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Protective Effect of Garlic against Diabetic Retinopathy in Adult Albino Rats

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ABSTRACT

Diabetes mellitus is the most common chronic endocrine disorder.Several recent studies have shown that diabetes associated with cardiovascular disease. Diabetic retinopathy is a common complication of diabetes, it may not have any symptoms or may not affect sight in the early stages but, as the condition progresses, and eventually the sight will be affected. Diabetic macular edema (DME) may occur where blood vessels leak their contents into the macular region of the retina and this may cause a more rapid form of vision loss.The present study is conducted to demonstrate the possible protective effects garlic in managing the effects and complications of diabetic retinopathy in streptozotocin-induced diabetic rats.60 male of albino rats were used and divided into three groups; the first group, control group; the second group, subjected to induction of diabetes; the third group diabetic rats treated with an extract of raw garlic by gastric gavage (0.4 g/100 g b.wt) for seven weeks. At the end of the trial the animals were killed and the retina was sampled and prepared for histopathological and ultrastructural examinations. Results revealed that diabetic rats showed marked decrease in their body weight with highlysignificant increase in their blood glucose and glycated hemoglobin levels. Retinal histopathological observation showed morphological changes of inner nuclear layer and outer nuclear layer. Moreover, administration of garlic leads to improvement in their body weight, blood glucose and an improving effect on the retina.

Keywords: Garlic, diabetes, retina, histopathology

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INTRODUCTION

Diabetes mellitus (DM) is a major public health problem worldwide; the incidence of the disease is gradually increasing in all regions of the world. The number of people suffering from DM is expected to rise to 2.21 billion by the year 2010. However, prevalence of DM in adults in Saudi Arabia is 23.7%.But, according to recent reports by the American Diabetes Association, people living in Saudi Arabia and the Gulf could face a lifetime risk as high as 60 per cent of developing diabetes [1]. It is recognized that long-standing diabetes to be an independent risk factor for cardiovascular disease, patients who suffer from diabetes type 1 or type 2 diabetes mellitus are prone to several heart disorders and blood vessels including coronary heart disease, stroke and peripheral arterial disease, cardiomyopathy and congestive heart complications failure.Cardiovascular now the main causes of morbidity and mortality caused by diabetes [2]. The diabetic retinopathy (DR) is one of the most common complications of diabetes mellitus, and also is the most common reason of blindness in diabetics. It is reported that nearly all people with type 1 and more than half with type two diabetes will develop retinopathy [3]. DR results from the damage of the small vasculature of the retina, multicellular and the light sensitive tissue at the back of the eye. It is a major cause of visual impairment worldwide [4].

The retinal capillaries are lined with endothelial cells responsible for maintaining the blood retinal barrier, and are surrounded by smooth muscle cells, pericytes, which provide tone to the vessels [5]. The vascular lesions that are identified at the early stage of DR include pericytes disappearance from capillaries resulting in pericyte ghosts, obliteration of capillaries and small arterioles, gradual thickening of vascular basement membrane, increased permeability of endothelial cells, and formation of microaneurysms (i.e. weakening of vessel walls that results in the projection of a balloonlike sac, vessel leakage, exudate, and hemorrhage) [6]. Medicinal plants provide therapeutic agents, in modern medicine and in traditional system. The efficacy and safety of the oral hypoglycemic agents have promoted a search for safer and more effective drugs for the treatment of diabetes [7].

Garlic (*Allium sativum* L.) belongs to the Alliaceae family is a common food spice in traditional medicine to enhance physical and mental health, which is consumed all over the world as a food flavoring agent [8].Garlic constituents prepared by various means have been shown to have diverse biological activities, including anticarcionogenic, antiatherosclerotic, antithrombotic, antidiabetic, and various other biological actions [9] were showed that ingestion of garlic juice resulted in better utilization of glucose in glucose tolerance test performed in rabbits. Also extracts of garlic produced a significant fall in blood sugar levels in rabbits.Thus; the antidiabetic action of garlic established in animal studies provided a background for further investigations concerning possible clinical implications for garlic-based preparations. We investigated the possible preventive effect of oral administration of garlic juice on the changes of biochemical factors and histopathological alterations in the retina caused by diabetes.

MATERIALS AND METHODS

Animals

60 male rats (weighing 250-300 gm) were fed and allowed free access to water in an air-conditioned room with a 12 hour light/12 hour dark cycle.

Chemicals

All chemicals and Streptozotocin (STZ) were used in this study was purchased form Sigma-Aldrich chemical Co., St. Louis. Mo, USA.

