



# The Possible Protective Role of Both, Rosiglitazone and Repaglinide on Liver and Kidney of Diabetic Guinea Pig (*Caviaporcellus*)

Ali Abd Alsalaam<sup>1, \*</sup>, Fathy M. Elshaer<sup>2</sup>, Hamdi Abdou Mansour<sup>1</sup>

<sup>1</sup>Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Assiut, Egyspt

<sup>2</sup>Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt

## Email address:

Dr.Ali4@yahoo.com (A. A. Alsalaam)

\*Corresponding author

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**Abstract:** Diabetes mellitus is one of the most costly and burdensome chronic disease of our time. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to resistance to the action of insulin, insufficient insulin secretion, or both. Diabetes mellitus causes nephropathy and fatty change in the liver and vascular changes. Rosiglitazone and repaglinide are new anti-diabetic agents. Rosiglitazone is a thiazolidinedione's agent acting as insulin-sensitizer. Repaglinide a non-sulphonyl urea insulin secretagogue, is a prandial glucose regulator. The present study is aimed to assess the potential protective role of Rosiglitazone and Repaglinide on Liver and Kidney tissues of diabetic guinea pig (*Caviaporcellus*). The used Guinea pigs were divided into five groups. Diabetes mellitus is induced in 4 groups of them by oral administration of fructose 50% concentration. One of the diabetic groups was served as diabetic non treated and the other 3 groups were treated by Rosiglitazone, Repaglinide and a combination of both drugs, respectively. Blood samples were collected for the biochemical studies. The animals were sacrificed and the liver and kidney were excised to be used for the histopathological studies. The present study showed that, the combination of rosiglitazone & repaglinide may have a synergistic protective effect against diabetes mellitus - induced renal and liver tissues damage. This study proved that the combination of rosiglitazone & repaglinide in treatment of diabetes mellitus is better than rosiglitazone or repaglinide alone in protection of the Liver and Kidney tissues of diabetic Guinea pigs, *Caviaporcellus*.

**Keywords:** Rosiglitazone, Repaglinide, Liver, Kidney, Guinea Pig, (*Caviaporcellus*)

## 1. Introduction

Diabetes mellitus is one of the most costly and burdensome chronic disease now and is a condition that is increasing in epidemic proportions in the world. It is a metabolic disorder characterized by resistance to the action of insulin, insufficient insulin secretion, or both. The major clinical manifestation of the diabetic state is hyperglycemia that is also associated with disturbances in lipid and protein metabolism [1].

Diabetes mellitus is classified into two types: Type-1 diabetes; previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, which is caused

by an absolute deficiency of insulin [2], and Type-2 diabetes which accounts for ~90-95% of those with diabetes, previously referred to as non-insulin-dependent diabetes or adult-onset diabetes. It is characterized by insulin resistance and /or a relative deficiency of insulin secretion [3]. There are other specific types of diabetes such as gestational diabetes mellitus (GDM), which is defined as glucose intolerance, which is first recognized during pregnancy [4], and diabetes due to genetic defects of the Beta-cells of pancreas which is characterized by onset of hyperglycemia at an early age [5].

Diabetes mellitus causing nephropathy in the form of glomerulosclerosis, non-specific chronic damage mostly related to vascular changes, glomerular hypertrophy probably

due to glomerular hyper filtration and Sub-endothelial deposition of hyaline material(6). Diabetes mellitus cause a noticeable increase in the numbers of glycogenated hepatocyte nuclei that present in the majority of diabetic livers. Fatty change is frequent, but is greatly affected by the presence of obesity [7].

Rosiglitazone is now available as thiazolidinediones [8, 9]. This agent is effective at lowering blood glucose level and is indicated as monotherapy in patients with type 2 diabetes [10], or as a combination therapy with a sulfonylurea or biguanides [11]. It is selective and potent agonist of peroxisome-proliferator-activated receptor (PPAR); an intracellular transcription factor that is expressed in target tissues for insulin action [12]. It increases the number of small adipocytes and the subcutaneous adipose-tissue mass, besides, the high level of PPAR expression in adipose tissue, have led to the hypothesis that Rosiglitazone exert their insulin-sensitizing actions either directly or indirectly, by means of altered adipokine release, modulating insulin sensitivity outside adipose tissue [13].

Repaglinide is non-sulphonylurea insulin secretagogue, is a prandial glucose regulator. Like the sulphonylureas; repaglinide acts by stimulating release of insulin from the cells of the islets of pancreas inhibiting ATP-sensitive K channels, activating the Ca channels with increase in intracellular calcium to release insulin [14]. However, repaglinide acts on a different binding site than the sulphonylureas [15]. It is not effective in the absence of functioning beta cells [16]. The oxidative stress in diabetic kidney is corrected by repaglinide [17].

The activity of repaglinide is dose dependent. Mean insulin levels begin to rise approximately 1.5 hours after the pre-prandial dose and declines towards baseline levels between meal time [18]. The rapid onset of action and the short duration of hypoglycemic effect of repaglinide makes it suitable for preprandial administration [19]. The present study is aimed to investigate the diabetes-induced renal and hepatic damage in Guinea pigs and to assess the potential protective role of Rosiglitazone, Repaglinide and their combination therapy against the diabetes induced Liver and Kidney tissue damage in guinea pig (*Caviaporcellus*).

## 2. Materials and Methods

### 2.1. Drugs and Chemicals

Two tested drugs were used; Rosiglitazone tablets (Avandia® 2mg, from Glaxo-Smithkline, Ireland) and Repaglinide (Novonorm®, 1mg, from Novo Nordisk, Denmark). D-Fructose as a white powder was purchased from EL-Gomhoria industries company (Egypt).

### 2.2. Preparation of Drugs and Doses

Dietary model of fructose induced insulin resistance [20], and its modification [21] was used. Animals were rendered diabetic by oral administration of fructose 50% concentration according to Beck-Nielsen et al., [22]. Fructose feeding was

shown to produce elevation in plasma triglycerides, insulin and blood glucose levels. Increased blood triglycerides have been shown to reduce the number of insulin receptors, thereby reducing insulin sensitivity.

