EVALUATION OF THE EFFECT OF FLAVONOID EXTRACT OF *Ginkgo biloba* LEAVES AND GLIMEPHAN ON OXIDATIVE STRESS AND RETINA DEGENERATION IN DIABETIC MALE RABBITS INDUCED BY STREPTOZOTOCIN

Wasan A. Jawad* , Muna H. AL-Saeed**

*Department of Optical-Techniques, College of Health and Medical Technology, Baghdad, University of Middle Technical.Baghdad,Iraq.

**Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Basrah.Basrah,Iraq.

(Received 15 september 2019,Accepted 6 October 2019)

Key words: Diabetes mellitus, *Ginkgo biloba*, Retina, Streptozotocin.

Corresponsing Author: mina31232@gmail.com

ABSTRACT

This study was carried out in animal house of Collage of Veterinary Medicine/ University of Basrah. An attempt has been done to induce diabetic by streptozotocin in male rabbits and investigation of the diabetes was induced oxidative stress and retina degeneration. Moreover, the purpose of this study was to isolate and evaluate the ameliorating effect of flavonoid extract of *Ginkgo biloba* leaves and glimephan in the prophylaxes or delay the development of diabetic retina degeneration and scavenging free radical induced oxidative stress and diabetic retina degeneration in male rabbits.The study was done on (32) adult male rabbits, their weight ranged between (2000-2500g) and aged between 7-8 months. The male rabbits were divided randomly into four groups, each group consists of eight rabbits as the following:

Group1:- Male rabbits at (Negative controls(-ve)) administrated normal saline1ml orally for 30 days.

Group2:- Male rabbits at(Positive control(+ve)) were given streptozotocin (65mg/kg B.W. dissolve in sodium citrate I.V.) for two days and remain for 30 days.

Group3:- Male rabbits were given streptozotocin(65mg/kg B.W. dissolve in sodium citrate I.V.) for two days, then treated with flavonoid extract of *Ginkgo biloba* leave (500mg/kg B.W. orally administration) for 30days.

Group4:- Male rabbits were given streptozotocin (65mg/kg B.W. dissolve in sodium citrate I.V.) for two days, then treated with glimphan drug (0.1mg/kg orally administration) for 30 days.

At the end of the experimental period, the blood samples were collected from heart by cardiac puncture for isolated serum and analysis biochemical parameters such as glucose, insulin, malonaldehyd (MDA),Superoxide dismutase (SOD) and glutathione peroxidase (GPx) concentrations and lipid profile. The results revealed a significant ($P \leq 0.05$) decrease of body weight, body weight gain and HDL concentrations in serum diabetic rabbits control(+ve) compared with control(-ve) while the results showed a significant ($P \leq 0.05$) increase the glucose, insulin cholesterol LDL,VLDL,MDA and GPx concentrations in serum diabetic male rabbits control(+ve) compared with control(-ve). The results obtained a significant decrease ($P \leq 0.05$) in body weight, body weight gain, SOD and HDL concentrations in serum diabetic male rabbits control(+ve). While male rabbits treated with flavonoid extract of *Ginkgo biloba* the results observed a significant ($P \leq 0.05$) increase in body weight and body weight gain compared with control(+ve). Whereas the results were revealed a significant($P \leq 0.05$)decrease of glucose, insulin, cholesterol, LDL,VLDL. MDA and GPx concentration rabbits treated with flavonoid extract of *Ginkgo biloba* leave compared with control(+ve) and glimephan also in addition, this extract improved the retina degeneration.Histological examination observed many pathological changes in pancreas and retina in diabetic group but in treated with flavonoid extract of *Ginkgo biloba*, the histological changes were near to the normal status. It is concluded that good anti-diabetic activity, hypoglycemia effect and regeneration of retina. Based on these results, we suggested the possible utilization of *Ginkgo biloba* as a therapy to prevent diabetic complication and improved the retina degeneration compared with another treated such as glimephan drugs.

INTRODUCTION

Diabetes mellitus is a metabolic disorders that is characterized by hyperglycemia and insufficiency in production or action of insulin produced by the pancreas within

the body [1]. Insulin is a hormone synthesized in β-cells of pancreas in response to various stimuli such as glucose, sulphonylureas and arginine however glucose is the major determinant[2]. The complications consist of both microvascular (neuropathy, nephropathy, and retinopathy) and macrovascular (atherosclerotic) disease [3]. There are several factors that play great role in pathogenesis of diabetes such as hyperlipidemia and oxidative stress leading to high risk of complications [4].

Diabetes in animals can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as Streptozotocin causes destruction of pancreatic β-cells which results in reduced insulin production. As a result, fatty acid is released from adipose tissues accompanied by increase ketone synthesis and progression to ketosis [5].

Ophthalmic complications of hyperglycemia are most effective in retina, represented by Diabetic retinopathy (DR) that is a vascular disease of the retina [6]. It is the most common cause of blindness in people over the age of 50 years [7].

The synthetic drugs for diabetes treatment are valuable in the treatment of diabetes mellitus, limited pharmacokinetic characteristic, secondary failure rate and accompanying adverse effects such as cardiovascular disease, hypoglycemia, coma and damage of the kidney and liver that can endanger the life of diabetic patient[8].

There are many anti-diabetic plants, which might provide useful source for the development of drugs in the treatment of diabetes mellitus, such as *Ginkgo biloba* [9]. *G. biloba* extract is used for treatment of diseases such as retinopathy, Alzheimer's disease, cardiovascular disease, dementia, memory loss, and cerebral ischemia [10]. The pharmacological modes of action include antioxidant effects, radical scavenging, inhibition of platelet activating factor, alterations in membrane fluidity (signal transduction), and inhibition of glucocorticoid synthesis[11]. also protect retinal tissue from oxidative stress. These might prevent progression of tissue degeneration in patients with D.M [12]. The study aimed to determine the effects of the flavonoid extract of *Ginkgo biloba* on oxidative stress and retinal degeneration in diabetic male rabbits.

MATERIAL AND METHODS

Experimental Animal Thirty two healthy adult male domestic rabbits with body weight ranged (2000g-2500g) about (7-8 months) age old were brought from local markets.). The animals were allowed to acclimatize in animal house in Veterinary Medicine Collage / Basrah University for two weeks before experimentation. The experimental animals were kept in well ventilated cages with (23±2 ºC) under controlled conditions about 12 hour light and 12 hour dark[13]. Provided with ration composed fodder which consists of dry bread and lettuce and drinking water.

Preparation of Flavonoid Extract of *Ginkgo biloba* Leaves.This method according[14]

Experimental Desig: Thirty two rabbits were divided randomly into four equal groups each group consistof eight animals as following:

The first group: served as (negative control)(-ve), administration of normal saline1ml daily for 30 days.