Induction of Diabetes

A single intraperitoneal injection dose (60 mg/kg b.wt) dissolved in 0.05 ml/l sodium citrate buffer; pH 4.5 was used for induction the diabetes. Hyperglycemia was confirmed 3 days after injection by measuring blood glucose level using an Accu-Check Sensor as a glucose meter. The animals with fasting blood glucose levels ≥250 mg/dl were considered diabetic.

Animal Groups

The animals were divided into three main groups:

Group I: Included twenty adult male albino rats considered as control group for all experimental groups fed on standard diet, each of them was keptwithout any treatment all over the experimentalperiods for 7 weeks.
Group II (Diabetic group) (STZ treated group): Consisted of twenty adult male albino rats. Diabeteswas induced by a single dose of STZ byintraperitoneal injection and untreated diabetic rats, (given normal saline solution) for 7 weeks.
Group III (garlic treated diabetic group): Consisted of twenty adult male albino rats. After diabetes confirmation test, the ratswere received 1 ml of garlic juice/100 g b. wt /day (equivalent to 0.4 g/100 g b. wt) by gastric gavages using ball-tipped needle for 7 weeks

At the end of the experimental period, the animals are killed under light anesthesia and the retina are sampled and fixed for further histological, immuno-histochemical and ultrastructural examination [10].

Preparation of Garlic Juice

The biology department, College of Science, Taif University was made the taxonomic identity of the plant. The fresh garlic purchased from the local market in Taif was peeled, washed, and chopped into small pieces.The garlic juice was prepared by adding 100 g of garlic with 250 ml of distilled water and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen at -10 °C until used [11].

Biomedical analysis

Estimation of Blood Glucose Level

Blood samples were obtained from the retro-orbital veins. The random blood glucose levels in all rats of each group were estimated at the end of the experiment by using timing schedule One Touch glucometer and strips and the mean blood glucose levels were calculated and subjected to statistical analysis.

Estimation of Glycated hemoglobin (HbA1%)

At the end of the experiment blood samples were obtained from the retro-orbital plexus. The hemoglobin variants in heparinized full blood samples were separated on a cation-exchange resin column, and the percentage of glycated hemoglobin (HbA1%) was determined in all rats of each group by a spectrophotometric assay using glycated hemoglobin kit. The mean glycated hemoglobin levels were calculated and subjected to statistical analysis.

Histological Study

Preparation of Paraffin Sections

The eyes were taken immediately and fixed in 10% neutral formaldehyde for 24 hours. The specimens were cut sagittaly then dehydrated in ascending grades of alcohol and xylol was used as a clearing agent. Impregnation was done in pure soft paraffin for two hours at 55Cº followed by embedding in hard paraffin. Sections from these paraffin blocks were stained by: Hematoxylin & Eosin, for routine histological examination.

Morphometric Study

The parasagittal region of the retina, near the midline, in five different stained sections obtained from five different rats (i.e. one section from each rat) was examined in each group.

Retinal thickness of different layers was measured at a 400× magnification, including: photoreceptor layer (PRL), outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL). Two measurements were taken on each section, at the two reference lines which were 1 mm away from the optic

nerve on both superior and inferior sides.This was performed using Image analyzer software (Image analyzer, in Anatomy and Histology Department, Faculty of Medicine, Taif University. The mean values were calculated per animal (n= 5) and the results were subjected to statistical analysis.

Electron Microscopic Study

The samples taken from eyes were cut into about 1mm3 specimens. Tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH7.3, for 3 hours at 4Cº and routinely osmicated in 1% osmium tetroxide. After dehydration with graded ethanol series, the samples were embedded in Araldite. Semithin sections were stained with toluidine blue stain and were examined under light microscope as a preliminary step. Ultrathin sections were stained with lead citrate and uranyl acetate and were viewed under transmission electron microscope.

RESULTS

Body Weight

Throughout the whole experimental periods, there was highly significant decrease in the body weight of the diabetic rats when compared with its corresponding control group. However, the garlic treated diabetic group showed highly significant increase in their body weight when compared with the diabetic non treated rats. Statistically, there was highly significant difference between the garlic treated diabetic group and its corresponding control one. The mean body weight 7 weeks after induction of DM was 264.75 ± 7.47, 109.48 ± 4.42 and 244.24 ±4.78 for control, diabetic and garlic treated diabetic group respectively **(Table 1)**.

Table 1: Comparison between body weight ofcontrol, diabetic and garlic treated diabetic groups.

