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Ameliorative effect of formulated chrysin administered in different routes on diabetic cataract in experimental animals

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Abstract

Diabetic cataracts are linked to oxidative stress, so to slow cataract progression, antioxidants should be used as therapy. Chrysin is a natural polyphenol has anti-inflammatory, antioxidant and antidiabetic effect with low bioavailability. Liposomes are nano-carriers for drug delivery. Our goal is to prepare Chrysin loaded liposome (CLL) ocular drug and study its potential as a topical medication, also compare with bioactivity of oral chrysin administration to treat diabetic cataracts. The thin lipid film method was used to synthesize CLL. Eighty male rats were divided into control, STZ- induced diabetic, Local and systemic treated groups. The systemic group received chyrsin orally once daily solubilized in 0.1% DMSO, whereas the treated group received CLL eye drop twice daily for 12 weeks. At end of experiment, slit-lamp imaging and lens examination were done. GSH, TSP, CP, Calpain, Caspase-3, and Ca were assessed in the lens tissue. There was significant difference of all parameters in treated groups compared to STZ diabetic group. An efficient CLL preparation was demonstrated by the results of DSC, TEM imaging, particle size, and zeta potential. In the lens tissue of diabetic rats, CLL demonstrated antioxidant effectiveness in oxidative stress-related parameters than systemic orally treatment. Lastly, the results showed that formulated chrysin might be suggested as a potential ocular drug for diabetes cataracts.

Keywords: Chrysin; Diabetic cataract; Liposomes; Oxidative stress.

1. Introduction

About 537 million people worldwide and 10.9 million people in Egypt suffer from diabetes. By 2030, that figure is predicted to increase to 13 million, and by 2045, it will reach 20 million **[1]**. One of the main processes in the development of diabetes mellitus is oxidative stress. **[2]**. Since the insulin does not regulate the diffusion of extracellular glucose into the lens, cataractogenesis is one of the first secondary consequences of diabetes mellitus. Worldwide, cataracts cause 51% of all cases of blindness **[3]**. Diabetic eye diseases comprise a number of pathological changes within the eye organ, which include, among others: cataracts, retinopathy, diabetic macular edema, dysfunctions of the tear film, and changes in corneal morphology **[4]**.

Due to the role of glucose-induced structural changes in lens proteins, diabetic status has been considered an important cause of accelerated cataractogenesis **[5]**.It has been found that one of the main causes of diabetes cataract development is glycation-induced lens damage, also, numerous routes, including increased osmotic stress, oxidative stress, or non-enzymatic glycation of lens proteins, have been used to study the pathophysiology of diabetic cataract **[6]**. Blood glucose management is required in diabetic patients to reduce the severity and progression of cataracts**[7]**.

Nowadays, the surgical removal of the cataract with intraocular lens implantation is currently the primary treatment for diabetic cataracts. About 20% of cataract operations are done on patients with diabetes **[8].** Despite the fact that cataract surgery is quite successful and implantation of an artificial lens eliminates the possibility of refractive changes, diabetic patients are regrettably more likely to experience intraoperative and postoperative problems than patients without the disease **[9].**

Flavonoids are being researched as potential medications that can lower the chance of developing cataracts**[7]**. Numerous disorders are associated with flavonoid advantageous biochemical and antioxidant characteristics **[10]**. Chrysin is a

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naturally occurring chemical with a range of pharmacological actions, including anti-inflammatory and antioxidant properties. Additionally, it had an antidiabetic effect comparable to that of insulin **[11]**. Chrysin's antioxidant activities, electron/hydrogen atom donation, singlet oxygen molecule quenching, and metal chelating potential are primarily responsible for its antioxidant effects **[12]**. Numerous studies have shown that chrysin has a very low oral bioavailability due to its rapid metabolism, rapid excretion, poor absorption, and poor solubility **[13]**.In recent years, the creation of ophthalmic formulations capable of restoring the preocular tear film and delivering hydrophilic and hydrophobic medications to the internal components of the eye has been one of the primary research issues. Liposomes are therefore a good way to achieve these goals when it comes to delivering drugs into the eyes **[14]**.

Liposomes are biocompatible, biodegradable, and nontoxic delivery systems that can include hydrophilic and hydrophobic medicinal substances. Phospholipids are the main component for creating encapsulated lipid bilayers, lipid–drug complexes, or sheet–drug complexes. Liposomes are essentially manufactured vesicles or colloidal particles **[15].** Furthermore, liposomes are an effective way to start and sustain drug release, and they may be used for ocular drug delivery **[16]**. This study's objective was toprepare biologically active nano formulation of chrysin liposomal ocular drug and Study the impact of it on the diabetic cataract of eye tissue of experimental diabetic rat models and also compare with bioactivity of oral chrysin administration to prevent or delay the diabetic ocular complications in type 1 diabetic rats.

2. Materials and Methods

2.1. Chemicals

Chemicals include: Chrysin, lecithin and cholesterol were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). EDTA, Sodium fluoride, Ethanol, NaCl, citrate buffer, diethyl ether, potassium phosphate from adwic Egypt Company. Streptozotocin from Tuko company. Phosphate buffer saline from Nasr Pharma company, Tris-HCl buffer from loba chemie company. Ketamine, Phosphotungstic acid, Soybean lecithin and EDTA from Advent Company. TCA from dop-ORGANIK sentex Company, and Folin ciocaltu reagent from Fisher scientific UK Company.Enzyme quantification kits were obtained from Bio diagnostic lab. Calpain and Caspase-3 Kits were purchased from SunLong Biotech Co, China.

2.2. Experimental animals.

Eighty Sprague Dawley male rats (160- 200 grams) were used in the experiment. They were acquired from the Research Institute of Ophthalmology's (RIO) laboratory animal research house. Experimental animals had unrestricted access to food and water in their stainless steel cages. Standard ventilation, temperature (25°C to 2°C), humidity (60–70%) and light/dark (12/12 h) were used to maintain the animals. The current work was approved by Fayoum University Supreme Committee for Scientific Research Ethics Committee (FU-SCSRE), (Code No. AEC 2427). Before induction of diabetes, the animals' eyes were checked with a Carl Zeiss slit lamp, and animals with any defect of lens were excluded.

2.3. Chrysin liposome preparation as eye drops

Liposome preparation as eye drops The method of **[17]** was utilized to create neutral multilamellar vesicles (MLVs) utilizing a soybean lecithin: chrysin molar ratio of 7:2. Briefly, 2.25 mg of chrysin powder and 20 mg of lecithin were added to 100 ml flask with a round bottom. At molar 1:7, cholesterol was added to the lipid make up. After adding 20 milliliters of ethanol, the flask was shaken to ensure that all of the lipids had dissolved. Using a rotary evaporator, the solvent was vacuum-evaporated until a thin, dry lipid layer developed. After that, the flask was placed under vacuum for an hour to be sure that any remaining ethanol had evaporated. After adding 10 ml of buffer (10 mM Trizma adjusted to pH 7), the flask was quickly stopped after being flashed through with a nitrogen stream. For half an hour, the flask was mechanically shaken at room temperature. over 50 degrees Celsius. Just aliquots of 20 mg of soybean lecithin were used to create control empty liposomes using the same traditional techniques as previously mentioned. It was determined that the chrysin/ml of trizma buffer concentration was 0.225 mg/ml.