The second group: served as(positive control) (+ve)injected of single dose of STZ (65mg/kg B.W)[15] into marginal ear vein after over fasted night. STZ dissolve in citrate buffer pH4.5 and administration D.W 1ml for 30 days. Then give after STZ injected water contain 5% glucose instead of drinking water to overcome the high insulin released to all rabbits injected with STZ and let it without treated for 30 days.

The third group: served as treated group injected of single dose of STZ (65mg/kg B.W) into marginal ear vein after over fasted night and dissolve in (1ml) of citrate buffer (pH4.5) Then give to all rabbits injected with STZ water contain 5% glucose instead of drinking water to overcome the high insulin released and after five day treated with flavonoid extract of *ginkgo biloba* orally (500 mg/kg/B.W) dissolved in 1ml normal saline for 30 days

The fourth group: served as treated group injected of single dose of STZ (65mg/kg. B.W) into marginal ear vein after over fasted night and dissolve in (1ml) of citrate buffer pH4.5 Then give to all rabbits injected with STZ water contain 5% glucose instead of drinking water to overcome the high insulin released and after five day then treated with glimephan (0.1mg/kg B.W) for 30days.

Studied Parameters

Body Weight and Body Weight gain Measurement: The weight of each animal was recorded in zero and at the end of the experiment by using balance

Biochemical Analysis: Analysis glucose, insulin, enzyme of oxidative stress such as MDA, SOD, GPx and Lipid profile.

Histological Techniques.: The organ samples were removed from animals after the end of experimental as pancreas and eyeball and this method according to [15].

Statistical Analysis :The mean values ± SD. Statistical analysis by using multivalent analysis of variance. All statistical analyzes were performed using SPSS statistical version 23 software package. Least significant different test (LSD) was used to test the difference between means (groups) $p \leq 0.05$ was considered significant [16].

RESULTS

Body Weight and Body Weight Gain of Diabetic Male Rabbits Induced by Streptozotocin treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan.

The results in Table (1) revealed a significant reduce ($P \leq 0.05$) in body weight and body weight gain of diabetic male rabbits compared with control(-ve) and treated with flavonoid extract of *Ginkgo biloba* while the results showed non-significant change ($P > 0.05$) body weight of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* and treated with glimephan compared with control (-ve) group. But the results exhibit a significant increase ($P \leq 0.05$) in body weight gain of diabetic rabbit treated with flavonoid extract of *Ginkgo biloba* and treated with glimephan compared with control(+ ve) and exhibit a significant decrease ($P \leq 0.05$) in body weight gain of diabetic rabbit treated with flavonoid extract of *Ginkgo biloba* and treated with glimephan compared with control(-ve).

Table 1: Mean Body Weight and Body Weight Gain of Diabetic Male Rabbits Induced by Streptozotocin treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan (Mean±SD) (n=8)

Parameters / Groups	Body Weight (g)		Body Weight Gain (g)
	0Days	30Days	
Control (-ve) Normal Saline(0.9% NaCl)	2218.80±194.45	2355.00±177.03 A	136.25±22.63 A
Control (+ve) Streptozotocin (65mg/kg)	2081.20±231.35	1736.20±229.28 B	-345.00±76.34 D
Streptozotocin+Flavonoid Extract of *Ginkgo biloba*	2175.00 ±236.03	2231.20±199.60 A	56.25±16.88 B
Streptozotocin+Glimephan (0.1mg/kg)	2162.50±330.83	2126.20±281.47 A	-33.75±8.67 C
LSD	NS	390.00	80.00

n=number of animals., Small letters denote differences between groups,P≤0.05 vs. control,

Glucose and Insulin Concentrations in serum of Diabetic Male Rabbits Induced by Streptozotocin Treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan.

The obtained results in Table (2) observed a significant rise (P≤0.05) in glucose concentration in serum of diabetic male rabbits compared with (-ve) control and another treated while the results showed non-significant change (P>0.05) glucose concentration in serum of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* compared with (-ve) control. The results showed a significant elevated (P≤0.05) in insulin concentration in serum of diabetic male rabbits compared with (-ve) control and another treated while the results showed non-significant change (P>0.05) in insulin concentration in serum of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* compared with (-ve) control.

Table 2: Mean Glucose and Insulin concentrations in Serum of Diabetic Male Rabbits Induced by Streptozotocin Treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan (Mean±SD (n=8)

Parameters / Treatments	Glucose mg/dl	Insulin (µl U/ml)
Control (-ve) Normal Saline(0.9% NaCl)	89.93±6.93 C	5.80±1.57 C
Control (+ve) Streptozotocin (65mg/kg)	216.82±48.34 A	20.18±3.42 A
Streptozotocin+Flavonoid Extract of *Ginkgo biloba*	82.46±4.96 C	4.81±0.63 C
Streptozotocin+Glimephan (0.1mg/kg)	125.48±3.45 B	11.00±1,41 B
LSD	35.54	5.20

n=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control.

Serum of Lipid Profile in Diabetic Male Rabbits Induced by Streptozotocin. Treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan.

The results in Table (3) revealed a significant elevate (P≤0.05) total cholesterol in serum of diabetic male rabbits (+ve) control compared with (-ve) control and another treated while the results showed a non-significant change (P>0.05) total cholesterol in serum of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leaves and treated with glimephan compared with (-ve) control. The results of triglyceride revealed non-significant change (P>0.05) in diabetic male rabbits (+ve) control compared with (-ve) control and another treated also the results showed non-significant change (P>0.05) triglyceride in serum of diabetic male rabbits treated with glimephan compared with (-ve) control and treated with flavonoid extract of *Ginkgo biloba* leaves.

The results of HDL revealed a significant decrease (P≤0.05) in diabetic male rabbits (+ve) compared with (-ve)control and another treated while the results showed a non-significant change (P>0.05) HDL in serum of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leaves and glimephan compared with (-ve) control.

The results of LDL revealed significant rise (P≤0.05) in diabetic male (+ve) control group compared with (-ve) control and another treated groups while the results showed a significant increase (P≤0.05) LDL in serum of diabetic male rabbits treated

with glimephan compared with (-ve) control and diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leaves but the results showed a non-significant change(P>0.05) LDL in serum of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leaves compared with (-ve) control.