Control gm	Diabetic group gm	Garlic diabetic group gm
264.15	109.41	244.14
270.25	110.42	245.20
265.35	111.93	246.39
266.45	112.85	247.48
267.55	113.37	248.57
268.65	108.69	241.66
269.75	107.52	242.75
260.85	106.43	243.84
259.71	105.34	240.93
258.72	104.24	241.12
257.73	102.14	242.21

Blood Glucose & Glycated Hemoglobin Levels

The diabetic rats revealed highly significant increase in their blood glucose & glycated haemoglobin levels after induction of DM when compared with its corresponding control group. Treatment of the diabetic rats with garlic markedly ameliorated these effects to be near the control levels.

The mean values for blood glucose level at the end of the experiment, after induction of diabetes, were 97.406, 544.20 and 243.60± 7.09 for control, diabetic and garlic treated diabetic groups respectively (**Table 2**). Moreover, the mean values for glycated haemoglobin level at the end of the experiment, after induction of diabetes, were 5.16, 12.08 and 7.80 for control, diabetic and garlic treated diabetic groups respectively (**Table 3**).

Table 2: Comparison between blood glucose levels of control, diabetic and garlic diabetic groups.

Control mg/dl	Diabetic group mg/dl	Garlic diabetic group mg/dl
93.81	497.21	213.61
94.82	499.22	214.64
96.83	491.24	215.65
98.84	492.27	216.67

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99.79	473.29	217.80
85.68	484.28	218.90
86.57	497.34	211.20
87.46	496.69	212.30
88.35	493.70	208.40
98.24	494.40	203.50
90.13	495.50	203.70

Table 3: Comparison between glycated hemoglobin levels of control, diabetic and garlic diabetic groups.

Control %	Diabetic group %	Garlic diabetic group %
5.01	12.08	7.90
5.02	12.18	7.95
5.14	12.22	7.94
5.13	12.31	7.93
5.10	11.98	7.92
5.11	11.88	7.89
5.12	11.91	7.88
5.08	11.81	7.87
5.06	11.87	7.86
5.04	11.94	7.85
5.16	11.95	784

Moreover, the mean values of fractal dimensions of analyzed digital retinal layers for control, diabetic groups and garlic diabetic group.

At the end of the experiment, after induction of diabetes, showed the thickness of photoreceptor layer (PL) were 1.78, 1.65 and 1.76 for control, diabetic and garlic treated diabetic groups respectively, the thickness of outer nuclear layer (ONL) were 1.82, 1.62 and 1.81 for control, diabetic and garlic treated diabetic groups respectively, the thickness of internal nuclear layer (INL) were 1.81, 1.71 and 1.83 for control, diabetic and garlic treated diabetic groups respectively in addition the thickness of ganglion cell layer (GCL) were 1.43, 1.32and 1.44 for control, diabetic and garlic treated diabetic groups respectively.

Histopathological Examination of Retina

Statistically, the diabetic group showed highly significant decrease in the mean retinal thickness when compared with its corresponding control group after the induction.On the other hand, diabetic rats treated with garlic showed highly significant difference in the mean retinal thickness when compared with that of diabetic group (Table 4 and Fig. 1).

Table 4: Results of the fractal dimensions of analyzed digital retinal layers for control, diabetic groups and garlic diabetic group.

Layer	GCL	INL	ONL	PL
Control group	1.43	1.81	1.82	1.78
	1.41	1.81	1.81	1.77
	1.44	1.81	1.83	1.77
	1.42	1.81	1.84	1.77
	1.42	1.81	1.81	1.78
	1.42	1.81	1.87	1.78
	1.42	1.81	1.81	1.79
	1.42	1.81	1.81	1.79
	1.42	1.81	1.82	1.79
	1.42	1.81	1.82	1.79
Diabetic group	1.32	1.71	1.62	1.65
	1.31	1.72	1.61	1.64
	1.33	1.72	1.63	1.66

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	1.32	1.72	1.61	1.65
	1.32	1.72	1.61	1.65
	1.32	1.70	1.61	1.65
	1.32	1.70	1.63	1.65
	1.32	1.70	1.63	1.65
	1.311	1.70	1.63	1.64
	1.31	1.70	1.63	1.64
Garlic diabetic group	1.44	1.83	1.81	1.76
	1.45	1.82	1.80	1.75
	1.43	1.84	1.80	1.74
	1.43	1.82	1.80	1.73
	1.43	1.82	1.82	1.72
	1.44	1.82	1.82	1.76
	1.44	1.84	1.82	1.77
	1.44	1.84	1.82	1.78
	1.44	1.84	1.82	1.79
	1.44	1.84	1.80	1.79

