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Comparison between the effect of the thiazolidinedione-rosiglitazone- & the sulphonylurea -gliclazide- and their combination on the liver of streptozotocin-induced diabetes mellitus in rats.

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Abstract

This study was conducted to compare between the possible effects of rosiglitazone "A new oral antidiabetic drug with selective PPAR-gamma agonistic effect" in a dose of 0.03 mg/kg BW and gliclazide " An oral antidiabetic sulphonylurea" in a dose of 10 mg/kg BW either used alone or in combination, for 6 weeks on the liver, serum glucose and lipid profile in streptozotocin diabetic rats.

Thirty rats were randomized into 5 groups (n=6). Group I; the control group was given saline orally daily for 6 weeks. Group II; the streptozotocin induced diabetic group. Group III received rosiglitazone, while group IV received gliclazide and group V received both drugs.
The results of the present study revealed that streptozotocin significantly ($P< 0.05$) elevated serum glucose, cholesterol and triglycerides in rats compared to the controls. The insulin sensitizer "rosiglitazone" either alone or combined with gliclazide decreased serum glucose significantly ($P< 0.05$) compared to the diabetic group. Gliclazide alone also had the same effect. Rosiglitazone alone decreased serum cholesterol and AST and in combination with gliclazide decreased serum ALT significantly ($P< 0.05$) compared to the diabetic group.

For histopathological study, liver tissue was prepared for both histological (H&E, PAS & Masson's trichrome) and immunohistochemical (alpha 1 antitrypsin expression) techniques. Both qualitative and quantitative analysis was done to assess the degree of hepatic damage. According to certain criteria, H&E stained sections were quantitatively examined to assess the degree of hepatocyte affection, beside other quantitative measurements (optical density & color area percentage) using the image analyser. Obtained results revealed that streptozotocin caused severe affection in 6% of hepatocytes, mild affection in 2% and moderate affection in 41%. The drug also resulted in significant increase in PAS stained glycogen granules in hepatocytes as well as collagen in portal tracts. Immunostaining of alpha 1 antitrypsin revealed increased expression in the lining of blood sinusoids including Kupffer cell cytoplasm and in the area around the central vein. Groups III, IV and V which were under the effect of rosiglitazone, gliclazide or both respectively, showed hepatocyte damage similar to that of diabetic control group; however the degree of that damage was only statistically significantly increased in case of group III.

When compared to diabetic control group, these groups (III, IV and V) showed no significant difference in both optical density of PAS positive reaction or mean color area percentage of collagen; however the mean optical density of immunostaining decreased significantly.

This indicated that rosiglitazone alone or when used concomitantly with gliclazide, in streptozotocin-induced diabetic rats resulted in improvement of their metabolic control, yet the potential of hepatotoxicity was still to be considered.

Introduction

Diabetes Mellitus is a chronic disease with the potential to cause severe complications. It is a major cause of heart disease and leading cause of blindness, kidney failure, and limb amputations.

Type I diabetes mellitus is a result of an absolute absence of insulin, while type II is a chronic, progressive, metabolic disorder that results from insulin resistance and defects in insulin production.

Although diet and exercise are considered the cornerstone of therapy for type II diabetics, many patients require pharmacological intervention. It can often be treated initially with an oral agent monotherapy, but may eventually require the addition of other oral agents to achieve targeted glycaemic levels (Defronzo, 1999).

Until recently, the only pharmacology available for insulin resistance was metformin, which enhances the sensitivity of both hepatic and peripheral muscle tissues to insulin. Thiazolidines are a new class of drugs for the treatment of type II diabetes. They act by improving insulin sensitivity in adipose tissue, liver and skeletal muscles (Mudaliar and Henry, 2001). They increase glucose transport and uptake and decrease insulin resistance by activation of a nuclear receptor; peroxisome proliferator-activated receptor-gamma (PPAR-gamma), found in adipose tissue, skeletal muscle, and the liver (Way-James et al., 2001).

Troglitazone, the prototype of glitazones was found to be hepatotoxic and was removed from the market (Bailey, 2000).

Rosiglitazone and pioglitazone are the newest agents in this class and therefore considered similar, but safer. However, due to the troglitazone experience, hepato-toxicity should be considered and liver function tests are still recommended before and during treatment with these agents (Al-Salman et al., 2000; Forman et al., 2000).

Gliclazide is a second-generation sulfon-ylurea that is widely used in the treatment of Type II diabetes mellitus. It was reported to be well-tolerated and generally safe. Its glucose-lowering effects are due to both enhanced insulin secretion and a decrease in insulin resistance (Alberti, 1994).

The aim of the present study was to clarify the effects of rosiglitazone versus gliclazide alone or combined with rosiglitazone on the liver and on some metabolic parameters in streptozotocin-induced diabetes mellitus in rats.