The results of VLDL revealed a significant elevate (P≤0.05) in diabetic male rabbits (+ve) control compared with (-ve) control and another treated while the results showed a significant increase (P≤0.05) VLDL in serum of diabetic male rabbits treated with glimephan compared with (-ve) and diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leaves but the results showed no changes(P≤0.05) VLDL in serum of diabetic male rabbits treated with flavonoid

Table 3 :Serum of Lipid Profile in Diabetic Male Rabbits Induced byStreptozotocin Treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan (Mean±SD) (n=8)

Parameters / Treatment	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control (-ve) Normal Saline(0.9% NaCl)	117.50±13.69 B	65.83±19.60	37.83±8.84 A	15.16±5.03 B	53.33 ±21.60 AB
Control (+ve) Streptozotocin (65mg/kg)	213.33±51.25 A	96.66±35.59	21.00±4.73 B	84.66±18.18 A	72.16±20.88 A
Streptozotocin+Flavonoid Extract of *Ginkgo biloba*	131.67±17.51 B	67.83±25.53	35.00±13.03 A	24.16±8.08 B	47.33± 16.02 B
Streptozotocin+Glimephan (0.1mg/kg)	144.17±19.60 B	65.66±27.47	32.16±5.81 A	26.33±3.82 B	51.50±17.61 AB
LSD	69.16	NS	11.16	58.33	24.83

n=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control.

Serum of Oxidative Stress Enzymes :MDA, SOD and GPx in Diabetic Male Rabbits Induced by Streptozotocin Treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan.

The obtained results in Table (٤) revealed a significant rise (P≤0.05) MDA in serum of diabetic male rabbits induced by strepozotocin (+ve) control compared with (-ve) control and another treated while the results showed a significant elevate (P≤0.05) MDA in serum of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leaves and glimephan compared with (-ve) control.

The results of SOD revealed a significant decline (P≤0.05) in diabetic male rabbits (+ve) control compared with (-ve) control and treated with flavonoid extract of *Ginkgo*

biloba leaves while the results showed a non-significant change (P>0.05) SOD in serum of diabetic male rabbits treated with glimephan compared with (+ve) control.

The results of Gpx revealed a significant height (P≤0.05) in diabetic male rabbits (+ve) compared with (-ve)control and treated flavonoid extract of *Ginkgo biloba* leaves while the results showed a non-significant change (P>0.05) GPx in serum of diabetic male rabbits treated with glimephan compared with (+ve) control.

Table 4: Mean MDA, SOD and GPx in Diabetic Male Rabbits Induced by Streptozotocin Treated with Flavonoid Extract of *Ginkgo biloba* Leaves and Glimephan(Mean±SD) (n=8)

Parameters / Treatment	MDA mg/dl	SOD mg/dl	GPx mg/dl
Control (-ve) Normal Saline(0.9% NaCl)	1.76±0.074 C	358.18±9.29 A	4.01±0.17 B
Control (+ve) Streptozotocin (65mg/kg)	3.59±0.16 A	158.72±19.62 B	5.10±0.55 A
Streptozotocin+Flavonoid Extract of *Ginkgo biloba*	3.27 ±0.18 B	366.82±15.83 A	3.92±0.14 B
Streptozotocin+Glimephan (0.1mg/kg)	3.15±0.12 B	164.85±9.01 B	4.15±0.04 A
LSD	0.31	193.33	0.94

n=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control.

Grossly Examination of lenses Opacification:

The results of grossly examinations in Table (5) and Fig. (1) shows the white clouds covering the whole eyes of (+ve) control treated with streptozotocin (Fig. b) as compared to (-ve) control (Fig. a) and indicated that 100% in streptozotocin-induced diabetic (+ve) control developed bilateral (stage 5) while all lenses were clear in control. In diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* (Fig. c), data showed lowered maturation of stretozotocin-induced diabetic to 25%, while 75% of the lenses still displayed stage 4. The data in Table (5) shows the percentage of diabetic treated with glimephan was 50%.

Table 5: Grossly Examination on the Clear of Lenses of Eyes

Parameters / Treatment	Number of Rabbits	Stage of Clouds Lenese					% of Clouds	% of Eyes Improvement
		1	2	3	4	5		
Control (-ve) Normal Saline(0.9% NaCl)	8	-	-	-	-	-	0%	-
Control (+ve) Streptozotocin (65mg/kg)	8	-	-	-	-	8	100%	-
Streptozotocin+Flavonoid Extract of *Ginkgo biloba*	8	2	-	-	-	-	25%	75%
Streptozotocin+Glimephan (0.1mg/kg)	8	-	-	4	-	-	50%	50%

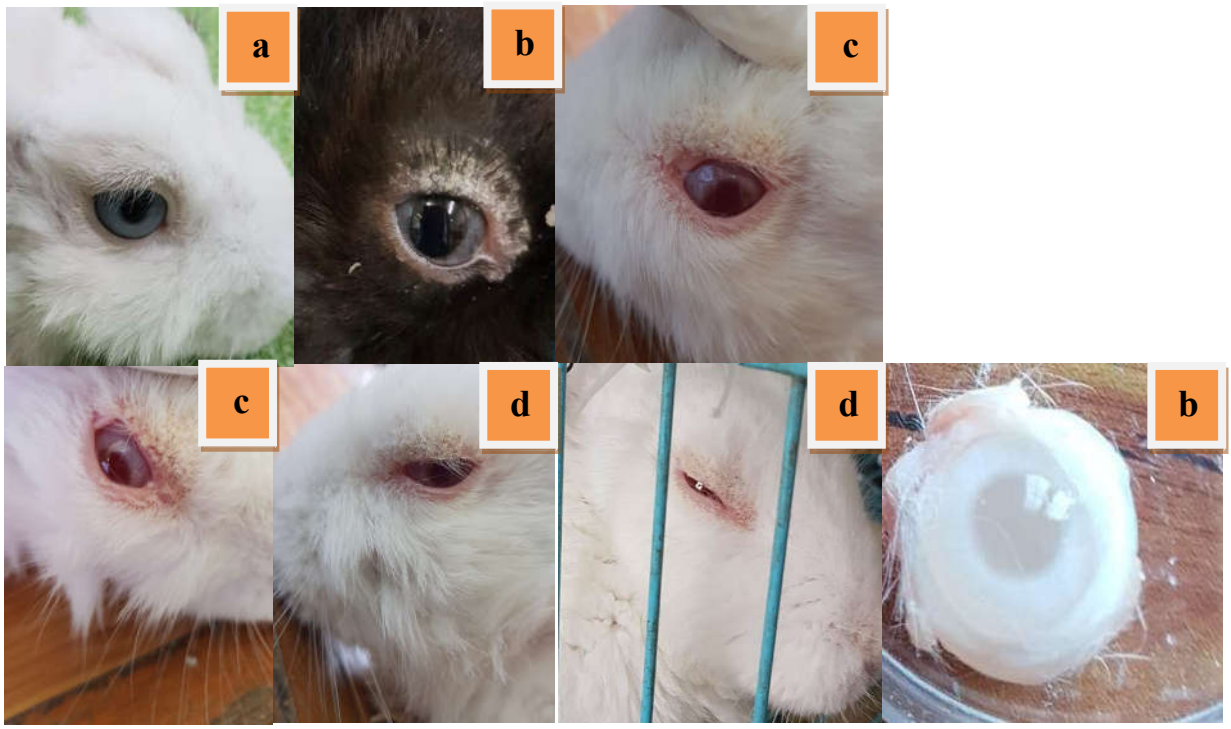

Fig.1:Representative gross photograph of eyes of rabbits for examination the lenses Opacification: a- (-ve) control; b-(+ve) control diabetic male rabbits; c-dibetic male rabbits treated with flavonoid extract of *Ginkgo biloba* Leaves. d- dibetic male rabbits treated with glimephan.