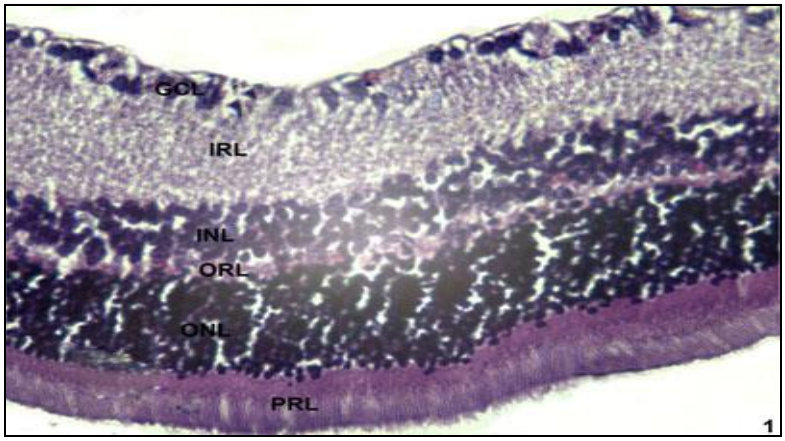

Fig. 1: Control group showing ganglion cells layer (GCL); inner reticular layer (IRL); inner nuclear layer (INL); outerreticular layer (ORL); outer nuclear layer (ONL); photoreceptors layer PRL. (H and E X 400).

Examination of the retina in the diabetic rats showed shrunken degenerated ganglion cells, wide spacing within the cells of the outer nuclear (ONL) and inner nuclear layers (INL) with disorganization of the retinal layers and even disappearance of discrimination marks between the different layers after the induction in addition to disorganization and marked destruction of the photoreceptor layer (PRL) after induction of DM with increased vascularity, leucostasis, and mild inflammation in outer nuclear layer (Table 4 and Fig. 2).

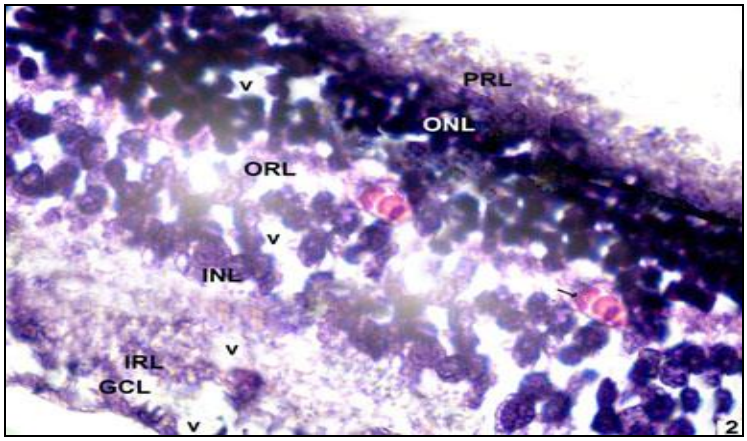

Fig. (2): A photomicrographs of retinal sections of the diabetic group: showing disorganization of the PRL, shrunken degenerated ganglion cells in the GCL, presence of vacuoles in all layers of retina (v), the ONL and increased in its vascularization (arrow) and wide spacing within the cells of INL. (H& E X 400).

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On the other hand, treatment of diabetic rats with garlic showed considerable improvement in the retinal appearance and organization that became nearly normal after the induction except .However, slight degenerative changes in the ganglion cells, very mildwidening of the intercellular spaces between the cells (Table 4 and Fig. 3).

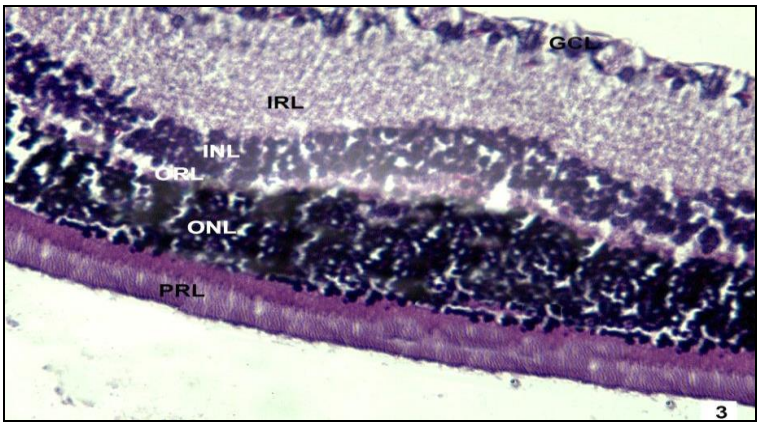

Fig. 3: A photomicrographs of retinal sections of the diabetic rats treated with garlic showing nearly normal inner reticular layer (IRL), inner nuclear (INL), outer reticular layer (ORL), outer nuclear (ONL) and photoreceptor (PRL). Ganglion cell Layer is apparently normal, (H& E X 400).