Materials And Methods

A) Animals:

This work was carried out on 30 male albino rats, weighing from 150-200 grams each. They were housed six per cage and allowed for acclimatization before the start of the work for one week. Animals had free access to food and water. They were kept under normal room conditions of temperature, humidity and normal light cycle.

B) Drugs:

Streptozotocin was obtained from (Sigma) as 1-gram pure white powder. The calculated dose (40 mg/kg BW) was given intraperitoneally in saline solution in two successive days to induce diabetes mellitus (Sato *et al*., 1986).

Rosiglitazone was obtained from (Smithkline Beecham pharmaceuticals) as 4 mg tablets. Tablets were chewed and given in a dose of 0.03 mg/kg BW suspended in saline by gavage daily for six weeks.

3- Gliclazide was obtained from (Amiriya Pharm.Ind.Alexandria /Egypt) as 80 mg tablets. It was given in a dose of 10 mg/kg BW daily for six weeks as in rosiglitazone.

C) Study design:

The animals were randomly divided into 5 groups (six animals each) as following:

Group I: Considered as the control. They were given 0.5 ml saline orally daily, for six weeks.

Group II: Diabetic non-treated group. Streptozotocin was used to induce diabetes.

Group III: Rosiglitazone group; diabetic rats received rasiglitazone as mentioned above.

Group IV: Gliclazide group; diabetic rats received gliclazide as mentioned.
Group V: Combination group; diabetic rats received both rosiglitazone and gliclazide at the same time in the same previously mentioned doses.

D) Biochemical data:

At the end of experiment, blood samples were obtained from retro-orbital venous plexus by capillary tubes without anaesthesia into clear centrifugation tubes. Serum was isolated by centrifugation for measuring the following parameters:

1-Serum glucose level.
2-Serum Alanine Amine transferase (ALT) and serum aspartate amine transferase (AST).
3-Serum cholesterol level.
4-High density lipoprotein level (HDL).
5-Low density lipoprotein level (LDL).
6-Serum triglycerides level (TGs).

E) Histological techniques:

After collection of blood samples, animals were sacrificed. The liver of each rat was dissected out, fixed immediately in 10% neutral buffered formalin solution, then processed to prepare 5µm thick paraffin sections suitable for performance of histological and immunohistochemical techniques. For histological techniques, sections were stained with H&E, Periodic acid Schiff (PAS) and Masson's trichrome stain.

In immunohistochemical technique, 5µm thick paraffin sections were used to localize alpha 1-antitrypsin in the liver tissue using the labeled streptavidin biotin technique. The primary antibody was rabbit polyclonal, obtained from Lab-Vision (USA). Sections from pancreas were used as a positive control. For negative control, a section of the liver from control group was processed but the primary antibody was omitted.

H&E stained sections were used for quantitative analysis to assess the degree of hepatocyte damage. According to Ahmed and Hosney (1992), the following degrees were used for quantification; no affection (no morphological changes), mild affection (cloudy swelling; swollen cells with pale cytoplasm), moderate affection (hydropic degeneration; vacuolated cytoplasm) and severe affection (necrosis; nuclear changes with cytoplasmic affection). The relative frequency of each degree was counted in 5 high power fields in 3 sections from each animal. Values were expressed as means and standard deviation.

Quantitative measurements were carried out using the image analyser (Super eye- Heidi soft) to measure:

1-The mean optical density of PAS positive glycogen granules in hepatocytes.
2-The mean optical density of alpha-1 antitrypsin immunostaining in Kupffer cells.
3-The mean color area percentage of the green color (collagen) in Masson's trichrome stained sections. The image analyser was calibrated for color measurement before using it. Thirty fields, at least, were captured and analyzed for each group.

F) Statistical analysis:

The data were analyzed using SPSS statistical software. Comparison of the mean and standard deviation (SD) of the above-mentioned, biochemical and histological parameters among diabetic and treated groups and diabetic and control groups was done using t-test. The significance level was considered at p value <0.05.

Results

A- measurements of serum glucose, ALT, AST and lipid profile (cholesterol, HDL, LDL and triglycerides) of adult male rats.

Effect of intraperitoneal (I.P.) injection of streptozotocin 40 mg/kg in two successive days on some metabolic parameters compared to the control group

The results of this study showed that serum glucose level was increased significantly ($P<0.05$) from 127.83 mg/dl in the control group to 536.5 mg/dl in the diabetic group. The same significant increase was observed also regarding the mean values of serum cholesterol and triglycrides (TGs), which increased from 57.5 mg/dl and 84.66 mg/dl respectively in the control group to 186.66 mg/dl and 199.83 mg/dl respectively in the diabetic group (Table 1).

There was non-significant difference (P>0.05) among mean values of serum ALT, AST, LDL and HDL in control and diabetic groups (Table 1).