Rosiglitazone solution was prepared and diluted by dissolving the tablets in distilled water and the solution was buffered with PH of 2.3. Repaglinide was available as microcrystalline powder. It was soluble in buffered aqueous solution. Doses given to experimental animals were corresponding to therapeutic doses in human, which calculated according to the method given by Paget and Barnes [23], who calculated the dose in relation to the surface area of each animal.

### 2.3. Experimental Design

30 guinea pigs (*Caviaporcellus*) of both sex, were used and divided into two groups:

Group I (Normal Group-6 animals): These animals were not subjected to induction of diabetes and did not receive any medications and served as control for 12 weeks. They were subjected to body weight and fasting plasma glucose measurement.

Group II (Diabetic insulin resistance): Twenty four guinea pig were subjected to diabetes induction by 50% fructose solution for 8 weeks. After 8 weeks animals were tested for diabetes by detecting glucose in urine using glucose test strips and confirmed by hem test. The animals in this group were subdivided into 4 groups of 6 animals each (a, b, c and d group):

Group-II a: Diabetic animal (control group) received 1ml distilled water by gastric gauge equal to the volume used as vehicle for drugs.

Group -II b: Diabetic animal received Rosiglitazone (3 mg/kg/day).

Group -II c: Diabetic animal received Repaglinide (1 mg/kg/day).

Group-II d: Diabetic animal received combination of Rosiglitazone (3 mg/kg/day) and repaglinide (1 mg/kg /day).

After 4 weeks treatment, all animals were tested for their body weight, fasting plasma glucose and fasting plasma insulin measurement.

#### 2.3.1. Estimation of Body Weight

Body weight changes in non-diabetic, diabetic control and anti-diabetic treated animals were recorded.

#### 2.3.2. Blood Sampling

Blood samples collected from the ophthalmic venous plexus through retro-orbital approach [24]. No food or fructose was allowed for 12 hours overnight, then animals anaesthetized by diethyl ether.

Steps: Apply pressure to the external jugular vein caudal to the mandible with thumb and gently elevate the upper eyelid with the index finger of the same hand. Break a small piece of the hematocrit tube into the conjunctiva of the mid dorsal globe; gently direct the hematocrit tube in a caudal and medial direction until blood is obtained. Discontinue the

external jugular pressure and remove the hematocrit tube. Finally, apply gentle pressure on the globe to provide hemostasis. All blood samples were centrifuged and the plasma was separated.

### 2.3.3. Estimation of Fasting Plasma Glucose

One ml was taken in a fluoride containing tube for the assay of the fasting blood glucose by an automated glucose oxidase method using Behring Diagnostic Reagents (SVR Glucose Test; Behring, La Jolla, CA) conversion from mg/dl to mmol/l by dividing on 18.2.

### 2.3.4. Estimation of Fasting Plasma Insulin

Samples of blood were allowed to clot and then centrifuged to separate the serum and was kept frozen at -20C for the assay of fasting Plasma insulin. Plasma insulin was assayed using Enzyme Linked Immune sorbent Assay (ELISA) by enzyme test insulin kits (MEDGENIX-INS-EASIA, Bio-source, Europe S.A.), which is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on micro titer plate. In the assay, standard control or samples were incubated with anti-insulin antibody for 30 minutes in the micro titration wells, which have been coated with another anti – insulin antibody. After incubation and washing, the wells were incubated with the substrate tetra methyl benzidine (TMB) for 15 minutes. A reagent (H<sub>2</sub>SO<sub>4</sub> 1.8 N) was added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurements of 450 and 490 nm. The absorbance is directly proportionate to the concentration of insulin present. A set of insulin standard is used to plot a standard curve of absorbance versus insulin concentration from which the insulin concentration in the samples can be calculated.

### 2.3.5. Estimation of Insulin Resistance Index [25]

Insulin resistance index were calculated from fasting plasma glucose (FPG) and fasting plasma insulin (FPI) using HOMA (Homeostasis Model Assessment):

Insulin resistance index (IRI) = FPG (mmol/l) x FPI (uU/ml) / 22.5. If IRI < 20 = insulin sensitive, If IRI > 20 = insulin resistance.

**Table 1.** Changes in body weight (gm) in non-diabetic (GI), diabetic control (GIIa), for 4 weeks with rosiglitazone (3mg/kg/day) (GIIb), repaglinide (1mg /kg / day) (GIIc) and their combination in guinea pig.

Animal group(n=6)	Normal(GI)	Diabetic (Insulin Resistance) GH			
		Control(GIIa)	Rosig.(G.IIb)	Repag.(GIIc)	Combination(GIIId)
Mean	285.16	239.65	322.75	256.65	326.8
± SEM	5.48	6.89	17.00	9.00	17.48
P		< 0.001			
P*			< 0.001	> 0.05	< 0.001
P**			> 0.05	< 0.001	
% change		- 15.9%	+ 34.7%	+7.09%	+36.4%

Rosi. = Rosiglitazone. Repagl. = Repaglinide. P=Test of significance between normal (GI) and diabetic control (GIIa) groups. P\*= Test of significance between diabetic control group (GIIa), rosiglitazone group (GIIb), repaglinide group (GIIc) and combination therapy group (GIIId). P\*\*= Test of significance between rosiglitazone group (GIIb) and repaglinide group (GIIc) compared to the combination therapy group (GIIId).

Analysis of data by ANOVA test showed that there was insignificant difference in increasing body weight between rosiglitazone group (GIIb) and the combination therapy

### 2.3.6. Histopathological Examinations

The control and treated guinea pig were sacrificed and Liver and kidney were excised out and immediately fixed in alcoholic Bouin's solution for 24 hours. These tissues were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Transverse sections were cut at 5µ, and stained with Harri'shaematoxylin and then counter stained with eosin. Finally, the slides were microscopically examined (XSZ-N107T) and photographed using camera (Toupcam) mounted on light microscope.

### 2.3.7. Statistical Analysis

Data were statistically analyzed using one way analysis of variance "ANOVA" and T-test [26]. Data were presented as mean ± SD. The statistical significance level was accepted at P< 0.05 and P< 0.001.