Histological Examination:

Pancreas :

The section of pancrease of (-ve) control male rabbits is composed of two major types of tissues, the *acini* and the *islets of Langerhans*. Fig.(2) and (6) normal of langerhan's islets. While the diabetic (+ve) control of male rabbits revealed

histological changes of both acini and *islets of Langerhans* of the pancreas represented by vacuolation and degeneration marked reduce of β-cells. Some exocrine acini revealed focal acinar damage represented by cytoplasmic vacuolation obvious as shown in Fig. (3) and(7).

But the pancrease of male rabbits treated with flavonoid extract of *Gingo biloba* leave showed amelioration of architecture of islets langerhan's compared to pancreas treated with streptozotocin alone as shown in Fig. (4) and (8). while the pancrease of male rabbits treated with glimephan showed clear revealed histopathological changes. The changes included vacuolation of langerhan's islets as shown in Fig. (5) and (9).

Retina :

The section of retina of **(-ve)** control male rabbits is composed of the innermost layer of the wall of the eye. The pigmented epithelium, which is adjacent to the choroid, absorbs light to reduce back reflection of light onto the retina. The ganglion cell layer contains cell bodies of ganglion cells. The optic nerve fiber layer contains axons of ganglion cells as shown in Fig. (10). While the diabetic (+ve) control of male rabbits revealed histological changes of retinal degeneration characterized by vacuolation of outer layer of ganglia, vacuolation of neuronal ganglia as shown in Fig. (11).

But the retina of male rabbits treated with flavonoid extract of *Gingo biloba* leaf showed retinal ganglia layers within normal layers as shown in Fig. (12). while the retina of male rabbits treated with glimephan showed clear revealed histological changes. The changes included partial vacuolation of ganglia in the inner layer of retina as shown in Fig. (13).

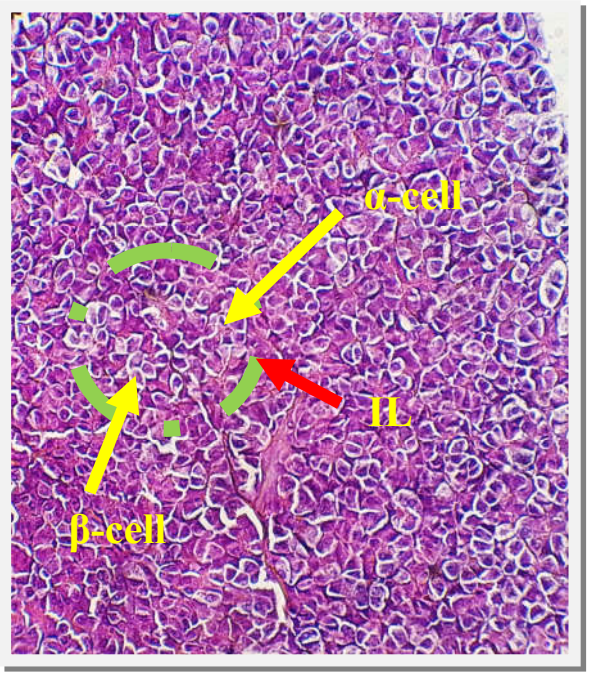

Fig.2: Section of pancreas of male rabbits ((-ve) control). Showing present normal islet of langerhans (IL) islet cell with light and large nuclei (β-cell) or with small, dark nuclei on periphery (α-cell)and normal acini (A), stained with H&E. (٤00X).

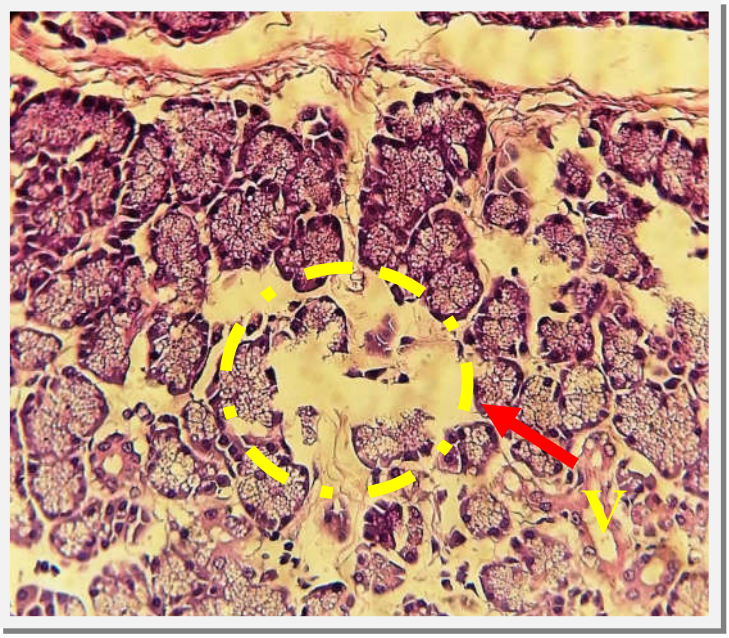

Fig.3: Section of pancreas of diabetic male rabbits ((+ve) control. Showing vacuolation (V) islets of langerhans (IL), Stained with H& E. (400X).