Effect of oral administration of rosiglitazone, gliclazide and combination of them for 6 weeks on some metabolic parameters in adult male diabetic rats compared to diabetic non-treated group

Biochemical results showed that the mean blood glucose level was decreased significantly from 536.5 mg/dl in the diabetic group to 139.5 mg/dl, 154.16 mg/dl and 129.16 mg/dl in groups that received rosiglitazone, gliclazide and combination of both respectively.

Regarding ALT serum level, it was also decreased significantly from a mean value of 81.3 mg/dl in the diabetic group to a mean value of 69.5 mg/dl in rats treated with combination of rosiglitazone and gliclazide as shown in table (2).

There was a significant difference (P<0.05) between serum level of cholesterol in diabetic and rosiglitazone groups, which was decreased from 186.66 mg/dl to 73.66 mg/dl respectively as shown in table (2).

Also, the results showed that TG level decreased significantly (P<0.05) from 199.83 mg/dl in diabetic group to 99.33 mg/dl in rosiglitazone group and more significantly (P<0.01) to 85.67 mg/dl in the combined group (table 2).

B- Histological results

Group I (Control group):

H&E stained sections of the control group showed normal histological structure of the liver. Hepatocytes were arranged in plates radiating from the central veins. They had normal polygonal shape with rounded vesicular nuclei and eosinophilic granular cytoplasm. Blood sinusoids with associated Kupffer cells were present between the adjacent plates of hepatocytes. Portal tracts were seen containing portal venule, hepatic arteriole, bile duct and lymphatic vessel (Fig. 1). The majority of hepatocytes (96%) appeared normal, however a small number showed mild (2%), moderate (1%) and severe (1%) affection according to the morphological criteria mentioned above. The percentage of each type of cellular affection was presented (Table 3). PAS stain showed glycogen granules in hepatocyte cytoplasm especially around the central vein (Fig. 2). The mean optical density of PAS positive glycogen granules in hepatocytes of control group was 0.30±0.05 (Table 5). Masson's trichrome stain showed scanty green colored collagen fibers in portal tracts and area around the central vein. Very scanty fibers were also detected around blood sinusoids (Fig. 3). The mean color area percentage of green stained collagen fibers in portal areas in this group was 0.09±0.03 (Table 5). Alpha 1-antitrypsin immuno-staining showed weak expression of the antibody mainly in the lining of the blood sinusoidal spaces in which Kupffer cells are associated (Fig. 4). The mean optical density of immunostaining in Kupffer cell cytoplasm was 0.28±0.04 (Table 5).

Group II (diabetic control):

H&E stained sections of the diabetic control group showed marked degenerative and necrotic changes in hepatocytes. These changes were less marked in both centrilobular and periportal areas (Fig. 5). There was swelling of hepatocytes as well as vacuolization of their cytoplasm. Necrotic changes occurred in 6% of the cells. They were in the form of nuclear pyknosis, karyorrhexis or karyolysis with either vacuolated or darkly eosinophilic cytoplasm. 41% of hepatocytes showed moderate affection in the form of vacuolization of their cytoplasm, while 2% showed mild affection and the rest (51%) appeared normal (Table 3). Dilatation and congestion of blood vessels was also observed in liver sections of some animals. No apparent changes were detected in Kupffer cells in H&E stained sections. PAS stained sections showed an increase in the amount of PAS positive glycogen granules (Fig. 6). The mean optical density of PAS positive granules in hepatocytes was 0.37±0.04 which was statistically significantly increased compared to control (Table 5). Sections stained with Masson' s trichrome stain revealed an increase in the amount of collagen fibers in portal areas

and in some sites between hepatocytes (Fig. 7). The mean color area percentage of green stained collagen fibers was 0.14±0.06 which was significantly increased compared to control (Table 5). An increased expression of Alpha 1-antitrypsin immunostaining was observed in the lining of blood sinusoids and in the area around the central vein in liver sections of this group. Kupffer cell cytoplasm was advantatiously stained (Fig. 8). The mean optical density of immunostaining in Kupffer cell cytoplasm in this group was 0.32±0.04 which was statistically significantly increased compared to control (Table 5).

Group III (rosiglitazone group):

H&E stained sections of this group showed changes similar to those of diabetic control group (Fig. 9), however a significant evidence of deterioration of liver condition was noted compared to that group. The percentage of normal hepatocytes dropped to 38±1.3 which was statistically significantly decreased compared to diabetic control (Table 4). 3% of hepatocytes of this group showed mild affection, while 50% showed moderate affection and 9% showed severe affection . The latter percentage was statistically significantly increased compared to the previous group (Table 4). Also the mononuclear cellular infiltration was marked in this group (Fig. 9). The mean optical density of PAS positive glycogen granules in hepatocytes was 0.37±0.06 which was statistically insignificant compared to diabetic control (Fig. 10; Table 6). The mean color area percentage of green stained collagen fibers was 0.14±0.05 which was also statistically insignificant compared to diabetic control (Fig. 11; Table 6). Immunostained sections showed weak expression of Alpha 1-antitrypsin immunostaining in blood sinusoidal lining as well as Kupffer cells cytoplasm (Fig. 12). The mean optical density of immunostaining in Kupffer cell cytoplasm in this group was 0.27±0.04 which decreased significantly compared to the previous group (Table 6).