## 3. Results

### 3.1. Body Weight

The difference between the final and initial weights of the guinea pig is shown in Table 1. In the control groups, the total body weight of the animal increased, while this weight gain was lower in the diabetic groups (p > 0.05). Diabetic control group (GIIa) showed a significant mean decrease in body weight from 285.16 ± 5.48 to 239.65±6.89gm compared to non-diabetic group (GI) with a percent change of -15.9%) (Fig. 1).

Four weeks treatment of diabetic animal with antidiabetic drugs, rosiglitazone (3mg/kg/day) alone (GIIb) or in combination with repaglinide (1mg/kg/day) (GIIId) compared to the diabetic control group (GIIa) showed a significant mean increase in body weights from 239.65± 7.04 gm to 322.75±17.35 & 326.86±17.84 gm, respectively, by a mean percent increase of 34.7%& 36.4%, respectively. However, the treated group with repaglinide (1mg/kg/day) alone (GIIc) showed an insignificant increase in body weight compared to the diabetic control group (GIIa) from 239.65±7.04 gm to 256.65± 9.19 gm with a mean increase of 7.09% (Table 1&Figs. 1,2).

group (GIIId), However, there was a significant increase in body weight in combination therapy group (GIIId) more than in repaglinide group alone (GIIc) (Table 1).

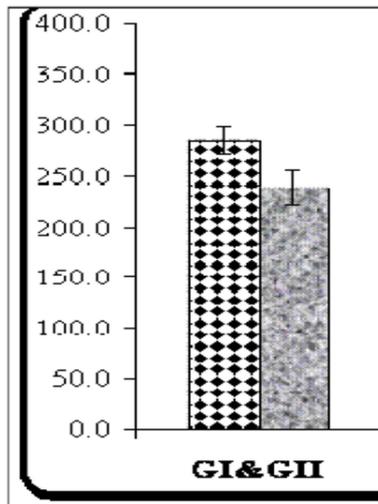


Fig. 1. Effect of oral administration of fructose 50% for 8 weeks on body weight of non-diabetic guinea pig *Caviaporcellus*. GI (Non-diabetic), GII (Diabetic).

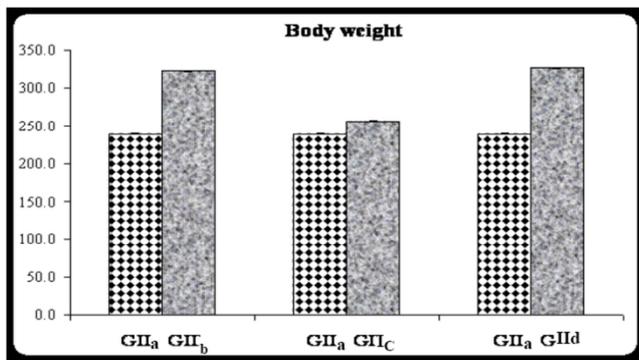


Fig. 2. Changes in body weight (gm) in non-diabetic guinea pig *Caviaporcellus*. (GIa), treated for four weeks with either rosiglitazone (3mg/kg/day-GIb), repaglinide (1mg/kg/day-GIc) and their combination (GIId).

### 3.2. Changes in Fasting Plasma Glucose (FPG)

It is clear from Table (2) that diabetic control group (GIIa) animals showed a significant elevation of fasting plasma glucose from a mean value of 6.22±0.12 mmol/l to 13.62±0.26 mmol/l compared to the non-diabetic (GI) by a mean percent increase of 118.9 (Fig. 3).

Four weeks treatment of diabetic animal with antidiabetic drugs, rosiglitazone (3mg/kg/day) alone (GIIb), repaglinide (1mg/kg/day) alone (GIIc) or a combination of rosiglitazone

(3mg/kg/day) with repaglinide (1mg/kg/day) (GIId) showed a significant reduction in elevated FPG from 13.62± 0.26 mmol/l to 7.49± 0.22 mmol/l, 9.03± 0.31 mmol/l, and 6.27± 0.26mmol/l, respectively, with a mean percent decrease of 44.9, 33.7 and 53.9, respectively (Table 2 & Figs. 3,4).

Analysis of data by ANOVA, showed that combination therapy of rosiglitazone and repaglinide group (GIId) reduced significantly the elevated FPG induced by insulin resistance more than that in rosiglitazone group (GIIb) or repaglinide group (GIIc) alone (Table 2).

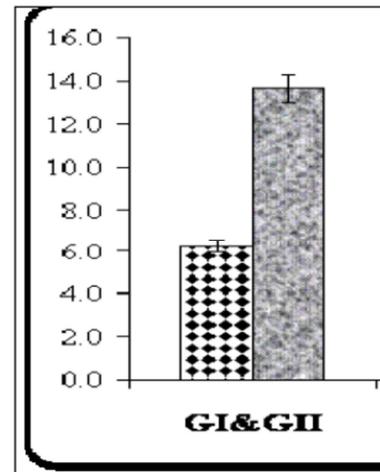


Fig. 3. Effect of oral administration of fructose 50% for 8 weeks on fasting plasma glucose in guinea pig *Caviaporcellus*. GI (Non-diabetic), GII (Diabetic).

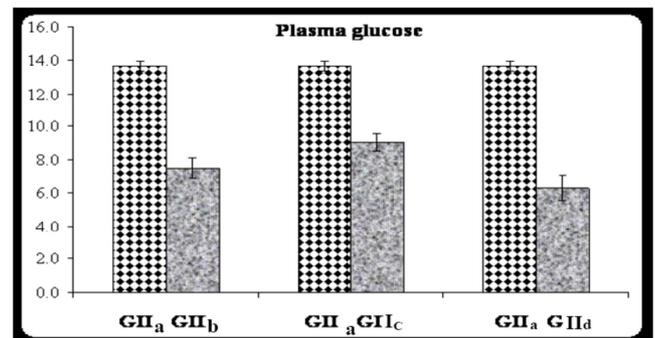


Fig. 4. Changes in fasting plasma glucose in non-diabetic guinea pig *Caviaporcellus* (GIa), treated for four weeks with either rosiglitazone (3mg/kg/day-GIb), repaglinide (1mg/kg/day-GIc) and their combination (GIId).