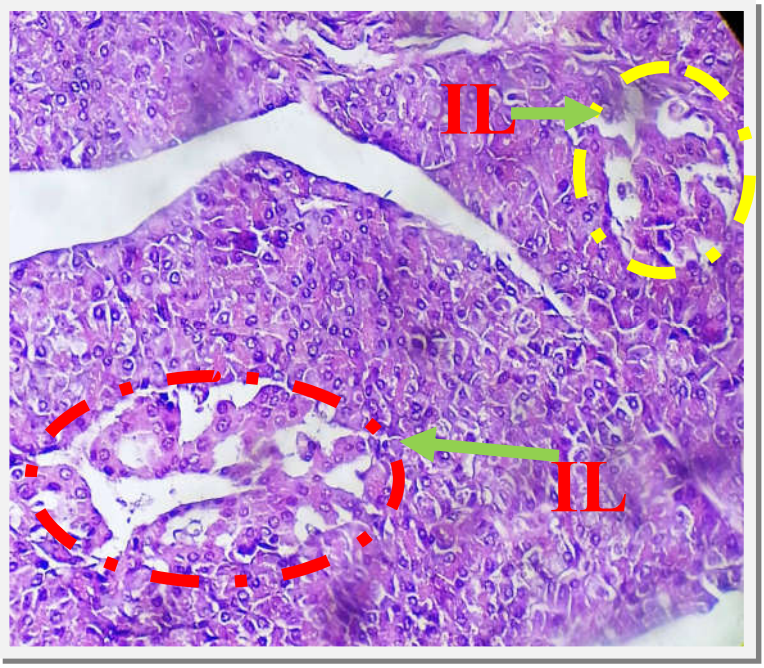

Fig.4: Section of pancreas of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba*. Showing within normal limits of islets of langerhans (IL), Stained with H& E. (400X

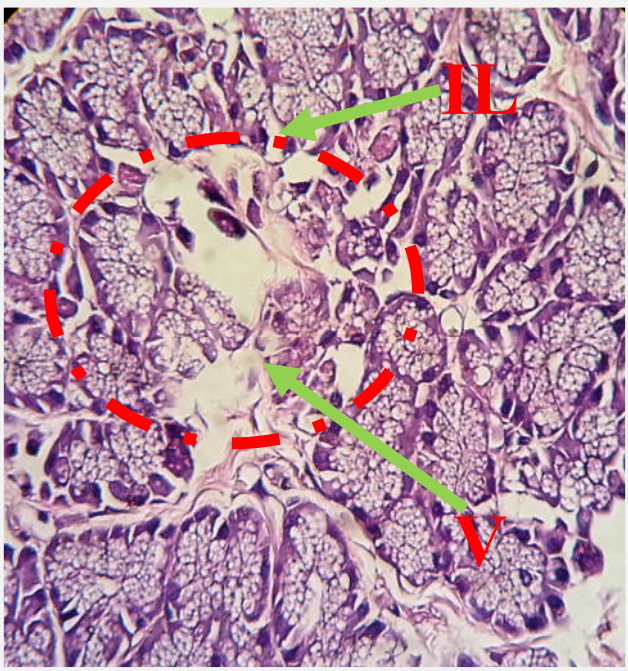

Fig.5: Section of pancreas of diabetic male rabbits treated with glimephan. Showing vacuolation (V) islets of langerhans (IL), Stained with H& E. (400X).

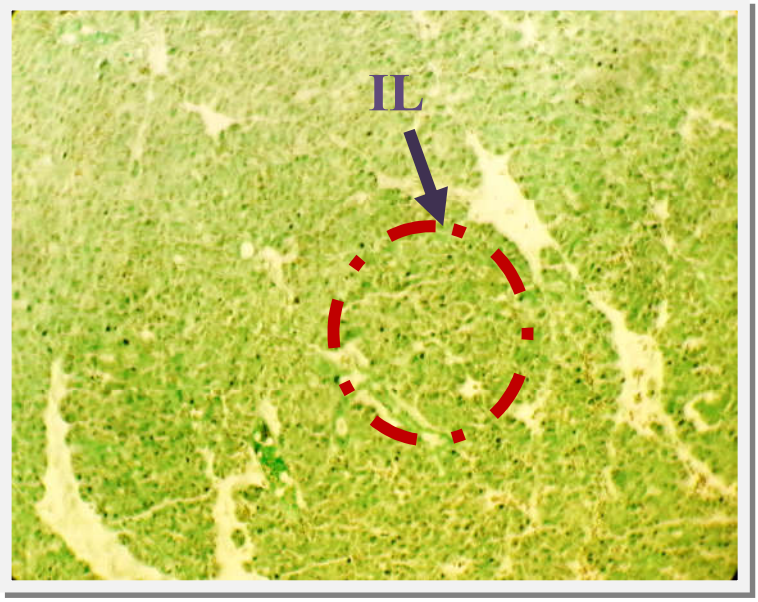

Fig.6: Section of pancreas of male rabbits ((-ve) control). Showing present normal islet of langerhans (IL), stained with Gomoroi aldehyde fucshion. (100X).

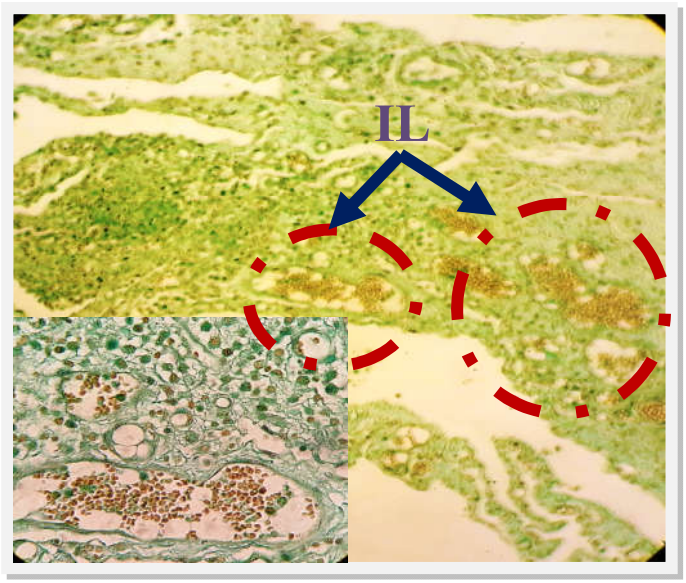

Fig.7: Section of pancreas of male rabbits ((+ve) control). Showing present degenerate, vacuolated islet of langerhans (IL), atrophic stained with Gomoroi aldehyde fucshion. (100X).