2.3.1. Liposome morphology by TEM

The size and shape of the empty and chrysin-encapsulated liposomes were examined using a negative stain transmission electron microscope HR-TEM (Tecnai, G20, FEI, Netherlands) running at 200 kV. An aqueous solution of 1% w/v. As a negative stain, phosphotungstic acid stain was applied. A 20 µl aliquot of the liposome samples was placed onto a carbon-covered copper transmission electron microscopy (TEM) grid after they had been diluted (1:10) in tris buffer pH 7.4 at 37°C. After the solution was left for a minute, the excess was taken out of the grid using filter paper. TEM pictures were taken and analyzed.

2.3.2 DLS and Zeta potential

The zeta potential, mean particle size, and size distribution of liposomes loaded with chrysin and blank liposomes were measured using the "Nanotrac Wave II, Microtrac, USA" which is particle sizing method for DLS (dynamic light scattering) with 7.4 PH of tris buffer at 25 °C. The experiment was conducted in triplicate, and the data are displayed as mean ± standard deviation.

2.3.3. Differential Scanning Calorimetry (DSC)

By using an indium-calibrated DSC, (model DSC-50, Shimadzu, California, USA), the thermal behavior of lyophilized samples of empty and chrysin-loaded liposomes is examined. 5-mg sealed samples are Tested in standard aluminum skillets. Each sample's thermogram spans the temperature range of 25 to 200 °C at a scanning rate of 3 °C per minute.

2.4. Preparation of chrysin for systemic treatment

Chrysin was dissolved in 0.1% DMSO at 100 mg/kg body weight once a day,orally for 12 weeks. The choice of doses was based on previous studies **[18]**. Chrysin at a dose of 100 mg/kg body weight effectively reduced the fasting blood glucose (FBG) as shown by **[19].**

2.5. Experimental Design and induction of diabetes mellitus

According to **[20]**, diabetes can be brought on by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight, diluted in 0.1 mol/L citrate buffered solution (pH 4.5). Each rat's blood glucose levels were determined three days later. Any blood glucose levels exceeding 250 mg/dl were regarded as diabetic in the animals. To confirm that the streptozotocin-injected rats had diabetes, the fasting blood glucose levels of the rats were measured after four days. The rats will be randomly assigned to four groups (20 rats per group) after a week of acclimatization.

Group 1: (N. control):animals were fed a typical diet.

Group 2: (STZ diabetic): received a single intraperitoneal injection of streptozotocin STZ shortly before use (55 mg/kg BW, dissolved in 0.05 M citrate buffer, pH 4.5).

Group 3: (Local treated): The rats were given an intraperitoneal injection of streptozotocin STZ (55 mg/kg body weight) and an eye drop (Chrysin loaded liposome) for a duration of 12 weeks.

Group 4: (Systemic Treated): The rats were given an intraperitoneal injection of streptozotocin STZ (55 mg/kg body weight) followed by an oral gavage of chrysin for a duration of 12 weeks.

2.6. Ophthalmological examination

An ophthalmologist inspected the animals' eyes once a week during the trial. A single drop of tropicamide 1% mydriatic was applied to the rats' eyes at least 30 minutes before to the fundus examination. After examining the pupils to confirm dilation, a second drop was given if necessary. The animals were anaesthetized with ketamine (20 mg/kg). The rat's thigh muscle was anesthetized ten minutes before to the fundus examination. Next, a single drop of proparacaine hydrochloride (0.5%), a local anesthetic, was applied to the rats' eyes. If required, an additional ketamine dosage of 5 mg/kg was given during extended testing. The fundus was examined with an indirect ophthalmoscope (Heine with 20 D Volk lens) after the room had been darkened.

2.7. Morphological assessment of cataract

The lenses were inspected and photographed using a bio-microscope (Horvitz, HS500, Shanghai, China) and a slit light. The cataract alterations were independently rated by three observers who were not aware of the animal grouping. Cataract ophthalmic assistants assess the lens opacity from 0 to V based on the ophthalmoscopic examination **[21]:**

1-Stage 0: display the transparent lens

1. Mild opacification is represented by stages I–II.
2. Moderate opacification is represented by Stages III–IV.
3. Stage V: denotes total opacification of the lens.

2.8. Blood and lens tissue collection.

Blood was collected once a week from orbital venous plexus for glucose estimation. At the end of experimentation period, rats were anaesthetized with diethyl ether. Blood samples were withdrawn for the estimation of the selected biochemical parameters, Glucose, MDA and GSH the tubes were divided into three test tubes: The first tube was coated by sodium fluoride for glucose estimation and plasma has been isolated after centrifugation of blood in tubes at 4000 r.p.m for 10 minutes. The second tube was coated by EDTA to determine reduced glutathione (GSH) in blood. After letting the third tube clot, the serum was separated using centrifugation for ten minutes at 4000 r.p.m. The serum was used for estimation of malondialdehyde. The eye ball was removed and lenses were separated from the eye using a sharp blade. The weight of the lenses was measured using a small clean glass tube and stored at -70 ºC for further investigation.

2.9. Assay of glucose level

Bio Diagnostic kit were used to measure plasma glucose enzymatically and this method outlined in **[22].**

2.10. Lens reduced glutathione assay

Reduced glutathione levels in lens tissue were measured using a method created by **[23]** and modified by **[24].**0.59 ml of Tris-HCl buffer, 0.01 ml of Ellman's reagent, and 0.2 ml of supernatant were combined, and 0.04 M EDTA was added to create 1 ml. The absorbance of the sample at 412 nm was measured using a Spectro UV-visible double beam. The following relation was used to determine the GSH concentration:

GSH in Lens (µmol/g.lens)=

$$\frac{\text{conc from st.curve (µmol/ml)}}{0.2 \times 307 \times \text{g tissue used}}$$

Where; 0.2 is the volume of the sample that was used in milliliters

The molecular weight of reduced glutathione is 307

2.11. Assay of total soluble protein in lens:

The lens's total soluble protein content was determined using the procedure described in **[25]**. 5 ml of standard samples were extracted from the lens homogenate and mixed with 1 ml of deionized water. They added 5 ml of alkaline copper reagent. After 10 minutes of standing, 0.5 cc of diluted foline reagent was added. For half an hour, the solution was let to stand. After 10 minutes, 0.5 ml of diluted foline reagent and a blank solution made up of 1 ml of distilled water and 5 ml of mixed alkaline copper reagent were added. A spectrophotometer (UVD-3200) set to 710 nm was used to measure the OD. The protein concentration in the sample was determined using the standard curve and expressed as mg/g wet weight.