Table 2. Changes in fasting plasma glucose (mml /l) in non-diabetic (GI), diabetic control (GIIa), for 4 weeks with rosiglitazone (3mg/kg/day) (GIIb), repaglinide (1mg/kg/day) (GIIc) and their combination in guinea pig *Caviaporcellus*.

Animal group(n=6)	Non diabetic(GI)	Diabetic (Insulin Resistance) GII			
		Diabetic Control(GIIa)	Rosig.(G.IIb)	Repag.(GIIc)	Combination(GIId)
Mean	6.22	13.62	7.49	9.03	6.27
± SEM	0.12	0.26	0.22	0.31	0.26
P		< 0.001			
P*			< 0.002	< 0.003	< 0.001
P**			< 0.013	< 0.002	
% change		+118.9%	- 44.9%	- 33.7%	- 53.9%

Rosi= Rosiglitazone. Repagl. =Repaglinide. P=Test of significance between normal (GI) and diabetic control (GIIa) rats P\*= Test of significance between rosiglitazone (GIIb) alone, repaglinide (GIIc) alone and combination group (GIId) therapy for 4weeks in comparison with diabetic control (GIIa). P\*\*= Test of significance between rosiglitazone group (GIIb) and repaglinide group (GIIc) in comparison with the combination therapy group (GIId).

**3.3. Changes in Fasting Plasma Insulin Level (FPI)**

Diabetic *Caviaporcellus* control group (GIIa) showed a significant increase in fasting plasma insulin level from 25.28± 0.60 μU/ml to 46.49±2.98 μU/ml compared to the non-diabetic (GI) with a mean percent increase of 83.9 (Fig. 5).

Four weeks treatment of diabetic rats (GIIa) with antidiabetic drugs, rosiglitazone (3mg/kg/day) alone (GIIb) and combination of rosiglitazone (3mg/kg/day) with repaglinide (1mg/kg/day) (GII d) showed a significant reduction in elevated FPI level, as the mean value decreased from 46.49 ± 1.24 μU/ml to 31.142 ± 0.86 μU/ml & 29.48 ± 0.86 μU/ml, respectively, with a mean percent decrease of

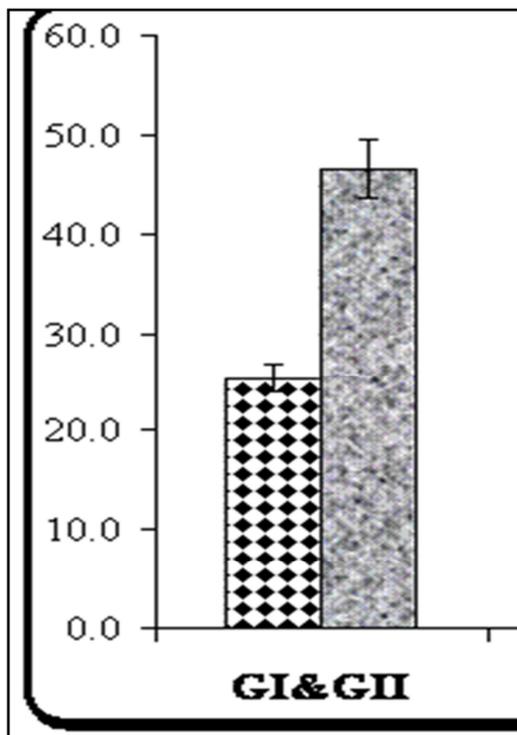
33.0 and 36.6 respectively. In contrast, treatment with repaglinide (1mg/kg/day) alone (GIIc) in a daily dose (1mg /kg/d) for 4 weeks produced insignificant reduction of the elevated FPI level by a mean percent decrease of 3.9 as the FPI level reduced from 46.49±1.24 μU/ml to 44.66± 1.33 μU/ml (Table 3 & Fig. 6).

Statistical analysis of data by ANOVA test showed that there was insignificant difference between rosiglitazone group (GIIb) and combination of rosiglitazone & repaglinide treated group (GII d) in reducing of elevated FPI. However there was a significant difference between combination of rosiglitazone & repaglinide treated group (GII d) that reduce FPI level more than repaglinide treated group (GIIc) alone (Table 3).

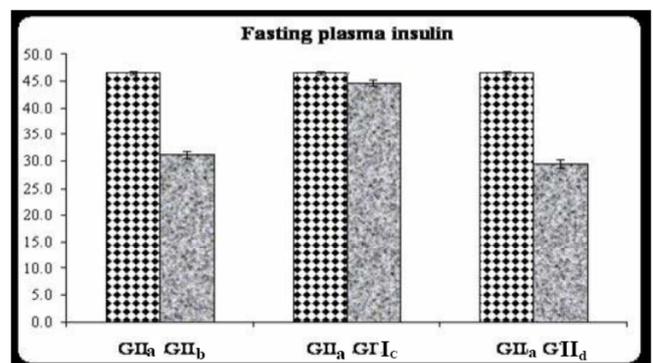
**Table 3.** Changes in Fasting plasma insulin (uU/l) in non-diabetic (GI), diabetic control (GIIa), for 4 weeks with rosiglitazone (3mg/kg/day) (GIIb), repaglinide(1mg /kg / day) (GIIc) and their combination in guinea pig *Caviaporcellus*.

Animal group(n=6)	Normal(GI)	Diabetic (Insulin Resistance) GII			
		Diabetic Control(GIIa)	Rosig.(G.IIb)	Repag.(GIIc)	Combination(GII d)
Mean	25.28	46.49	31.14	44.66	29.48
± SEM	0.60	1.24	0.86	1.33	0.86
P		< 0.001			
P*			< 0.002	> 0.05	< 0.001
P**			> 0.05	< 0.001	
% change		+ 83.9%	- 33.0%	- 3.9%	-36.6%

Rosi= rosiglitazone. Repagl.=repaglinide. P=Test of significance between normal (GI) and diabetic control(GIIa) animals. P\*= Test of significance between rosiglitazone (GIIb) alone, repaglinide (GIIc) alone and combination group(GII d) therapy for 4weeks in comparison with diabetic control (GIIa). P\*\*= Test of significance between rosiglitazone group (GIIb) and repaglinide group (GIIc) in comparison with the combination therapy group (GII d).