Ultrastructure Examination of Retinae

The ultrastructure examination of the control retinae showed the nuclei of rods and cones in the ONL (Fig. 4). Identified of the cone cells were by their presence just inner to the outer limiting membrane and their euchromatic nuclei. They are distinguished from the rods that are more numerous and present at different levels within the ONL. Rods possess heterochromatic nuclei with characteristic aggregation of their chromatin in the center.

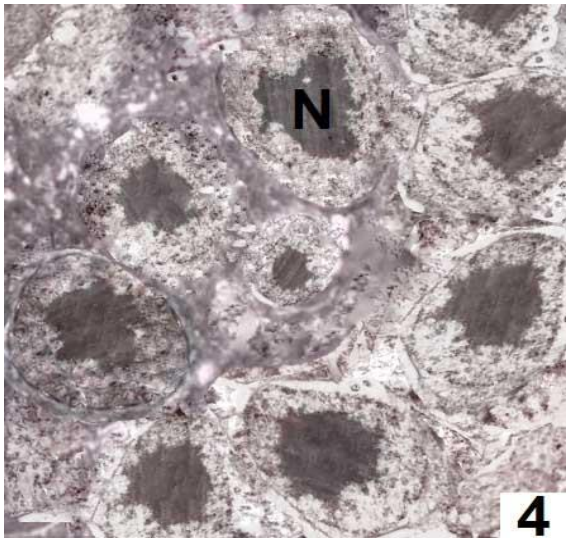

Fig. (4): A photomicrograph of a section in the ONL of retina of control rats showed adense chromatin of the nucleus (N), (TEM X4000).

In the INL, horizontal, bipolar, amacrine and Muller cell were observed (Fig. 5).In the GCL showing ganglion cells with large euchromatic nuclei and moderate amount of organelles were also detected (Fig. 6).

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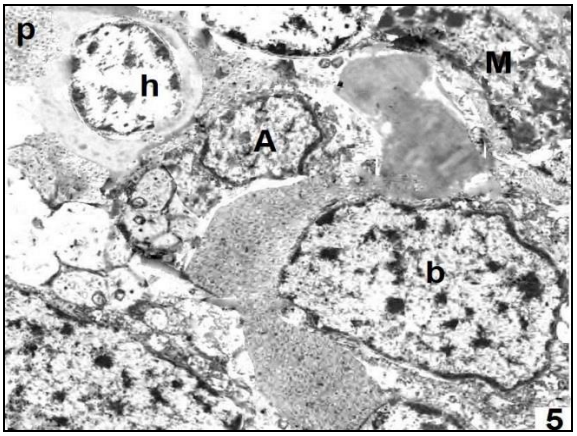

Fig. (5): A photomicrograph of a section of the INL. of retina of control groupshowed: horizontal cell (h) with its horizontal process (p), bipolar cell (b), amacrine cell (A) with its large rounded euchromatic nucleus and Muller cell (M) with irregular outline and electron dense nucleus, (TEM X6000).

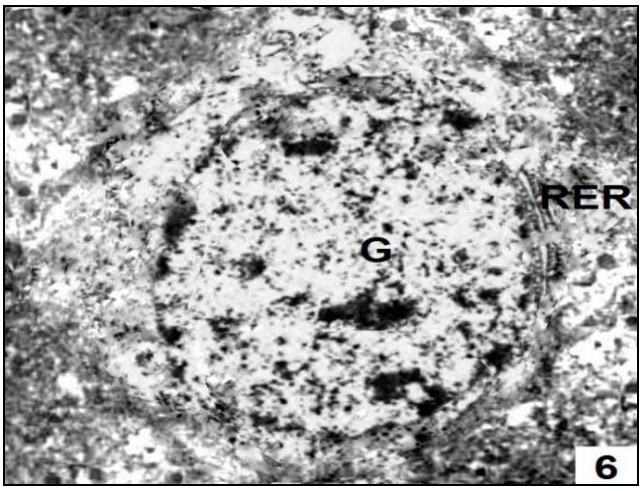

Fig. (6): A photomicrographs of a section in the GCL of control group showed ganglion cell (G) with a large euchromatic nucleus and rough endoplasmic reticulum (RER), (TEM X6000).

Examination of the retina of diabetic rats revealed membrane-bound electron-dense cytoplasmic inclusion bodies within the cone cells cytoplasmic vacuoles (Fig. 7). In the INL, distorted bipolar cell nucleus and shrunken nucleus of Muller's cell and cytoplasmic vacuoles (Fig. 8) and In the GCL showing the ganglion cells had swollen mitochondria with loss of structural details, lipofuscin granules with cytoplasmic vacuoles (Fig. 9).