2.12. Assay of Carbonyl Protein (CP) in lens

CP was evaluated using **[26].** After mixing 200 µl of lens protein samples with 800 µl of 10 mM DNPH in 2.5 M HCl, the mixture was vortexed and allowed to sit at room temperature for one hour. After centrifuging at 8000 r.p.m. for 10 minutes at 4°C, the supernatants were collected and combined with 1 ml of 10% (w/v) Trichloro-acetic acid (TCA) before spinning down at 8000 r.p.m. for 10 minutes at 4°C. One milliliter of 20% (w/v) TCA was added prior to incubation on ice for five minutes.The protein pellet was centrifuged at 8000 r.p.m. for 10 minutes at 4°C after being cleaned three times with 1 milliliter of 1:1 (v/v) ethanol: ethyl acetate. Following a final washing, the protein pellet was centrifuged at 8000 r.p.m. for 5 minutes at 4°C after being re-suspended in 500 µl of 6 M guanidine hydrochloride. Using control as a blank, the supernatant was gathered and measured at 370 nm. Applying the formula:

Carbonyl Protein µmol /ml = (A370/0:022 µM - 1) (500 µl /200µl)

Aliphatic hydrazone has an extinction coefficient of 22 mM/cm. µmol/mg protein was the expression of carponyl protein in the lens.

2.13. Assays of Caspase-3 and Calpain in lens tissue

Sun Long Biotech kits (Biotech Co., LTD, Zhejiang, China) were used to detect the levels of Calpain and Caspase-3 in the lenses in accordance with the manufacturer's instructions.

2.14.Assay of Calcium in lens

Using an Agilent atomic absorption spectrometer equipped with a 10-cm air-acetylene burner and Agilent single-element hollow cathode lamps, the calcium ions were found.

2.15. Statistics

The mean ± S.D. was used to present the results. A one-way analysis of variance (ANOVA) was used to assess the data. The differences between the groups were compared using post hoc analysis with the least significant difference (LSD). P-values below 0.05 were regarded as significant in statistics.

3. Results

3.1. Chrysin liposome eye drops preparation results

3.1.1. Liposome morphology by TEM

All of the liposomes produced for this investigation had an approximately spherical form, were evenly distributed, and were less likely to combine for empty and encapsulated vesicles, as shown by the TEM results in Figure 1 (Figure 1A).

According to TEM data, chrysin can physically interfere with the liposomes' ability to pack their membranes on their surface (Figure 1B). When Chrysin was present in liposomes, the distance between adjacent bilayers grew, making the liposomes bigger than the controls.

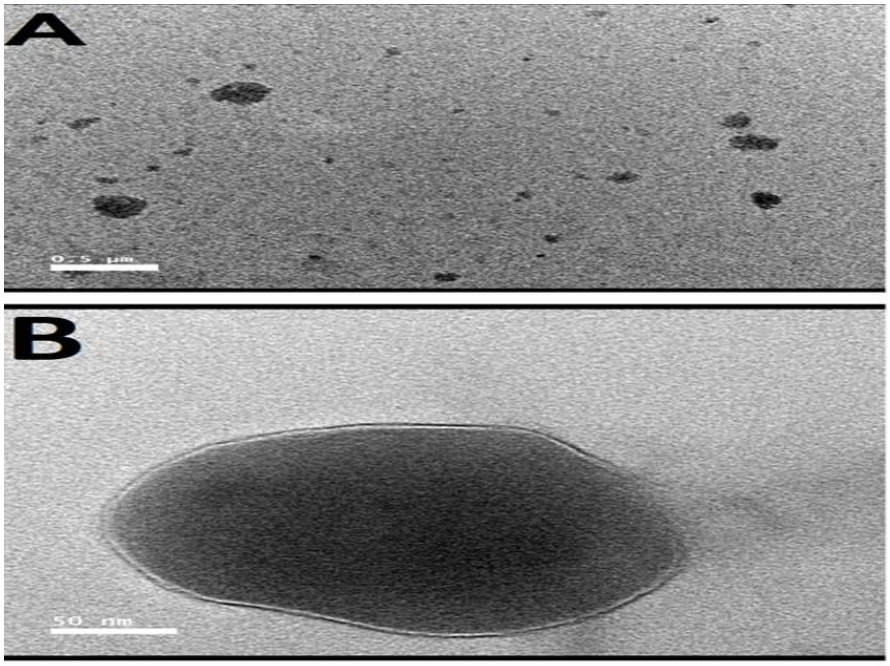

Figure 1: Transmission electron micrographs with magnification (a=0.5 µm, b= 50nm) to see chrysin molecules encapsulated in liposome, (a) empty liposomes (control), (b) Chrysin loaded liposomes (CLL)

3.1.2. Liposomes size distribution by DLS

The polydispersity index (PDI) efficiently accounts for the homogeneity of colloidal suspension particles. Values greater than 0.7 indicate that the dynamic light scattering approach is not stable since the sample has a very wide size range. A pure soy lecithin lipid sample with a mean size diameter of 152.1±99.70 nm and a PDI of 0.333 is shown in Figure 2A. When chrysin is encapsulated into pure soy lecithin lipid, Figure 2B shows that the mean size diameter of pure soy lecithin increases to 605.9±71.57 nm with 0.610 PDI.

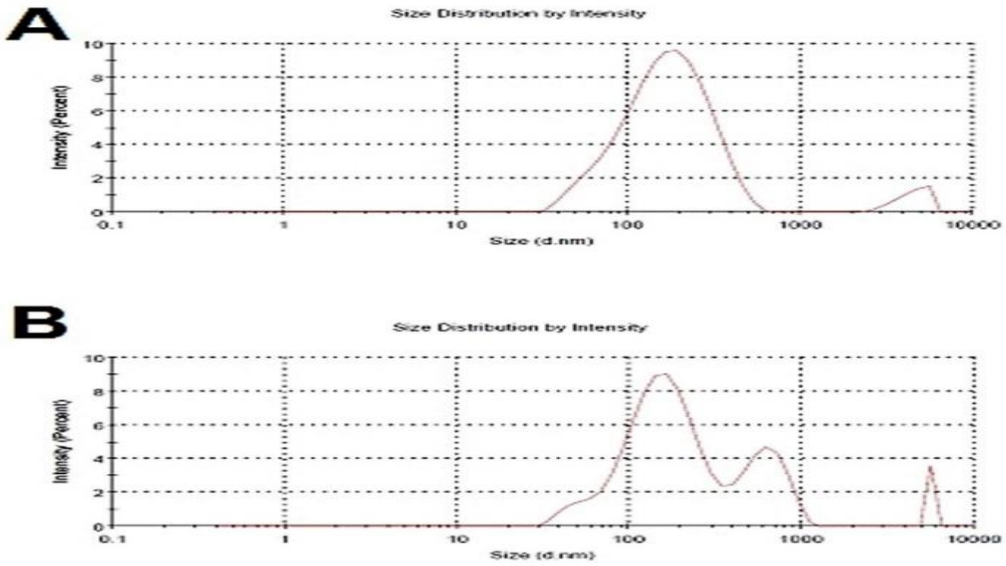

Figure 2: Liposomes size distribution measured by dynamic light scattering (DLS) for (A) empty Soy lecithin liposomal sample, and (B) Chrysin-encapsulated liposomes.