**Fig. 5.** Effect of oral administration of fructose 50% for 8 weeks on fasting plasma insulin in guinea pig *Caviaporcellus*. GI(Non diabetic), G II(Diabetic).



**Fig. 6.** Changes in fasting plasma insulin in diabetic guinea pig *Caviaporcellus* (GIIa), treated for four weeks with either rosiglitazone (3mg/kg/day-GIIb), repaglinide (1mg /kg /day- GIIc) and their combination (GII d). GII a(Non-diabetic), G IIa, b,c(Diabetic).

**3.4. Changes in Insulin Sensitivity Induced by Drinking 50% Fructose Solution**

Diabetic animals control group (GIIa) showed a significant increase in insulin resistance index (IRI) from 7.28 ± 0.36 to 28.12 ± 0.62 compared to non-diabetic (GI) by a mean percent increase of 286.3, (Fig. 7).

Four weeks treatment of diabetic rats with antidiabetic drugs rosiglitazone (3 mg / kg / day) alone (GIIb), repaglinide (1mg/ kg/ day) alone (GIIc) and combination of

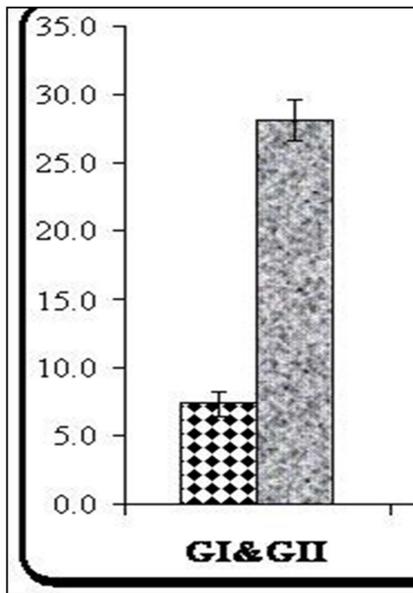
rosiglitazone (3mg/kg/day) & repaglinide (1mg/kg/day) group (GIId), showed a significant decrease in insulin resistance index from  $28.12 \pm 0.62$  to  $11.67 \pm 1.47$ ,  $17.87 \pm 0.21$ , respectively, with a mean percent decrease of 58.5, 36.4 & 70.9, respectively (Table 4 & Fig. 8).

Analysis of data with ANOVA test showed that treatment with combination therapy of rosiglitazone (3mg/kg/day) and repaglinide (1mg/kg/day) group (GIId) produced a more reduction in mean insulin resistance index than the other two groups(GIIb, GIIc) (Table 4).

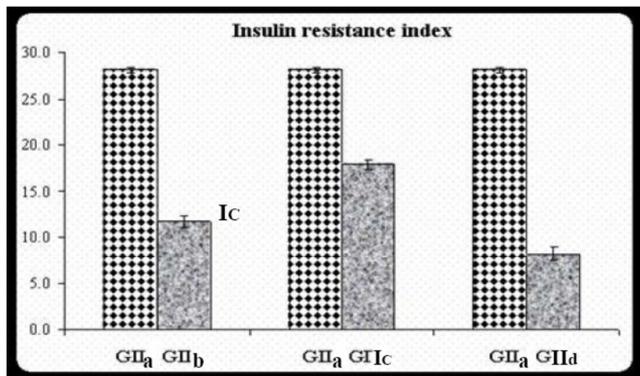
**Table 4.** Changes in insulin resistance index in non-diabetic (GI),diabetic control (GIIa), for 4 weeks with rosiglitazone (3mg/kg/day) (GIIb), repaglinide (1mg/kg / day) (GIIc) and their combination in guinea pigCaviaporcellus.

Animal group(n=6)	Non diabetic(GI)	Diabetic (Insulin Resistance) GII			
		Diabetic Control(GIIa)	Rosig.(G.IIb)	Repag.(GIIc)	Combination(GIId)
Mean	7.28	28.12	11.67	17.87	8.19
± SEM	0.36	0.62	1.47	0.48	0.21
P		< 0.001			
P*			< 0.001	< 0.002	< 0.001
P**			< 0.035	< 0.003	
% change		+ 286.3%	- 58.5%	- 36.4%	- 70.9%

Rosi= rosiglitazone. Repagl. =repaglinide.P=Test of significance between normal (GI) and diabetic control(GIIa) animals. P\*= Test of significance between rosiglitazone (GIIb) alone, repaglinide(GIIc) alone and combination group(GIId) therapy for 4weeks in comparison with diabetic control (GIIa). P\*\*= Test of significance between rosiglitazone group (GIIb) and repaglinide group (GIIc) in comparison with the combination therapy group (GIId).



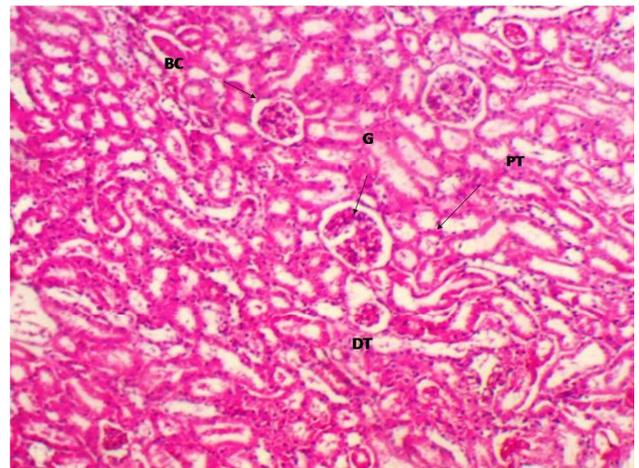
**Fig. 7.** Effect of oral administration of fructose 50% for 8 weeks on insulin resistance index in guinea pigCaviaporcellus.GI(Non diabetic), G II(Diabetic)