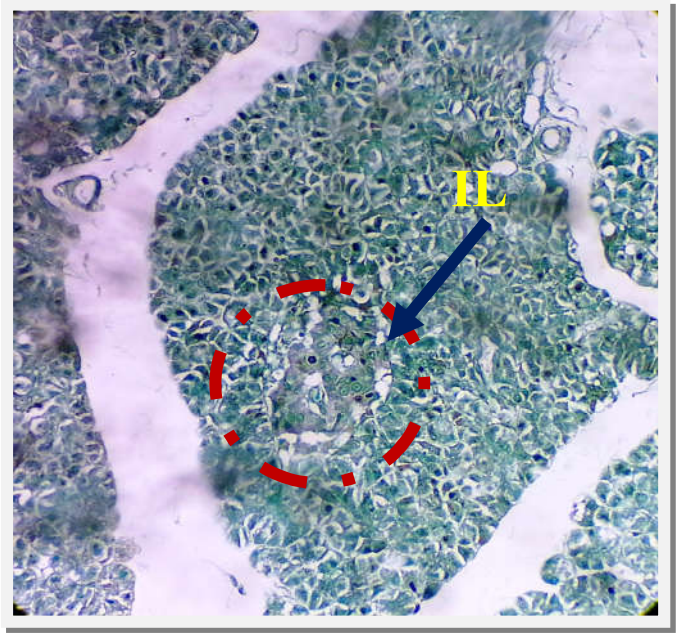

Fig.8: Section of pancreas of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba*. Showing within normal limits of islets of langerhans (IL), Stained with Gomoroi aldehyde fucshion. (400X).

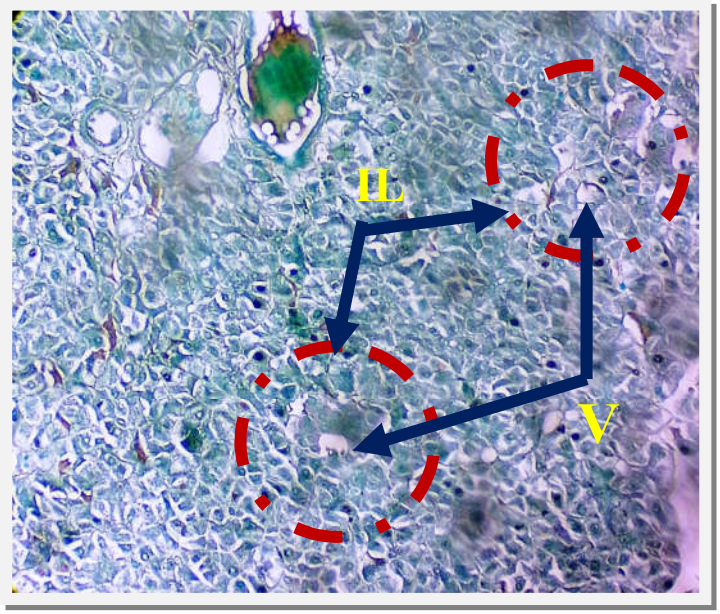

Fig.9: Section of pancreas of diabetic male rabbits treated with glimephan. Showing vacuolation (V) islets of langerhans (IL), Stained with Gomoroi aldehyde fucshion. (400X).

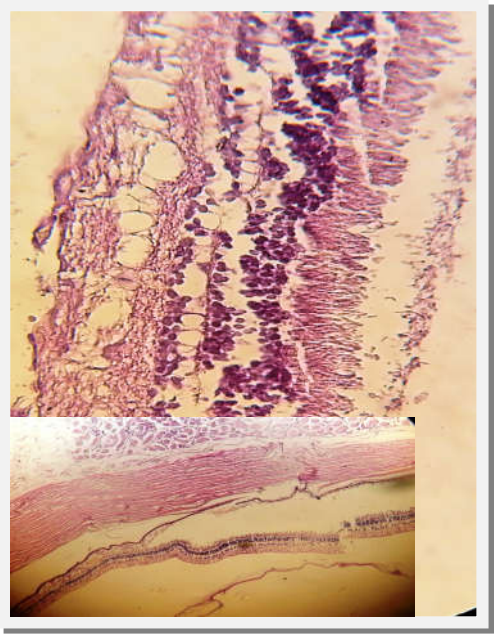

Fig.10:Section of retina of male rabbits((-ve) control). Showing present normal stained with H&E. (400X).

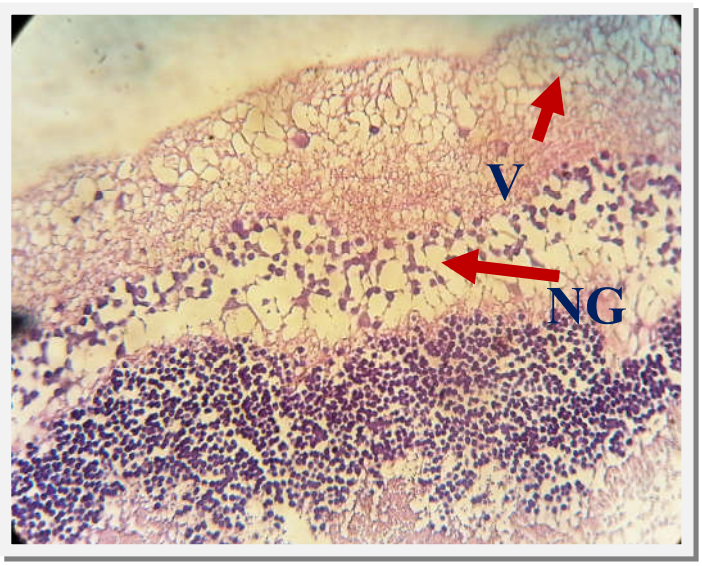

Fig.11: Section of retina of male rabbits((+ve) control). Showing retinal degeneration characterized by vocuolation of outer layer of ganglia, vacuolation of neuronal ganglia(NG). stained with H&E. (400X).

Fig.12: Section of retina of diabetic male rabbits treated with flavonoid extract of *Ginkgo biloba* leave. Showing Showing retinal ganglia layers within normal layers stained with H&E. (400X).

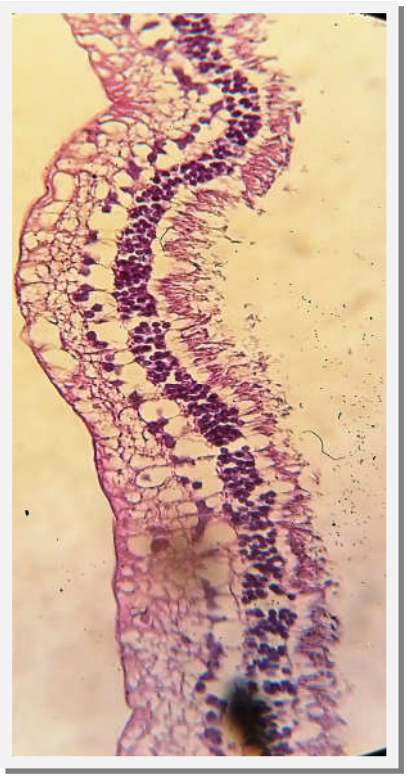

Fig.13: Section of retina of diabetic male rabbits treated with glimephan. Showing partial vacuolation of ganglia in the inner layer of retina, stained with H&E. (400X).