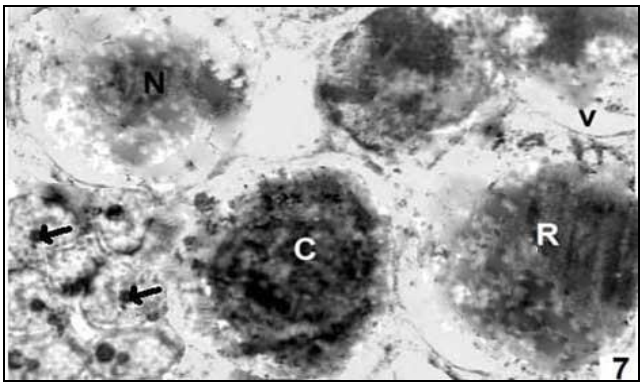

Fig. (7): A photomicrographs of sections in ONL of diabetic rats showed nuclei of rods (R) and cones (C). Note the electron dense inclusions (arrow) in the cytoplasm of cones, dense chromatin of the nucleus (N) and cytoplasmic vacuoles (v), (TEM X6000).

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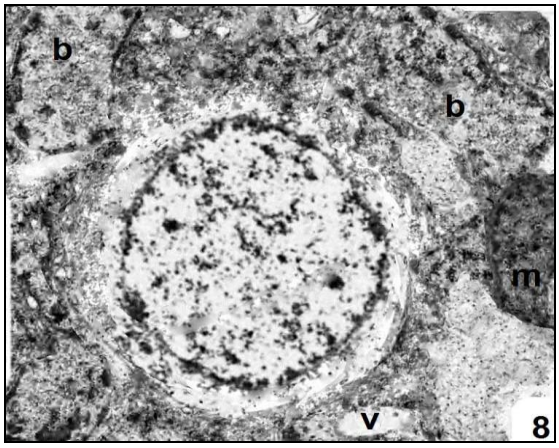

Fig. (8): A photomicrographs of section in the INL of retinaof rat of diabetic rats showed a distorted bipolar cell nucleus (b) and shrunken nucleus ofMuller's cell (m) and cytoplasmic vacuoles (V), (TEM X6000).

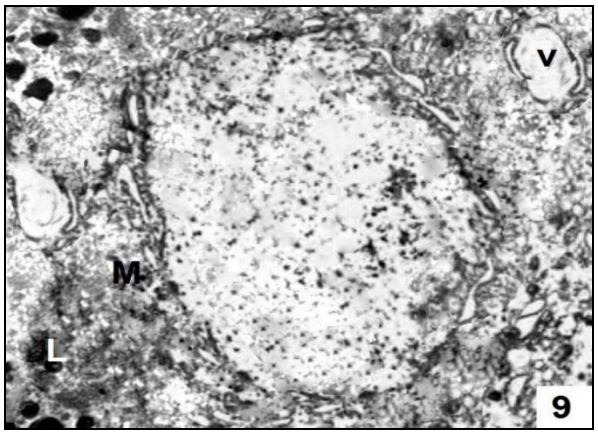

Fig. (9): A photomicrographs of sections in the GCL of retina of diabetic rats showed a swollen mitochondria (M) with loss of structural details and lipofuscin granules (LF) and cytoplasmic vacuoles (v), (TEM X6000).

Treatment of diabetic rats with garlic showed significantly improvementof nuclei of rods and cones in the ONL when compared with treated diabetic group (Fig.10). In the INL, showed significantly improvement of horizontal, bipolar, amacrine and Muller cell were observed (Fig.11). In the ganglion cell layer showed significantly improvementof ganglion cells with large euchromatic nuclei and moderate amount of organelles were also detected (Fig. 12).

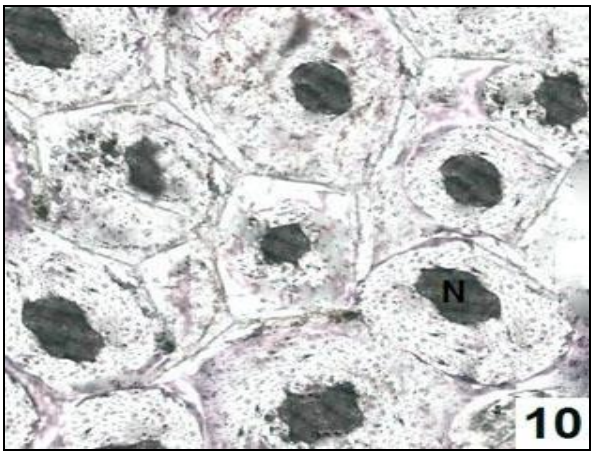

Fig. (10): A photomicrograph of a section in the ONL of retina of garlic treated rats showed significantly improvement of nuclei (N) of rods and cones when compared with diabetic group, (TEM X4000).