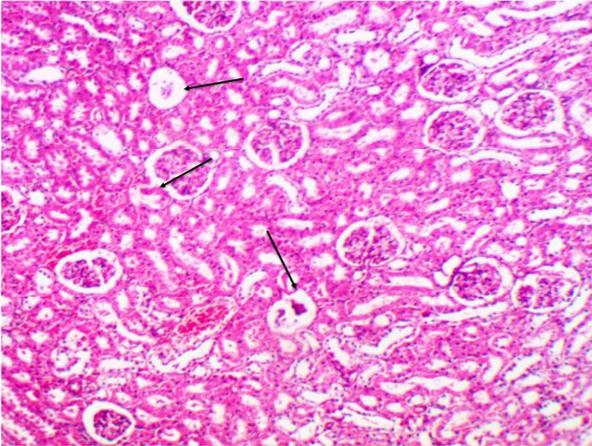
**Fig. 8.** Changes in insulin resistance index in diabetic guinea pigCaviaporcellus (GIIa), treated for four weeks with either rosiglitazone (3mg/kg/day-GIIb), repaglinide (1mg /kg /day- GIIc)and their combination (GIId). GII a (Non-diabetic) G IIa, b,c(Diabetic).

### 3.5. Histopathological Examinations

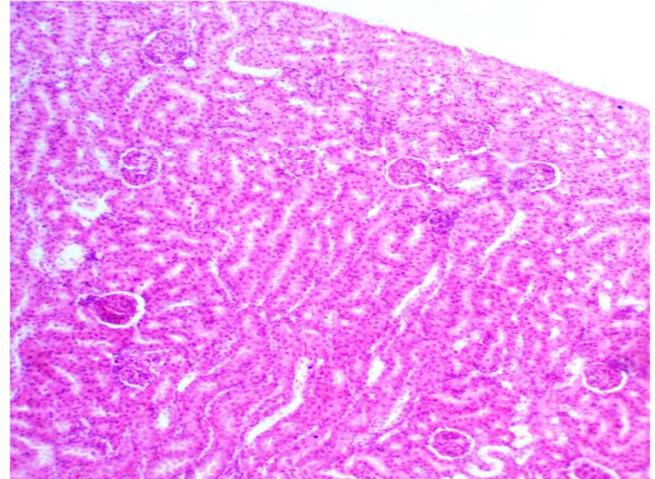
In both groups of control guinea pig, the kidney presented a typical histological structure (Fig. 9). In the kidneys of the untreated diabetic guinea pig (Fig. 10), many pathological alterations were found; including partial cellular lesion with acidophilic material in the glomerulus, distortion of the tubular wall and enlargement of the tubular lumen (Fig. 10). Also, vacuolization of the cytoplasm of the tubular epithelium cells was observed. The diabetic guinea pig treated with rosiglitazone displayed no glomerular or tubular pathological alterations (Fig. 11). The diabetic guinea pig treated with repaglinide showing the absence of Inflammatory processes (Fig. 12), but the combination of both rosiglitazone + repaglinide have better improvements on renal tissue compared to each drug when used alone (Fig. 13).



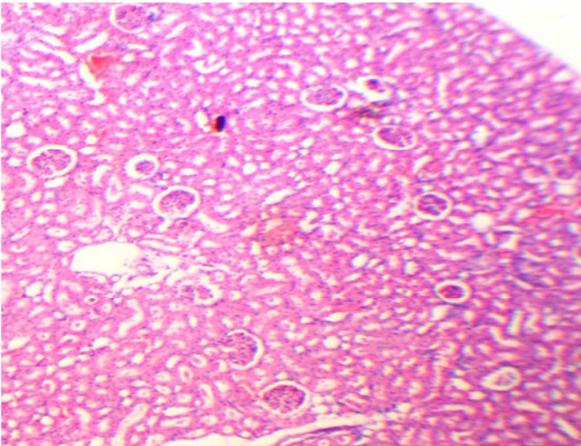
**Fig. 9.** A Photomicrograph of kidney section from non-diabetic control guinea pigCaviaporcellus stained with (H. &E. stain, X100) showing normal Bowman's capsule (BC), glomeruli (G), proximal tubules (PT) and distal tubules (DT).



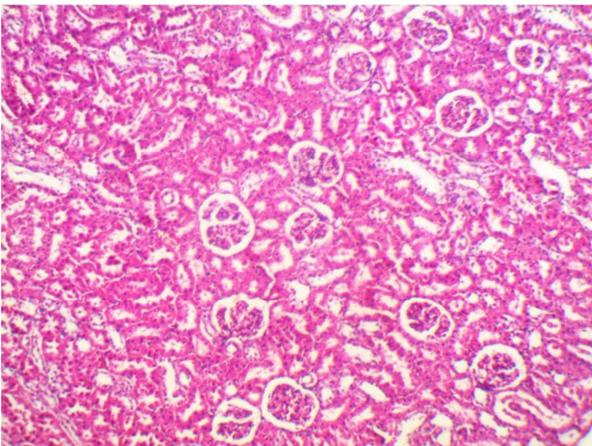
**Fig. 10.** A Photomicrograph of kidney section from non-treated diabetic control guinea pig *Caviaporcellus* stained with (H. &E. stain, X100) showing moderate damage in the renal tubules, tubular atrophy, also glomerular shrinking and necrotic areas was also observed



**Fig. 13.** A Photomicrograph of kidney section from diabetic guinea pig *Caviaporcellus* treated with rosiglitazone and repaglinide for 4 weeks stained with (H. &E. stain, X100) showing: Most histological structures of kidney still normal like in the control group or rosiglitazone and repaglinide produced more improvement in renal tissue than do each drug alone.

