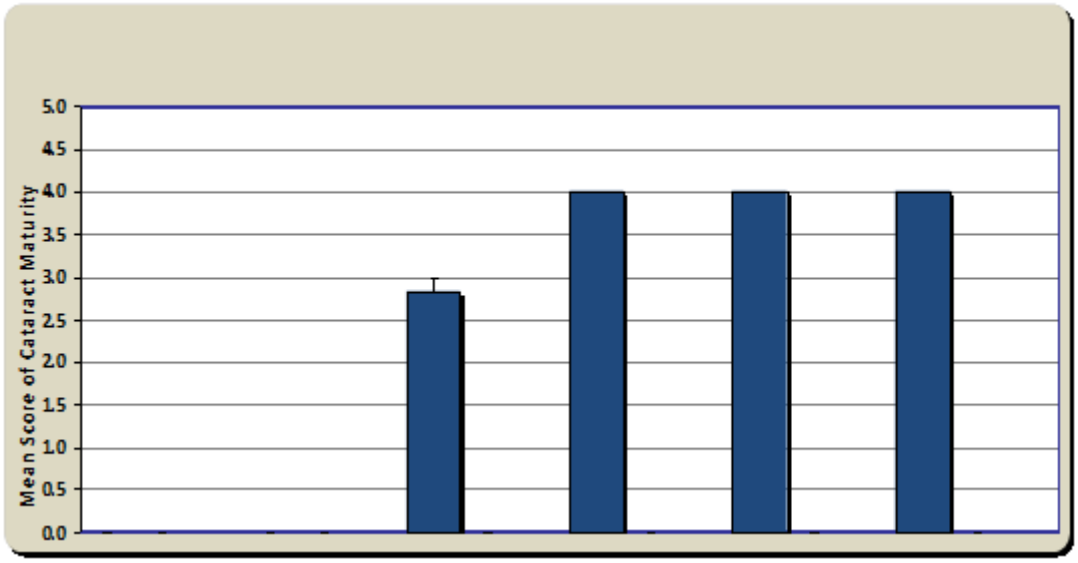


***Figure (5)*** *.Electron micrograph; Longitudinal section of the cataractous (control) lens shown the darkly stained aggregations (yellow arrows) which surrounded by the clear areas (blue arrows) in the fibers and losing the homogenous state of cytoplasm of the cataractous lens fibers. (13500 x).*




| | (Pre-Induction) (Day) | | | | | (Post-Induction) (Day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 5 | | | 1 | | 7 | | 14 | | 21 | |
| | Rt | Lt | Rt | Lt | | Rt | Lt | Rt | Lt | Rt | Lt | Rt | Lt |
| Reduction | 0.0 | 0.0 | 0.0 | 0.0 | HS | 2.8 | 0.0 | 4.0 | 0.0 HS | 4.0 | 0.0 | 4.00 | 0.00 |
| ±SEM | 0.00 | 0.00 | 0.00 HS | 0.00 | | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

***Figure (6):*** *Effect of DW on Mean Score of Cataract Maturity in Rabbits pre and post induction of cataract (n=6), SEM = Standard Error of Mean, HS = high significant difference (p < 0.01) compared to corresponding preinduction mean score of cataract maturity.*