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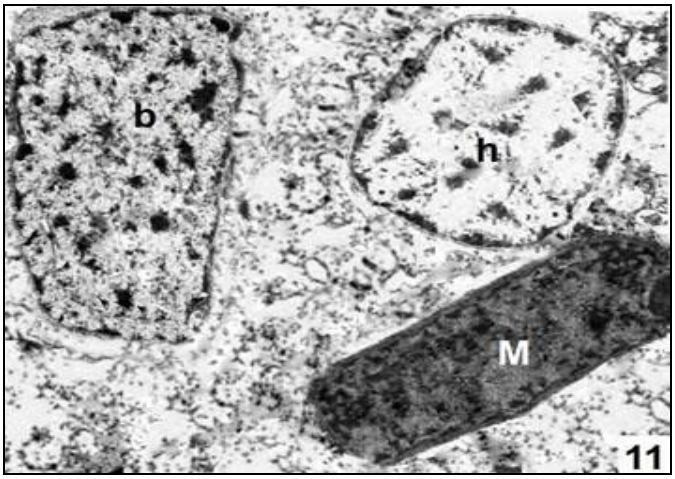

Fig. (11): A photomicrograph of a section of the INL of retina of rat of third group showed significantly improvement of horizontal cell (h) bipolar cell (b), and Muller cell (M) with electron dense nucleus, (TEM X6000).

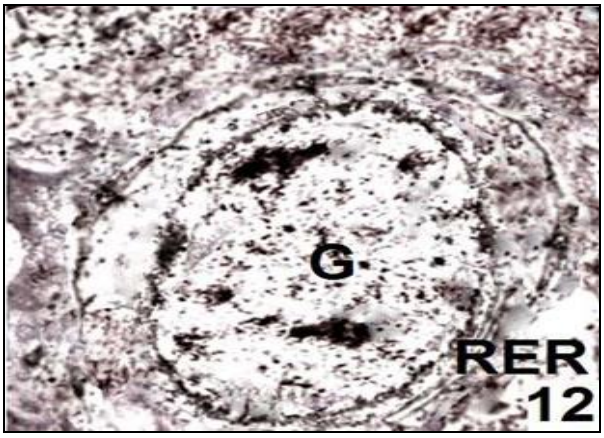

Fig. (12): A photomicrographs of a section in the GCL of third group showed significantly improvement of ganglion cell (G) with a large euchromatic nucleus and rough endoplasmic reticulum (RER), (TEM X4000).

DISCUSSION

Streptozotocin exerting cytotoxic effect on pancreatic β-cells, possiblyby generating lipid peroxides and excess reactive oxygenspecies (ROS), interfering with glucose transporter GLUT-2and causing DNA damage either by alkylation or peroxynitrite formation [12, 13]. In the present study the garlic reduced the plasma glucoselevels in STZ induced-diabetic rats. The hypoglycaemic action of garlic could be due to an increase in pancreatic secretion of insulin from β-cells, release of bound insulin or enhancement of insulin sensitivity. It has been suggested that garlic can enhance serum insulin by effectively combining with compounds like cysteine, which would spare insulin from SH group reactions which are a common cause of insulin inactivation. The antioxidant effect of S-allyl cysteine sulfoxide, an isolated product from garlic, may contribute to its beneficial effect in diabetes [14].

In the present study, the measurements of the diabetic rats' body weight revealed a highly significant decrease in their body weight in comparison with the control group, these results were in parallel with the results of Obrosova *et al.* [15], who contributed that the decrease of body weight either to the increase of the urine output causing dehydration and loss of valuable fluids or to the breakdown of muscles caused by high blood sugar.

We also observed that the weight loss that occurred in STZinduced diabetic rats was attenuated by garlic treatment compared to the control and diabetic group .These changes may be a reflection of the improved health of the garlic-treateddiabetic animals these results were in agreement with the results of Thomson *et al*. [14].

In this study, blood glucose showed a highly significant increase in its level in experimentally induced diabetic rats. These results were in agreement with results of Menon *et al.* [16]. In addition, there was a highly significant increase in the level of glycosylated hemoglobin, which is considered as a marker of protein glycation, these were in agreement with Sheykhzade *et al.* [17].