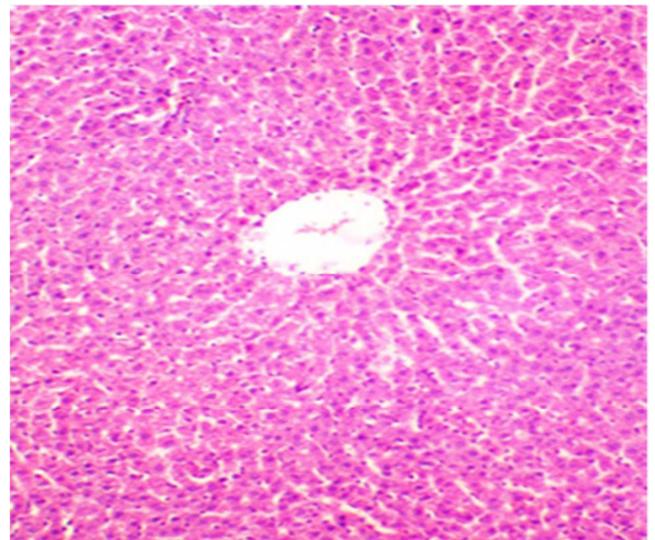


**Fig. 11.** A Photomicrograph of kidney section from diabetic guinea pig *Caviaporcellus* treated with rosiglitazone for 4 weeks stained with (H. &E. stain, X100) showing: The glomeruli are lined with flattened endothelial cells; the mesangio epithelial cells are cubical. - The vasculature is normal. - The renal tubules are lined with cubical epithelium. - The interstitium is formed of c.t. stroma.

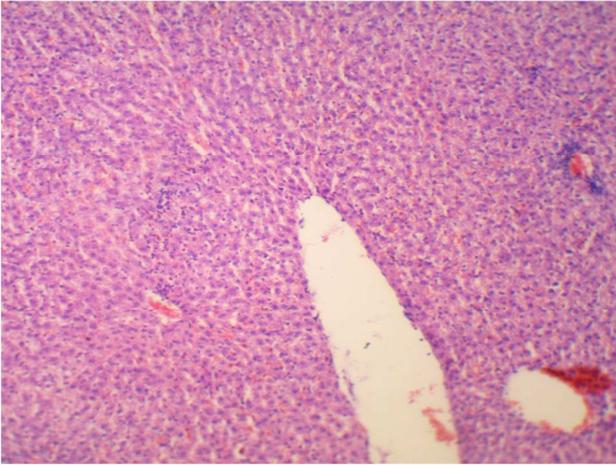


**Fig. 12.** A Photomicrograph of kidney section from diabetic guinea pig *Caviaporcellus* treated with repaglinide for 4 weeks stained with (H. &E. stain, X100) showing: -The glomeruli are lined with flattened endothelial cells; the mesangio epithelial cells are cubical. Most histological structures of kidney still normal like in the control group.

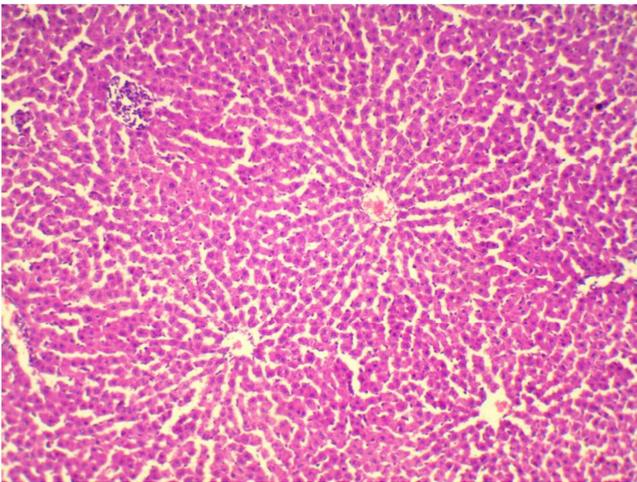
The liver of the control guinea pig (Fig. 14) presented typical histological organization, matching the description of [28]. In the liver of the untreated diabetic animals, widely dilated central vein was observed. There was moderate cellular necrosis at some hepatic cells, and infiltration of lymphocytes was also recorded (Fig. 15). The diabetic animals treated with rosiglitazone showing mildly dilated central vein with mild diffuse necrosis (Fig. 16). The diabetic guinea pig that treated with repaglinide showed moderate histopathological lesions (Fig. 17), but the combination of both rosiglitazone + repaglinide on have better improvements on renal tissue compared to each drug when used alone (Fig. 18).



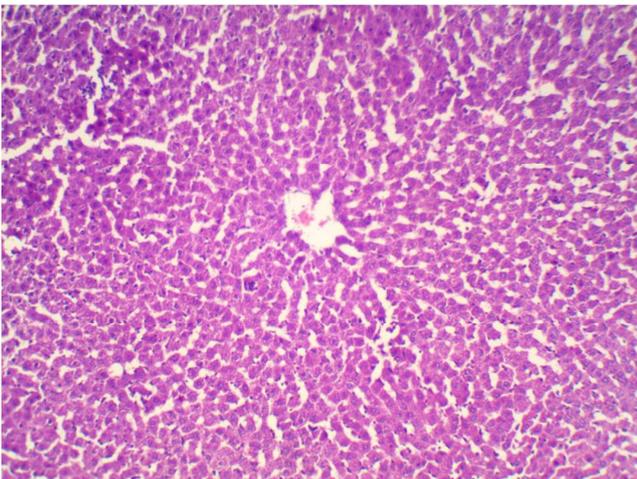
**Fig. 14.** A photomicrograph of liver section from non-diabetic guinea pig *Caviaporcellus* stained with (H.&E. stain, X100) showing: central vein surrounded by appearing normal hepatocytes, normal blood sinusoids & bile canaliculi.



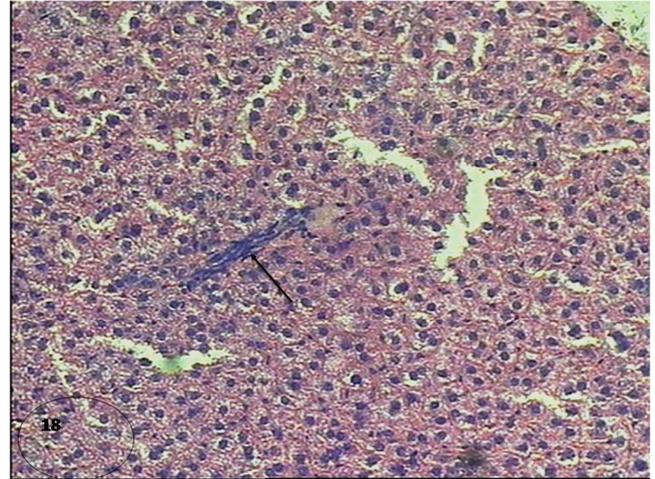
**Fig. 15.** A Photomicrograph of liver section from a none treated diabetic control guinea pig *Caviaporcellus* stained with (H. & E. stain X100) showing: Widely dilated central vein. There was moderate cellular necrosis at some hepatic cells, and infiltration of lymphocytes also recorded.