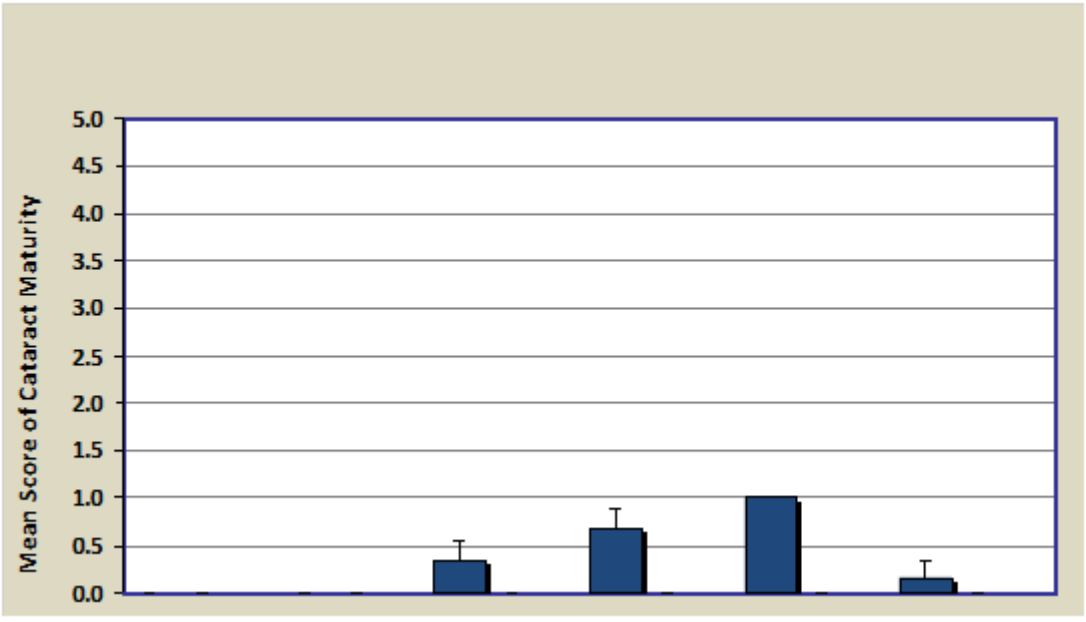



***Figure (7):*** *Effect of Heparin eye drop * on Mean Score of Cataract Maturity in Rabbits pre and post induction of cataract (n=6) SEM = Standard Error of Mean, NS = Not Significant difference (p > 0.05) compared to corresponding preinduction mean score of cataract maturity, S = Significant difference (0.01 ≤ p< 0.05) compared to corresponding preinductiont mean score of cataract maturity, HS = high significant difference (p < 0.01) compared to, corresponding preinduction mean score of cataract maturity*

***Table (1):*** *Significance of differences between heparin sodium (5000 IU/ml) and distilled water groups regarding the mean score of cataract maturity of right eyes of rabbits.*

| | Pre induction (Day) | | Post induction of cataract (Day) | | |
|---|---|---|---|---|---|
| **Group** | **0** | **5** | **7th** | **14th** | **21st** |
| **Distilled Water** | **No** | **No** | **HS (H)** | **HS (H)** | **HS (H)** |

0 = Baseline (Pre-treatment), No = no difference (normal eyes), HS = Highly significant difference ($P \leq 0.01$), H = the lowest value belongs to heparin sodium group.

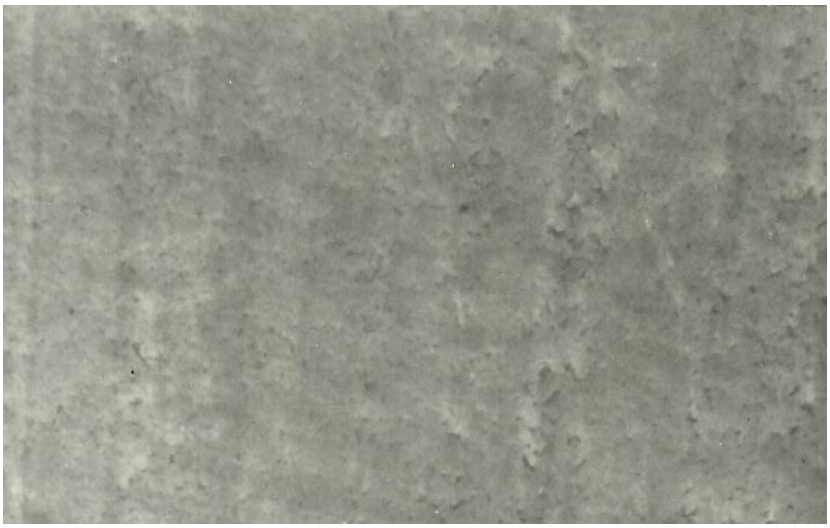


***Figure (8).****Electron micrograph; Longitudinal section of the lens protected with heparin sodium (5000IU/ml) eye drop, shown the homogenous cytoplasm without any darkly stained aggregations. (10500 x).*

***Table (2).*** *The levels of GSH and MDA which are measured at the end of prophylaxis. (the values in µ Mol/l).*

| µMol/l | Normal group | DW group | Heparin sodium group |
|---|---|---|---|
| MDA | 0.034±0.00042 | 0.21±0.0025 | 0.035 ± 0.00019 |
| GSH | 0.00126±0.0000017 | 0.000465±0.000005 | 0.00125±0.0000013 |

***Table (3).*** *The Significance of differences in GSH levels, between the heparin sodium group , DW group, and normal group.*

| GSH | DW | Heparin sodium |
|---|---|---|
| Normal | HS (N) | NS |
| DW | | HS (H) |

HS = highly significant difference ($P \leq 0.01$), NS = Non Significant difference ($p > 0.05$), N= the highest level belong the normal group, H= the highest level belong the Heparin sodium group.

***Table (4).*** *The Significance of differences in MDA levels, between the heparin sodium groups, DW group, and normal group.*

| MDA | DW | Heparin sodium |
|---|---|---|
| Normal | HS (N) | NS |
| DW | | HS (H) |

HS = Highly significant difference ($P \leq 0.01$), NS = Non Significant difference ($p > 0.05$), N= the lowest level belong the normal group, H= the lowest level belong the Heparin sodium group.