3.1.3. The zeta potential magnitude

Empty liposomes had a negative zeta potential of magnitude (-41.3±8.31 mV), as seen in figure 3. Because chrysin is integrated into the liposomal membranes, chrysin-loaded liposomes displayed a larger negative zeta potential (-42.8±5.91 mV) than blank liposomes. In general, particles are regarded as stable if their zeta potentials are +30 mV or more than -30 mV for positive ethanol.

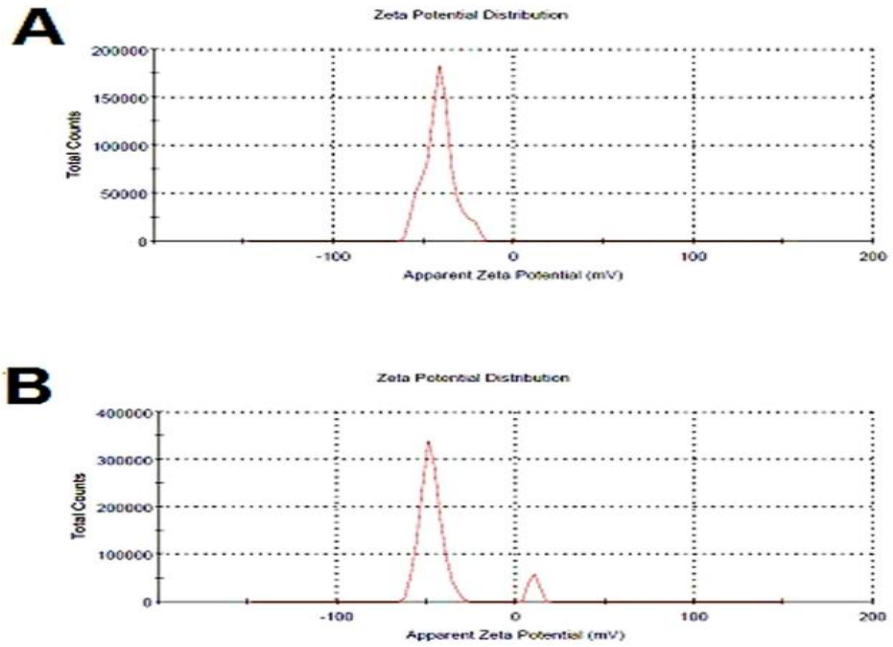

Figure 3: Zeta potential for (A) empty Soy lecithin liposomal sample, and (B) Chrysin-loaded liposomes.

3.1.4. DSC results

Because soy lecithin vesicles may imitate many properties of biological membranes, they were employed as a model membrane. When submitted for DSC analysis, pure soy lecithin vesicles showed a notable main endothermic peak (Tm) at 73.92 °C following dehydration (Figure 4). When chrysin was added to soy lecithin liposomes, as illustrated in figure 4, the temperature shifted to 98.22 °C, higher than the major endothermic peak (Tm) of empty soy lecithin, which appears at 73.92 °C.

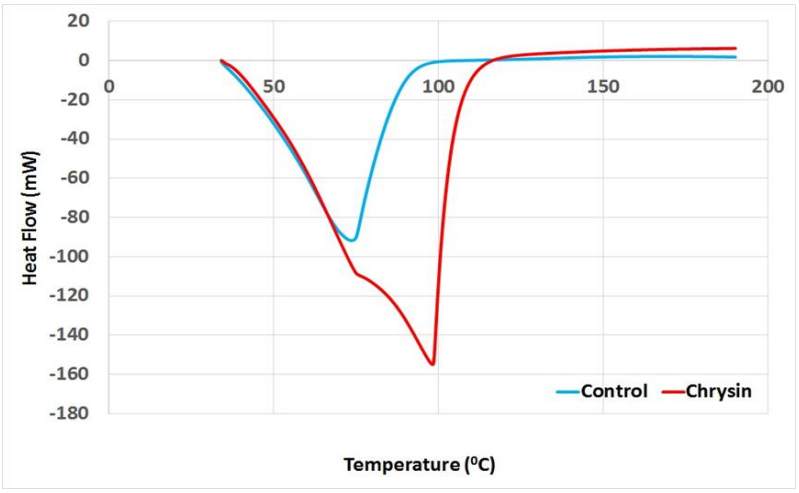

Figure 4: DSC diagrams of liposomes made of empty soy lecithin, liposomes doped with chrysin.

3.2. Fasting blood sugar level

Both the STZ diabetic group and the local and systemic treatment groups showed a considerable rise in plasma F.B.S. concentrations as compared to the control group. Comparing the systemic treatment to the STZ diabetic group revealed

a highly significant change in blood glucose concentration, while the local treatment demonstrated an improvement in blood glucose concentration as shown in **table (1).**

Table 1: The mean values of fasting blood glucose in different studied groups

Group		**FBS (mg/dl)**
N. Control (n=20)	**Range** **Mean± SD**	**78.3 – 91.5** **84.9± 14.1**
STZ diabetic (n=20)	**Range** **Mean± SD** **% change**	**187.9 – 211.6** **200± 25.2** [a#] **↑ 135.4 %**
Local treatment (n=20)	**Range** **Mean± SD** **%change** **%change***	**132.3 – 153.3** **142.8 ± 22.4** [a#, b#] **↑ 68.2 %** **↓ 28.5 %**
Systemic treatment (n=20)	**Range** **Mean± SD** **%change** **%change***	**121.9 – 138.2** **130 ± 17.4** [a#, b#] **↑ 53.2 %** **↓ 34.9 %**

%change vs control group%change* vs diabetic group

[a]: *p* valuevs. N. control group [b]: *p* value vs. Diabetic group*, $p< 0.05$; #, $p< 0.001$.