In the garlic treated diabetic group, there was highly significant decrease in both blood glucose level and glycated haemoglobinas compared to the diabetic group. These results were in agreement with Eidia *et al*. [18], who reported that garlic alcoholic extract significantly decreased serum glucose, triglycerides, cholesterol, urea, uric acid, AST and ALT, while increased serum insulin levels in treated diabetic rats as compared with diabetic rats. Theyalso added that the garlic extract might enhance glucose utilization because it significantly decreased the blood glucose level in glucose-loaded rats. It may be due to restoration of delayed insulin response or due to inhibition of intestinal absorption of glucose [19], also contributed that the hypoglycaemic effect of garlic, attributed mainly toallicin-type compounds.

In diabetes several signs appear, one of which is retinopathy [20]. The main cause of visual impairment and blindness throughout the world is diabetic retinopathy [21]. The retina, as part of thecentral nervous system, uses glucose as anexclusive energy source for dynamicactivities such as for capturing images andfor primary visual processing [22].Therefore, systemically impairedglucose metabolism causes dysfunction inthe neural retina soon after the onset of diabetes [23].

Photoreceptors of the outer retina have an important in the pathogenesis of early diabetic retinopathy, and the retinal microvasculature that is affected by diabetes.Recent studies suggest that photoreceptors might play a critical role in the diabetes induced degeneration of retinal capillaries, and thus can no longer be ignored. The present study suggested that diabetes-induced alterations in photoreceptor structure, these results were in parallel with data of Kern and Berkowitz [24]. In the present work the outer nuclear there were vacuolated cytoplasm in addition, there was apparent decrease in their number. This was in accordance with Faried *et al.* [25], who noticed degeneration of cones with decrease in the number of the cells in outer nuclear layer. Also in the inner nuclear layer of the retina in the present work, spacing between their cells with apparent vacuolated cytoplasm was noted. In addition, there was apparent decrease in their number. This was in accordance with Szabadfi *et al.* [26], who noticed that the degeneration of cones with decrease in the number of the cells with degenerative changes in cells of the inner nuclear layer and with Lu *et al.* [27], who reported that the inner nuclear layers of the retina decreased from 3-4 rows to 2 rows.

By transmission electron microscope especially the outer nuclear layer the present study showed that vacuolated rarified cytoplasm in the different layers of the retina of diabetic rats [29]. This was in agreement with Salido *et al.* [28], who reported that the presence of vacuolated cytoplasm within the diabetic retinas to the occurrence of hyperosmolarity state as a result of hyperglycemia. As regard the ganglion cell layer, the present study showed marked degeneration of the ganglion cells. Faried *et al.* [30], attributed that this degeneration due to the presence of apoptosis in ganglion cells. Atrophy or loss of retinal ganglion cell demonstrates the retinal damage in almost all strains of diabetic rats induced by the toxic chemical streptozotocin.

The reduction in the thickness of inner plexiform layer of retina also proves the retinal ganglion cell loss or damage. Various researchers have demonstrated retinal ganglion cell death or loss in diabetic rats, the duration of their studies extended from 8weeks to 1 year [30].These results were in coincidence with the histological observation in our study revealed atrophy or loss of retinal ganglion cell in the streptozotocin induced diabetic rats retina. A gradual decrease in the thickness of the inner retina, to well below that observed in the normal state, seems more likely due to the emergence of the decay of the neural components affected by the altered biochemical environment, including the glucose transport system [31].

In the present study, there was a significant decrease in the thickness of the sensory retina with the progression of the disease; these results were parallel with Faried *et al.* [25], who reported that decrease in the thickness and degenerative changes that involved the sensory retina as a result of DM.

In the present study, degenerative changes were present in the retina of the diabetic groups after induction of DM. These degenerative changes affected nearly all layers of sensory retina, these was in agreement with Martin *et al*. [32], who reported that the histological changes to the inner retina seemed to

be more severe [25], added that significant decrease in the retinal functions within 4 to 5 weeks after STZ treatment.

According to the longer duration of its development, DR is generally divided into two stages: non-proliferative stage, also named early stage, which is characterized by the leakage of vessels; and proliferative stage or late stage, where proliferation of retinal vessels will be induced by various growth factors [28, 33]. These results were in parallel with our results where long term of diabetes induces proliferation of retinal blood vessels.

In the present work, it was noted that garlic markedly delayed the onset of DR in comparison to the diabetic untreated rats along morphometric, histological, and electron microscopic levels.In the present study, garlic ameliorated the damaging effect of DM on the retina along the morphometric and histological levels. This was in agreement with Shiju and Viswanathan [34], who conclude that aged garlic extract has the ability to ameliorate kidney damage in diabetic rats and the renoprotective may be attributed to its antiglycation and hypolipidemic activities.

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