**Fig. 16.** A Photomicrograph of liver section from diabetic guinea pig *Caviaporcellus* treated with rosiglitazone for 4 weeks stained with (H. & E. stain, X100) showing: mildly dilated central vein with mild diffuse necrosis.



**Fig. 17.** A Photomicrograph of liver section from diabetic guinea pig *Caviaporcellus* treated with repaglinide for 4 weeks stained with (H. & E. stain, X100) showing: The central vein was moderately dilated. There was moderate centrilobular necrosis of the hepatocytes.



**Fig. 18.** A Photomicrograph of liver section from diabetic guinea pig *Caviaporcellus* treated with rosiglitazone and repaglinide for 4 weeks stained with (H. & E. stain, X100) showing: mild focal cellular necrosis. Occasional cells showed ground glass appearance. There was mild early fibroplastic proliferation (arrow).

## 4. Discussion

Diabetes mellitus is a syndrome of abnormal glucose metabolism characterized by hyperglycemia resistance to the action of insulin. Type-2 diabetes has become epidemic in the past few decades, with a dramatic increase in its incidence worldwide [29]. It is associated with insulin deficiency along with varying degree of peripheral

Insulin resistance is a condition in which increased insulin is required to produce a normal biological response (i.e. a normal blood glucose level). Insulin resistance is caused by both acquired (weight gain, reduced exercise) and genetic factors. It is often accompanied with other cardiovascular risk factors, including increased abdominal fat, hypertension, elevated glucose levels and dyslipidemia - a constellation of features known as the metabolic syndrome [30-31].

Fructose induced insulin resistance remains a well-recognized and reliable method for induction of diabetes in experimental animals. Drinking 50% fructose solution instead of tap water for 8 weeks resulted in the development of an insulin resistance syndrome in guinea pig *Caviaporcellus* which manifested by a rise in fasting plasma glucose level, fasting plasma insulin level and increased insulin resistance index as assessed by homeostasis model assessment (HOMA) [32].

In the present work induction of diabetes in experimental animals by 50% fructose solution resulted in the development of an insulin resistance syndrome which manifested by a rise in fasting plasma glucose level, fasting plasma insulin level and increased insulin resistance index.

In the present work addition of rosiglitazone to repaglinide resulted in a significant reduction of FPG, FPI levels, and reduction in insulin resistance index, this finding is in agreement with the description of others [33, 6, 34].

We notice that the total body weight of the guinea pig increased while this weight gain was lower in the diabetic

groups. Diabetic control group showed a significant mean decrease in body weight in comparison to non-diabetic group. The present work suggested that the beneficial effect of rosiglitazone / repaglinide combination therapy in improving glycemic status could be to the stimulation of glucose metabolism and also through the antioxidant effects, which protected the liver and kidney from damage. These results are in agreement with that recorded by Cavalli *et al.* [35].

The histopathological examination showed a picture of diabetic nephropathy in the form of proliferation of mesangio epithelial cells with lobulations, proliferation of the renal tubules with hydropic degeneration of their epithelial cells; renal arterioles show thick walls and narrow lumen due to vascular hyalinosis.

According to Raskinet *al.* [36], who stated that diabetes mellitus causing nephropathy in the form of glomerulosclerosis, non-specific chronic damage mostly related to vascular changes, glomerular hypertrophy probably due to glomerular hyperfiltration and Sub-endothelial deposition of hyaline material.

Rosiglitazone treated group in our study showed that renal vasculature and glomeruli were changed in diabetic guinea pig and these changes were regenerated to reach to the normal state. These findings suggest that treatment with Rosiglitazone may have an anti-inflammatory effect on the kidneys. Rosiglitazone can reduce nephropathy in diabetic fatty guinea pig. There is hypothesis that the beneficial effects of TZDs in animal models of diabetic kidney (and other organs) are predominantly due to a reduction in oxidative stress and anti-inflammatory actions of these PPAR- agonists [37].

Repaglinide in this study showed a mild improvement in nephropathy that caused by induction of diabetes. These results were supported by Gumieniczek [17], who reported that the oxidative stress in diabetic kidney corrected by repaglinide. The drug does not affect glucose concentration and its antioxidative effect is not secondary to its action on hyperglycaemia. This study suggests an additional advantage of repaglinide which contributes to its effectiveness in therapy.

On the other hand combination therapy of rosiglitazone and repaglinide produced more improvement in renal tissue than do each drug alone. Histopathological examination of liver tissue demonstrated changes in the form of widely dilated central vein with moderate cellular necrosis at the centrilobular area. Hepatocytes showed ground glass appearance of the cytoplasm.

In accordance Frank and Mitros [7], diabetes mellitus cause a noticeable increase in the numbers of glycogenated hepatocyte nuclei that present in the majority of diabetic livers. Fatty change is frequent, but is greatly affected by the presence of obesity; it seems to be closely related to the phenomenon of insulin insensitivity. Biliary tree disease may also occur; this may in part be related to the frequent production of lithogenic bile in these patients.

Rosiglitazone in this study showed mildly dilated central vein with mild diffuse necrosis, further more combination

therapy of rosiglitazone and repaglinide produced more changes in liver content that was reported in our study in the form of diminished areas of necrosis and decreased picture of ground glass appearance of the cytoplasm.

In conclusion, these data indicated that the treatment of Type 2 diabetes using rosiglitazone / repaglinide combination therapy is more effective than either agent used as monotherapy and should be considered a promising treatment option in appropriate patients.

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