$p> 0.05$ is non- significant, $p \leq 0.05$ is significant,

$p \leq 0.001$ is highly significant

3.3. Progression of Cataract

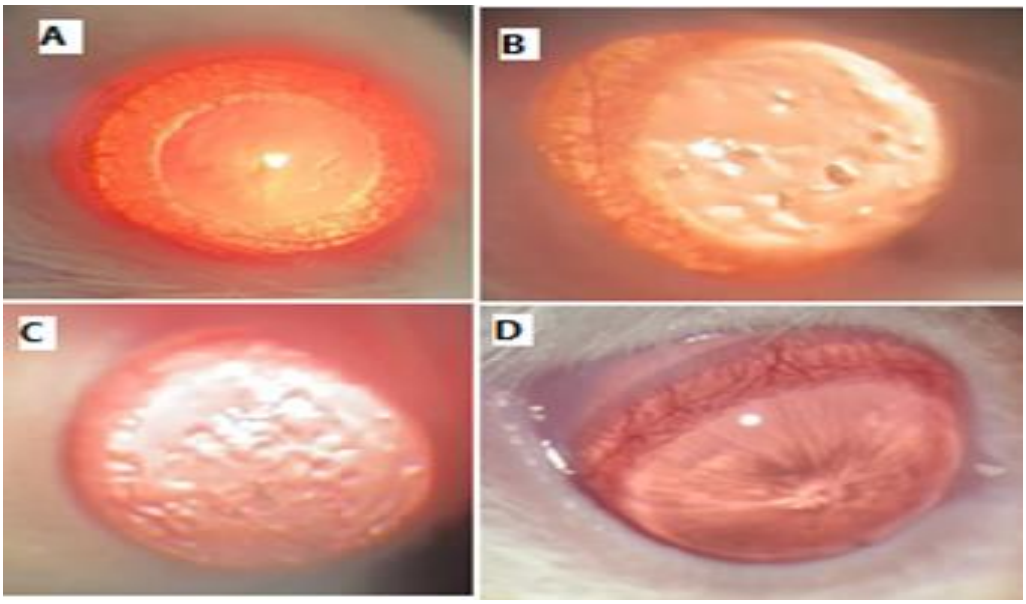

Figure 5: displays representative images of lenses in different stages of diabetic cataract at the end of the study (A: Normal clear eye lens,B: Stage I, C: Stage II and D: Stage III)

Figure 5 displays representative images of lenses in different stages of diabetic cataract at the end of the study. Twelve weeks after the diabetes induction, a slit-lamp examination was done and revealed the onset of cataracts. All of the lenses in the N. control group, however, were clean and normal (stage 0). Moreover, about 15% of the diabetic rats developed mild cataracts (stage I), 45% had mild opacification (stage II), and 5% had clear lenses (stage 0). Moreover, about 35% of the diabetic rats showed significant opacification.However, in rats administered CLL eye drops locally, about 25% of the lenses represented stage I (minor opacification) and 25% represented stage II (mild opacification), with the rest of the lenses in stage 0 (50%) of the animals. On the other hand, in rats administered chrysin dissolved in DMSO systemically, only 10% of the lenses displayed evidence of stage II (mild opacification), while 35% of the lenses displayed signals of stage I (mild opacification). The bulk of the lenses (55%) were in stage 0 **(table 2)**.

Table 2: percent of different stages in diabetic cataract and clear lens in all experimental groups

Group	Stage of Cataract			
	Clear	Stage I	Stage II	Stage III
N. Control(n=20)	20 (100%)	0	0	0
STZ-Diabetic (n=20)	1(5%)	3 (15%)	9 (45%)	7 (35%)
Local treatment(n=20)	10 (50%)	5 (25%)	5 (25%)	0
Systemic treatment(n=20)	11 (55%)	7 (35%)	2 (10%)	0

3.4. Biochemical parameters in lens tissue

3.4.1. Total soluble lens protein (TSP in lens)

The group with STZ diabetes showed a noticeably reduced level of lens total soluble protein than the control group, and both the local and systemic treatment groups had significantly lower total soluble lens protein concentrations than the control group. As shown in **figure (6)**, the systemic treatment did not significantly alter the total soluble protein concentration when the STZ diabetic group, while the local treated group had a considerably greater concentration of total soluble lens protein than the STZ diabetic group.

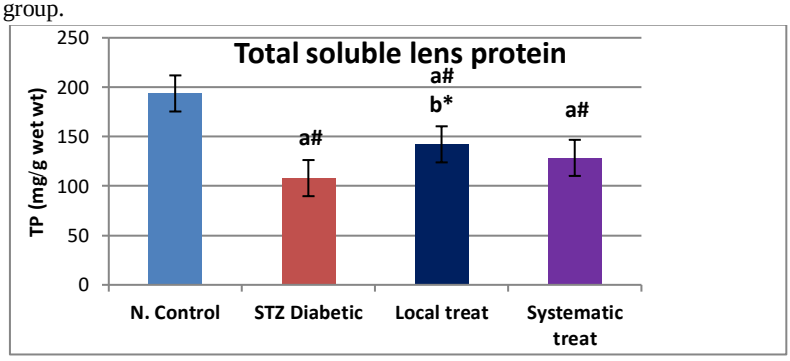

Figure 6: Total soluble lens protein concentration in the different groups. Results are presented as arithmetical means ± SD. "a" indicates the significance of the data that compares group N. control vs. all groups. "b" indicates the significance of the data that compares STZ diabetic group vs. treated groups of rats.*, $p < 0.05$; #, $p < 0.001$.

3.4.2. GSH and carponyl protein in lens:

The group with STZ diabetes had a noticeably decreased GSH concentration than the control group, and the local and systemic treatment groups both had significantly lower GSH concentrations than the control group. Comparing the local and systemic therapy groups to the STZ diabetic group, **Figure (7)** shows that neither group's GSH levels changed significantly.

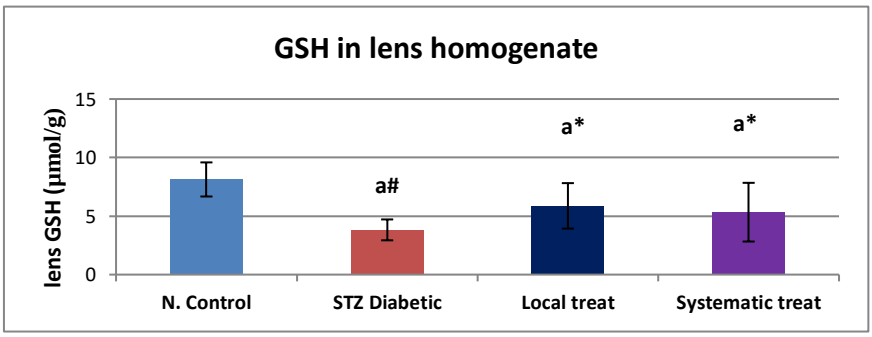

Figure 7: Levels of lens GSH in the different groups. Results are presented as arithmetical means ± SD. "a" indicates the significance of the data that compares group N. control vs. all groups. "b" indicates the significance of the data that compares STZ diabetic group vs. treated groups of rats.*, $p < 0.05$; #, $p < 0.001$.