DISCUSSION

Diabetes mellitus was caused by dysfunction in pancreas in male rabbits [17] . This leads to partial or complete insulin deficiency in diabetic rabbits that appears to have side effects on all systems, including eyes lead to retina degeneration [18] . The present study concludes that sterptozotocine at 65mg/kg B.W weight induces the diabetes and the rabbits are shown higher glucose levels and reduced body weight and organs weight. This is associated with catabolism of fats and proteins due to proteolysis on muscular tissues occurred in insulin deficiency states[19] .

The animals treated with STZ appeared very weak with loss of their body weights because of injurious effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions. Present observations are in agreement with the findings of [20, 21,22,23] . Our results also showed that treated with flavonoid extract of *Ginkgo biloba* lead to improve the body weight and body weight gain in male rabbits. Through its contents which were flavonoids several flavonoid subclasses have been shown to decrease energy intake, increase glucose uptake in muscle *in vivo*, and decrease glucose uptake in adipose tissue in vitro.

In this study, glucose concentration of serum diabetic male rabbits elevate concentration above the normal concentration, and this elevation in glucose concentration was approximately constant through the course of diabetes. Reduction in pancreatic β-cell mass is associated with development of diabetes [24]. Treatment with the flavonoid extract of *Ginkgo biloba* revealed reduction in blood glucose concentration diabetic male rabbits, due to the presence of flavonoid in *Ginkgo biloba* leaves. These compounds can decline glucose level by stimulating pancreatic β-cells to secret more insulin in blood circulation [25,26] . It is possible that the flavonoid extract of *Ginkgo biloba* may increase glucose removal from blood, through reducing the release of glucagon or elevate that of insulin.

The changes of pancreas in diabetic male rabbits were showed by the vacuolation of islet. While the pancreas of male rabbits treated with flavonoid extract of *Ginkgo biloba* showed improvement of architecture of islets langerhan's compared to diabetic pancreas and treated with glimephan. The pancreas of rabbits treated with the

flavonoid extract of *Ginkgo biloba* showing nearly normal structure of islets of langerhans. The protective effects of *Ginkgo biloba* as antioxidant on streptozotocin induced β-cells destruction [27].

Flavoinod extract *Ginkgo biloba* is one of the most widely used herbs in the world, and is known for its numerous health benefits [28]. Previous studies showed that *Ginkgo biloba* leaves contains high amounts of narengin (193.15 ppm), rutin (108.49 ppm), hisperdin (107.21ppm) and quercitrin (94.86 ppm).

Ophthalmic examinations revealed diabetic rabbits only developed retina degeneration caused by oxidative stress[18]. The flavonoid content of *Ginkgo* enables the compound to scavenge the free radicals more effectively. The flavonoids in *Ginkgo* help protect cells against free radical contact. In addition, these flavonoids provide protection to blood vessels against the damaging effect of plaque build-up. The *Ginkgo* leaf extract is also known to improve coronary blood flow through antiplatelet activity and by improving contractile functions which are due to elevate release of catecholamines from endogenous liver tissue reserves by flavonoids [29]. In this study found significantly elevated in the level of MDA and GPx but significantly reduced in SOD (+ve) control treated with streptozotocin. After male rabbits treated with flavonoid extract *Ginkgo biloba*, the result showed gradually higher level in SOD and lower level in MDA as increased in the concentrations of *Ginkgo biloba* extract.

This study confirm that diabetes have an adverse effect on retina function as proposed by others[18] also this study demonstrates a protective effect of the flavonoid extract of *Ginkgo biloba* recovery of diabetes and retina degeneration eminent in diabetic state. The best results obvious clearly in retina degeneration treated with500mg/kg B.W. flavonoid extract of *Ginkgo biloba*. Our results concluded that *Ginkgo biloba* in a dose of concentrations 500mg/kg bw *Ginkgo biloba* supplementation protected the eye and inhibited clouds formation.

The pharmacological activity of flavonoid extract *Ginkgo biloba* was attributed to synergistic action of flavonoids such as terpene and trilactones [30]. Previous studies indicated the use of the *Ginkgo* extract in the treatment of heart, short term memory loss, diabetes, depression and dementia [31-33]. Also, acting as a powerful antioxidant [34,35]. In addition, *Ginkgo biloba* extracts were found to protect rats against different eye diseases such as age related macular degeneration [36]. diabetic retinopathy [37].

Gingko biloba leaves contain are full of flavonoids, which act as antioxidants and these flavonoids are known to help with retinal problems [38].

The major causes of blindness are diabetic retinopathy and trauma. Among these alternations, cataract is the foremost cause of blindness globally and is responsible for 50, 51% of total blindness [39]. Therefore an alternative medicine treatment for the control/delay of diabetic retina degeneration could make a great impact in these parts of the world. Recognition of the role played by natural antioxidant in delaying the onset of diabetic retina degeneration has opened new avenues for treatment retinpathy [40] Recent investigations have shown that phytochemicals antioxidants can scavenge free radicals and prevent various diseases like retina degeneration. Both of synthetic and plant origin that could be effective in a delaying or preventing the formation of retina degeneration [41].

The present study was designed to determine the possible protective effects of *Ginkgo biloba* against oxidative damage induced by streptozotocin. The excess oxidative stress was previously reported to induce extensive oxidative modifications on lens proteins especially α-crystalline protein, a major protein component of the lens resulting in the enhanced lens opacity. *Ginkgo biloba* extract is considered an alternative medicine for the treatment and / or the prevention of different eye diseases. Flavonoid extract *Ginkgo biloba* can enhance the antioxidant ability of retina and partially inhibit the apoptosis of photoreceptors (42) . *Ginkgo biloba* has also been shown to prevent diabetic retinopathy in diabetic rats. *Ginkgo biloba* may act as a neuroprotective and prevent damage to retinal ganglion cells, this plant extract would be an interesting component for prevention and treatment of ocular diseases and other major neurodegenerative retinal pathologies [43] .

The results revealed an increase in the serum of total cholesterol and LDL and VLDL in male rabbits treated with streptozotocin. In streptozotocin-induced diabetes, the rising in serum cholesterol and reducing in HDL concentrations is usually associated with an increase in glucose concentration[44-46].