The STZ diabetic group had a considerably higher concentration of CP in lens tissue homogenate than the control group, while the systemic treatment group had a significantly higher concentration than the control group. Comparing the local treated group to the control group, however, revealed no discernible changes. CP concentration improved with both local and systemic therapy when compared to the STZ diabetic group as shown in **figure (8)**

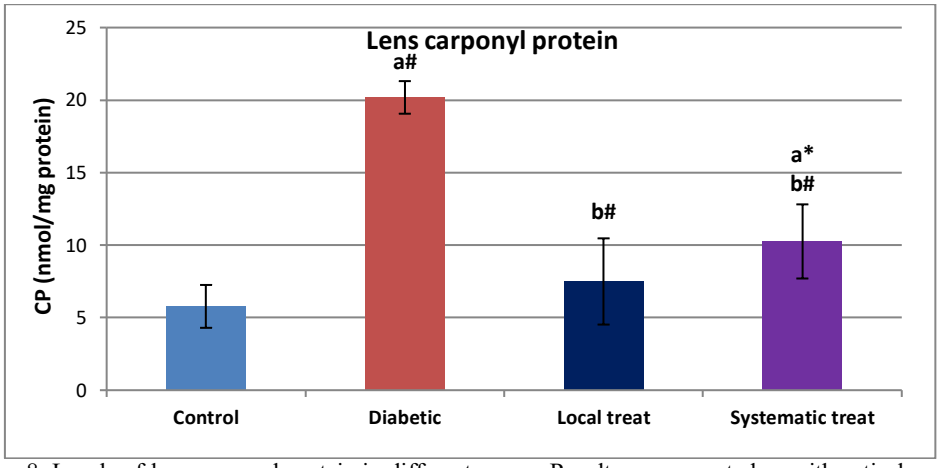

Figure 8: Levels of lens carponyl protein in different groups.Results are presented as arithmetical means ± SD. "a" indicates the significance of the data that compares group N. control vs. all groups. "b" indicates the significance of the data that compares STZ diabetic group vs. treated groups of rats.*, $p < 0.05$; #, $p < 0.001$.

3.4.3. Level of Caspase-3 as apoptotic marker in lens tissue:

Compared to the control group, the STZ diabetes group had noticeably greater levels of lens caspase-3. Comparing the local and systemic treatment groups with control also revealed a significant increase. However, when comparing the local and systemic treated groups with the STZ diabetic group, the levels of lens caspase-3 significantly decreased, as seen in **figure (9).**

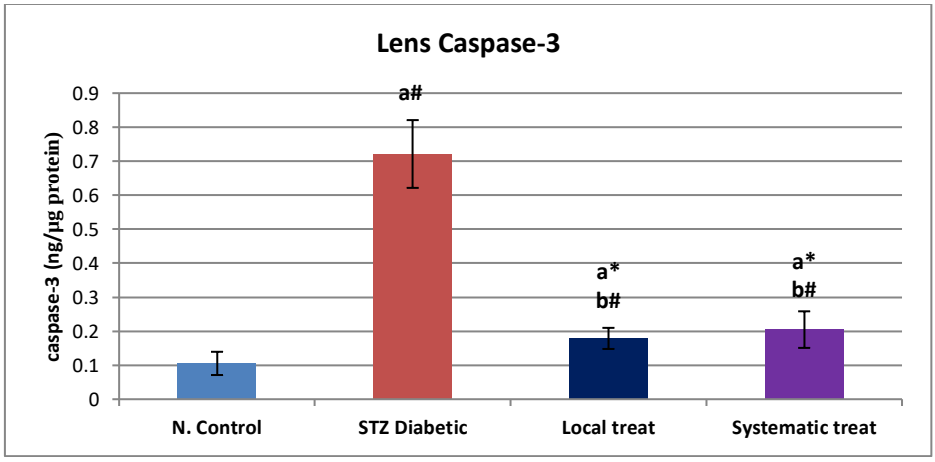

Figure 9: Lens caspase -3 concentrations in the different groups. Results are presented as arithmetical means ± SD. "a" indicates the significance of the data that compares group N. control vs. all groups. "b" indicates the significance of the data that compares STZ diabetic group vs. treated groups of rats,*, $p < 0.05$; #, $p < 0.001$.

3.4.4. Levels of Calpain and Calcium in lens tissue:

The concentration of lens calpain-1 increased significantly and highly significantly in the STZ diabetic group, and it increased significantly in the systemic treated group and significantly in the local treated group when compared to the control group. However, as shown in **figure (10)**, there was a notable drop in the concentration of lens calpain-1 when comparing the STZ diabetic group with the local treated and systemic treated groups.

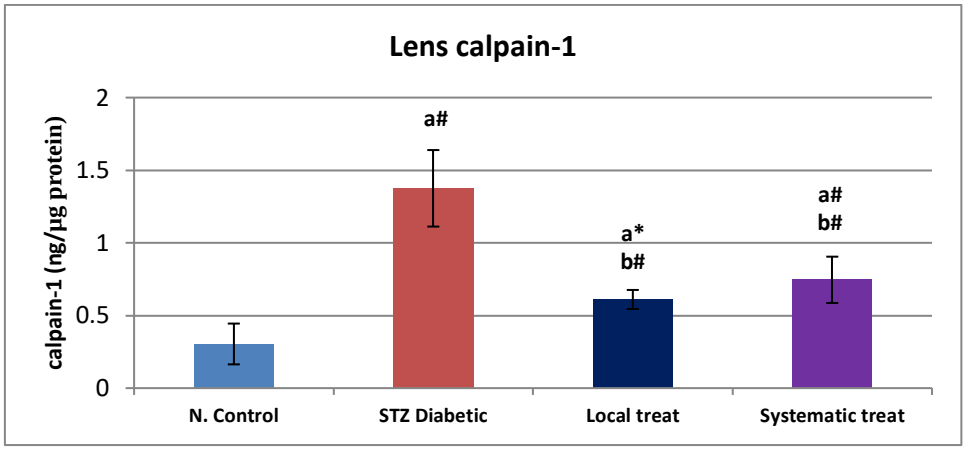

Figure 10: Total lens calpain-1 concentration in the different groups. Results are presented as arithmetical means ± SD. "a" indicates the significance of the data that compares group N. control vs. all groups. "b" indicates the significance of the data that compares STZ diabetic group vs. treated groups of rats.*, $p < 0.05$; #, $p < 0.001$.

The calcium content in the lenses of the STZ diabetic group was noticeably greater than that of the control group. the calcium concentrations in the lenses of the systemically and locally treated groups were both noticeably greater than those of the control group. When compared to the STZ diabetic group, it was discovered that both the local and systemic therapies improved calcium concentrations **(figure 11)**.

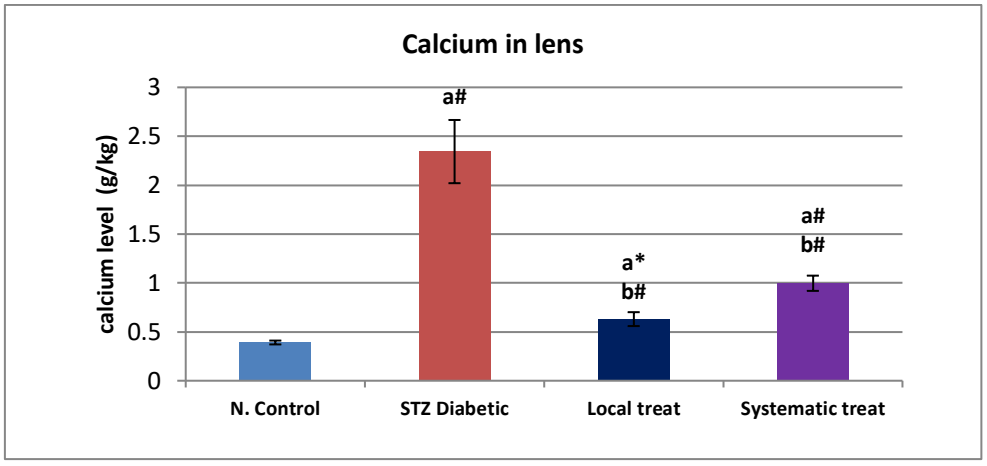

Figure 11: Levels of calcium in lens of different groups. Results are presented as arithmetical means ± SD. "a" indicates the significance of the data that compares group N. control vs. all groups. "b" indicates the significance of the data that compares STZ diabetic group vs. treated groups of rats.*, $p < 0.05$; #, $p < 0.001$.

4. Discussion

Diabetes mellitus (DM) is a dangerous, long-term condition brought on by either insufficient insulin synthesis or the body's inability to use the insulin that is generated**[27].**Type 1 diabetes, in which the immune system kills the insulin-producing β-cells in the pancreas, is caused by a lack of insulin. Over time, diabetes can have a detrimental effect on the eyes, leading to serious vision problems such diabetic retinopathy, glaucoma, and cataracts. People with diabetes are twice as likely to acquire cataracts **[28]**. Lens opacity occurs when oxidative stress levels exceed the lens's natural antioxidant capability. Antioxidants are among the compounds that have been demonstrated to slow the formation of cataracts. Chrysin is a naturally occurring flavones and has a number of pharmacological actions, also has low water solubility and limited bioavailability **[29]**. Encapsulation of chrysin in liposomal systems has allowed improved biological action against diabetes and better formulation in aqueous formulations.

In this study, we looked into the impacts of oral and topical chrysin liposomal administration on the oxidative stress markers of the lens as part of cataract treatment in type 1 diabetic rats. As chrysin's low bioavailability limits its use, so new materials have been discovered for coating currently available drugs, which can enhance treatment by increasing

bioavailability, decreasing toxicity, improving tissue adherence, accurately delivering medication, and prolonging its duration of action **[30]**. Transmission electron microscopy (TEM), dynamic light scattering methods (DLS), and differential scanning calorimetry (DSC) are used to assess the physicochemical characterizations of the chrysin liposomal eye drop formulation. Our current strategy was justified by the idea of using liposomes to increase the duration of chrysin's ocular contact. We demonstrate that each produced liposome was nearly spherical. Nanomaterials and nanodrug delivery techniques boost the entrapped drug's bioavailability due to their small size. It is widely accepted that size distribution is an important topic in the therapeutic application of liposomes. It is thought to be a crucial standard describing the quality of liposome suspensions. They also play a crucial role in the physical properties and biological fate of liposomes and the molecules they entrap in vivo. For parenteral administration, for instance, the ideal size range is 70–400 nm. Liposomes in this size range promote accumulation of medicines in certain target organs including the liver, spleen, and bone marrow. Additionally, their blood stability is good, which leads to consistent medication release rates.

Calorimetric methods are widely used to investigate drug-bio-membrane interactions. DSC is a technology utilized in such research. The thermotropic shifts that eventually occur in a liposome sample in the presence of a pharmacological molecule are detected and measured in DSC tests. According to the DSC concept statement, phospholipid vesicles undergo a reversible phase transition, caused by increasing temperature, from a 'gel' state, in which the acyl chains are orderly packed within the bilayers, to a 'liquid crystal' state, associated with an increase in spatial disorder in the bilayers. Because such a transition is associated with heat intake, it is an endothermic process. The temperature at which the gel phase transitions to the rippling phase is known as the pre-transition temperature (T_p) and it is mostly connected to the polar area of phospholipids. Following that, the bilayer melts from the rippled phase to the liquid phase at the main transition temperature (T_m). The melting point (T_m) is the endothermic peak temperature for the lipid gel-to-fluid phase transition measured during the heating scan. Changes in the lipid structure have a significant impact on all of the aforementioned phases.

When Chrysin was present in liposomes, the distance between the surrounding bilayers widened, making the liposomes bigger than the controls. The increased particle size could be the result of stronger hydrogen bonding interactions between the pharmacological agent and the lipid bilayer of liposomes. These findings are in line with the DSC data and support the possibility that chyrsin might be inserted into the hydrophobic region of the bilayer. The polydispersity index (PDI) effectively takes particle homogeneity into consideration in colloidal solution. Values larger than 0.7 indicate that the dynamic light scattering technique is unstable when the sample has a very large size range. The mean size diameter of pure soy lecithin increased upon encapsulation of chrysin into pure soy lecithin lipid, most likely due to the electrostatic repulsive force between the negative charge of the soy lecithin PO_2^-group and the negative charge of the chrysin OH-group. This is because the chrysin drug is primarily thought of as a lipophilic drug and could be trapped in the hydrophobic core of the bilayer. Liposomes became larger than the control ones when chrysin was added because it increased the space between nearby bilayers.

The magnitude of the zeta potential can be used to determine the colloidal system's potential stability. The colloidal dispersion would become more stable as the zeta potential increased because there would be greater repulsion between the particles. If all of the suspended particles have a strong negative or positive zeta potential, the particles will seem to resist each other and not have a tendency to combine **[31]**. In this work, particles are considered stable if their positive ethanol content is larger than -30 mV and their zeta potentials are +30 mV or higher. Chrysin appears to increase the The zeta potential turned negative when it was due to a density of negative charge presented. Because of changed interactions between the liposomes and the encapsulated medications, DSC characterization was employed to investigate alterations in the lipid bilayer phase change **[32, 33]**. As a model membrane, soy lecithin vesicles were employed because Numerous properties of biological membranes can be imitated by this phospholipid. The vesicles may be impacted by a substance present in the soy lecithin membranes. thermotropic parameters of the transition. Chrysin's incorporation into soy lecithin In contrast to the primary endothermic, liposomes displayed a shift to a higher temperature. peak (Tm) of empty soy lecithin, demonstrating that chrysin significantly impacted the acyl chain bilayer of soy lecithin, which establishes a conformational order inside the phospholipids and enhance the lipid acyl chains' cooperative transition **[34]**. The elevated temperature of empty soy lecithin's primary endothermic peak (Tm) demonstrated that chrysin incorporation promotes the formation of acyl chains that are loose and disorganized.