These results indicate the relationship between hypercholestermia and retina degeneration. The most significant ocular findings, such as the accumulation of lipids in the choroid, retinal disorganization, and lipid keratopathy. With respect to the retinal macroglia, the synthesis of the apoE by the Müller cells, its subsequent secretion in vitro, and its being taken up by the axons and transported by the optic nerve enabled the detection of apoE in the latter geniculate body and in the superior

colliculus [47] . These optically empty spaces, with an elongated or needle shape, were previously occupied by crystals of cholesterol monohydrate or crystals of cholesterol esters [50] In other studies, the analysis in the form adopted for the crystallizations of the different types of lipids revealed that the needles corresponded to esterified cholesterol, and the short, thin ones to triglycerides [49] . It had been recently reported that hypercholesterolaemic rabbis had a build-up of lipids (foam cells and cholesterol clefts) mainly at the suprachoroidea and to a lesser extent at the choroidal vascular layers. This lipid compressed the choroidal vessels and causes hypertrophy of the vascular endothelial- and vascular smooth-muscle cells. The ultra-structural analysis of these vascular structures demonstrated numerous sings of necrosis and a severe damage of the cytoplasmic organelles and caveolar system [48,51] Recently, it has been reported that in comparison with normal control animals, hypercholesterolaemic rabbits had a reduction of the amplitudes of the first negative peak of the visually evoked potentials, the density of the RGCs, and the thickness of the INL and photoreceptor-cell layer. Enhanced activity of iNOS in hypercholesterolaemic rabbits might be involved in impaired visual function and retinal histology. Down regulation of eNOS activity might be one of the causes for impairment of the autoregulation [52]. The formation of foam cells is a consequence of phagocytes from the macrophage-oxidized LDL (48) , with the retention of cholesterol in the vascular wall and the activation of ACAT (acetyl-cholesterol-acyl-transferase) [53] .

تقييم تأثير المستخلص الفلافونويد لاوراق نبات الجنكو *Ginkgo biloba* *leaves* وعقار Glimephan على الاجهاد التاكسدي والتنكس الشبكي في ذكور الارانب المستحثة داء السكري بوساطة االستريبتزوتوسين

*وسن عباس جواد * ، منى حميد السعيد**

*قسم التقنيات البصرية، كلية التقنيات الصحية والطبية/ بغداد، جامعة التقنية الوسطى، بغداد . العراق

**فرع الفسلجة والأدوية والكيمياء ،كلية الطب البيطري ، جامعة البصرة. البصرة . العراق.

الخلاصة

تمت الدراسة في البيت الحيواني التابع الى كلية الطب البيطري- جامعة البصرة لمحاولة استحداث داء السكري باستخدام الستريبتوزوتوسين ولمعرفة ما يعكسه داء السكري من مضاعفات على شبكية العين في

ذكور الارانب فضلا عن دراسة التأثير العلاجي لمستخلص الفلافونيد لاوراق نبات الجنكو بايلوبا بجرعة (٥٠٠ملغم / كغم من وزن الجسم) بالمقارنة مع عقار glimephan (المخفض لمستوى السكر في الدم بجرعة ٠.١ ملغم/كغم من وزن الجسم) ومدى فاعلية هذا المستخلص في تخفيض مستوى السكر و وتجنب اثاره الضارة. وتضمنت الدراسة استخدم ٣٢ ذكر من الارانب البالغة المحلية ، تتراوح أوزانها ما بين (٢٠٠٠-٢٥٠٠غم) وبأعمار تتراوح (٧- ٨ شهر) قسمت عشوائيا بالتساوي إلى اربع مجاميع (٨ ارنب/ مجموعة) .

الأولى:- مجموعة السيطرة(السالبة) جرعت ذكور الارانب (٠.٩ %) من المحلول الفسيولوجي (١مل)لمدة ٣٠ يوم.

والمجموعة الثانية:- مجموعة سيطرة (موجبة) حقنت ذكور الارانب في الوريد الاذني بالستريبتوزوتوسين (٦٥ ملغم/كغم) بعد أذابته ب(١مل)من بفر الصوديوم ستريت لمدة ٢ يوم لاستحداث داء السكري وتركت لمدة ٣٠ يوم بدون إي معاملة.

المجموعة الثالثة :- حقنت ذكور الارانب في الوريد الاذني بالستريبتوزوتوسين (٦٥ ملغم/كغم)بعد أذابته ب(١مل) من بفر الصوديوم ستريت لمدة ٢ أيام + عقار glimephan لمدة ٣٠ يوم.

المجموعة إلرابعة:- حقنت ذكور الارانب في الوريد الاذني بالستريبتوزوتوسين ٦٥ ملغم/كغم بعد أذابته ب(١مل)من بفر الصوديوم ستريت لمدة ٢ أيام+ (٥٠٠ ملغم/ كغم) من المستخلص الفلافونويد لاوراق نبات الجنكو مدة ٣٠ يوم المجموعة.

بعد انتهاء فترة التجربة تم سحب عينات الدم(١٠مل) من قلب الحيوانات حيث وضعت في أنابيب غير حاوية على مانع للتخثر لغرض الحصول على مصل الدم لإجراء بعض قياسات المعايير الكيموحيوية كقياس تركيز السكر وهرمون الانسولين في مصل الدم وقياس تراكيز بعض الانزيمات SOD,MDA,GPxو صورة الدهون كاملة.لوحظ هناك انخفاض معنوي ($p\leq0.05$) في اوزان والزيادة الوزنية للحيوانات المصابة بداء السكر المستحث بواسطة الستربتوزوتوسين. كما لوحظ ارتفاع معنوي($p\leq0.05$) في مستوى السكر والانسولين و الكوليسترول و.LDL,VLDL,MDA, GPx, بينما لوحظ انخفاض ($p\leq0.05$) في مستوى HDL,SODللحيوانات المصابة بداء السكر مقارنة مع مجموعة السيطرة السالبة غير المعاملة، بينما لوحظت النتائج العكس عند معاملة للارانب المصابة بداء السكر بالمستخلص الفلافونويد لاوراق نبات الجنكو اي ان هناك انخفاض معنوي ($p\leq0.05$) في مستوى السكر والانسولين و الكوليسترول و ,LDL,VLDL,MDA, GPx وارتفاع معنوي ($p\leq0.05$) في مستوى HDL,SOD.لوحظ ايضا وجود تغيرات نسجية في البنكرياس وشبكية العين لذكور الارانب المصابة بالسكري ولكن هذه التغيرات النسجية اختفت في الحيوانات المعاملة بالمستخلص الفلافونويد لاوراق نبات الجنكو وكانت اقرب الى الطبيعي.نستنتج أن مستخلص الفلافونويد لاوراق نبات الجنكو بايلوبا له فعالية مضادة لداء السكري وامن مقارنة بالعلاجات الاخرى المستخدمة مثل عقار glimephan إي له تأثير مخفض للسكر.

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