In the present study, wistar rats were injected with a single dose of streptozotocin (STZ) at 55 mg/kg body weight **[35]** in order to induce type 1diabetes in three groups; diabetic, local treated (local delivery with chrysin liposome eye drops) and systemic treated groups. According to **[36]** Type 1 diabetes can be induced in rodents by a single STZ injection. Diabetic cataractogenesis is a complicated process including oxidative stress, polyol pathway activation, and protein glycation **[37]**. Our ocular examination findings show that chrysin is capable of preventing lens opacification. As a result, in rats treated locally with liposomal chrysin eye drops, most of the lenses were in stage 0 (50%) and about 25% of the lenses were represented mild opacification (stage I) and 25% of the lenses were represented stage II (mild opacification). Also, in rats treated systemically with chrysin dissolved in DMSO, most of the lenses were in stage 0 (55%) and 35% of the lenses were

represented mild opacification (stage I) while only 10% of the lenses were represented stage II (mild opacification). It was observed that no rats' lenses complete lens opacification (stage v) in all experimental groups at the end of the experimental period. Our findings are consistent with the findings of **[38]** who found that chrysin appears to prevent induced cataractogenesis by maintaining the redox system components at near-normal levels**.**

The current study found that after three months of the trial, the body weight of the STZ diabetic group was lower than that of the control group, whereas the body weight of the rats that received both local and systemic treatment rose relative to the STZ diabetic rats. These findings align with those of **[39&40]**. This implies a polyphagic condition and weight loss caused by an overabundance of tissue protein degradation **[41]**. Chyrsin-treated groups demonstrated a discernible effect in controlling the body weight loss of the diabetic rats. Additionally, our results showed that diabetic rats administered chrysin either systemically or locally (eye drop) had lower blood glucose levels at the end of the trial, reaching 142 and 130 mg/dl, respectively, but rats with STZ injections had considerably higher blood glucose levels. These results aligned with those found in **[42]**.

Hyperglycemia raises aerobic glycolysis and the mitochondrial electronic chain (ETC), which in turn causes superoxide generation and mitochondrial malfunction. The polyol pathway competes with glutathione reductase for NADPH, which lowers reduced glutathione (GSH) and the cellular antioxidant response **[43]**. In our investigation, the STZ diabetic and chrysin-treated (locally and systemically) groups had significantly lower GSH levels than the normal control group. However, both diabetic and chrysin-treated rats showed a significant rise in GSH levels (both locally and systemically). These findings are consistent with **[42]**. The pathogenesis of diabetic eye diseases is significantly influenced by oxidative stress, according to **[44]**.

In the current study, there were notable changes in serum MDA levels, as when comparing the local and the systemic treated groups with STZ diabetic group there was a high significant reduction in MDA concentration. So, treatment with Chrysin effectively restored serum MDA levels. This finding is consistent with previous research of **[45]**. Chrysin treatment led to a reduction in MDA levels and an increase in GSH levels, indicating its positive effect on the antioxidant system and its potential as a therapeutic agent for managing oxidative stress in diabetes. In the present study, the mean level of lens GSH was significantly lower in the diabetic cataract group than in normal rat lenses; this finding agreed with **[46]**. While chrysin-treated animals showed a substantial increase in lens GSH parameter compared to diabetic group rats. This result was in accordance with other research that revealed that chrysin as a flavonoid has antioxidant action raising GSH in lens **[47]**.

The insolubilization of lens proteins was considered as the key changes that result in lens opacification during the development of cataract. So, in the current study we assessed the total soluble protein concentration of lenses from all studied groups. Our results showed that both STZ diabetic rats and chrysin-treated rats showed a decrease in total soluble lens protein when compared to normal control rats, but chrysin-treated rats showed a highly significant increase in this parameter when compared to the STZ diabetic group, this result agreed with **[48].** Chrysin administration to rats delayed the loss of total soluble protein in the lens, which was closely linked with the delay in cataract maturation in treated groups. To assess oxidative damage in proteins, we looked at the amounts of soluble proteins as well as protein carbonyl groups (PCG). Protein carbonyl group (PCG) buildup is believed to be associated with oxidative stress and protein dysfunction **[49]**. In this study, the STZ diabetic group's level of lens carbonyl protein was significantly higher than that of the control group. Furthermore, the carponyl protein concentration in the systemic treated group was considerably higher than in the control group, although there was no detectable difference in the local treated group. Carponyl protein concentrations improved with both local and systemic therapy when compared to the STZ diabetic group. This result is consistent with that of **[47]**, who discovered that chrysin reduces the overall amount of oxidants in diabetic rats' lenses, which is connected to a drop in ROS levels in the lens environment.

It was reported that, caspase-3 is up-regulated and activated after cataractogenesis. So, when measured in this study it was found that; the lens caspase-3 levels of the STZ diabetic group were considerably greater than those of the control group in our investigation. Furthermore, there was a noticeable increase when comparing the systemic and local treatment groups to the N control. However, comparing the STZ diabetic group to the local and systemic treated groups revealed a significant decrease in lens caspase-3 levels. We think that this might be due to chrysin's antioxidant qualities and capacity to scavenge free radicals. These outcomes concurred with those of **[50]**.

Calcium-activated proteases (calpain), were used as a marker for intracellular proteolysis. In cataract regions, most lens cells lose their lysosomes and nuclei, leaving only calpain and cytoplasmic proteases to remove damaged proteins and maintain lens function **[51]**. Calcium imbalance was considered to be a significant crucial event associated with the ultrastructural abnormalities in cataracts **[52]**. The lens's protease, calpain, can also become abnormally activated, leading to cataracts. According to the current study, the systemic treated group had significantly greater calcium and lens calpain-1 concentrations than the control group, and the local treated group had significantly higher concentrations as well. In comparison to the STZ diabetic group, the calcium and lens calpain-1 concentrations were considerably lower in the systemically and locally treated groups. These findings were consistent with those of **[53]**, who asserted that chrysin's capacity

to modify or prevent cataractogenesis was due to its antioxidant properties, capacity to regulate lenticular Ca2+ levels, and capacity to inhibit calpain activity and the cascade of apoptotic processes.

5. Conclusion

According to the findings, the TEM imaging showed the favorable formulation of Chrysin liposomes. The DSC measurements, zeta potential, and particle size revealed a fair value for the flavonoid Chrysin as a lipophilic molecule. In the eye tissue of diabetic rats, CCL demonstrated greater antioxidant activity than oral chrysin in a number of oxidative stress-related parameters. Additionally, the formula's ameliorative effect on the levels of Caspase-3 and Calpain in diabetic rats was evaluated and diabetic rats' lens opacification has improved. The strong point of the current study was the novelty in the preparation of CLL as eye medication. The limitation of the present study is that not examining the cytotoxicity of the synthesized CLL, which was one of its drawbacks. Also the use of male rats restricts the applicability of the findings, so future research should include both male and female rats.

6. References

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