Group IV (gliclazide group):

In this group, liver affection was insignificant compared to diabetic control (Table 4) as shown in H&E stained sections (Fig. 13). Table (4) showed that the percentage of the apparently normal hepatocytes was 41±7.5 while those showing mild affection were 2±0.8%, moderate affection were 50±8.2% and those showing severe affection were 7±8.5%. The mean optical density of PAS positive glycogen granules in hepatocytes was 0.36±0.06 which was statistically insignificant compared to diabetic control (Table 6). The mean color area percentage of green stained collagen fibers was 0.16±0.06 which was again statistically insignificant compared to group II (Table 6). The mean optical density of immunostaining in Kupffer cell cytoplasm in this group was 0.29±0.06 which decreased significantly compared to diabetic control (Fig. 14; Table 6).

Group V (combined group):

In group V, a slight significant evidence of deterioration of histopathological condition of the liver was observed compared to diabetic control in the form of rise of percentage of the moderately affected hepatocytes to 53±10.7 while that of normal, mildly affected and severely affected were 40±12.1, 1±0.8 and 6±3.5 respectively (Table 4;. Fig. 15) showed congestion, degenerative and necrotic changes. The mean optical density of PAS positive glycogen granules was 0.35±0.06 which was statistically insignificant compared to diabetic control (Table 6). The mean color area percentage of green stained collagen fibers was insignificant compared to the same group (0.15±0.06), however the mean optical density of immunostaining in Kupffer cell cytoplasm was 0.28±0.04 which decreased significantly compared to diabetic control group (Fig. 16; Table 6).

Table (1) shows some metabolic parameters expressed in mg/dl in diabetic group compared to control group

	Control Group N=6	Diabetic Group N=6
Glucose		
Mean	127.83	536.5*
S.D. (+)	27.33	283.99
t-value		3.616
ALT		
Mean	66.5	81.83
S.D. (+)	30.74	13.17
t-value		1.372
AST		
Mean	140.17	148.67
S.D. (+)	49.91	32.12
t-value		0.639
Cholesterol		
Mean	57.50	186.67*
S.D. (+)	10.11	62.76
t-value		1.014
HDL		
Mean	28.33	37.00
S.D. (+)	20.45	12.82
t-value		1.175
LDL		
Mean	11.00	11.27
S.D. (+)	8.99	11.86
t-value		0.047
TGs		
Mean	84.83	199.83*
S.D. (+)	19.29	22.51
t-value		1.863

*** Significant (P<0.05 compared to the control group).**

Table (2) shows some metabolic parameters expressed in mg/dl in diabetic treated groups compared to diabetic non-treated group

	Diabetic Group N=6	Diabetic treated Groups		
		Rosiglitazone Group **N=6**	**Gliclazide Group N=6**	**Rosi+ Glic. Group N=6**
Glucose Mean S.D. (+) t-value	536.5 283.99	139.5* 40.31 3.329	145.17* 56.12 3.327	129.17** 17.74 3.88
ALT Mean S.D. (+) t-value	81.83 13.17	100.33 42.76 1.103	89.17 28.72 0.488	69.5* 12.00 3.067
AST Mean S.D. (+) t-value	148.67 32.12	124.67 26.79 1.135	126.83 22.12 1.168	149.83 18.58 0.071
Cholesterol Mean S.D. (+) t-value	186.67 62.76	73.67* 8.36 1.689	100.00 7.24 1.523	80.33 8.09 1.362
HDL Mean S.D. (+) t-value	37.00 12.82	31.69 9.35 1.843	36.3 21.9 0.098	36.5 6.38 0.083
LDL Mean S.D. (+) t-value	11.27 11.86	12.5 7.23 2.81	11.67 6.44 0.79	15.17 10.11 1.121
TGs Mean S.D. (+) t-value	199.83 22.51	99.33* 19.46 1.951	106.33 29.41 1.992	85.67** 41.44 0.013

*Significant ($P<0.05$ compared to diabetic non-treated group).
**Significant ($P<0.01$ compared to diabetic non-treated group).

Table (3): shows the frequency distribution of histopathological changes in the liver in diabetic control group compared to control group

Group	No affection (%)	Mild affection (%)	Moderate affection (%)	Severe affection (%)
Control (±SD)	96±1.5	2±1	1±0.5	1±0.01
Diabetic(±SD)	51±15.6	2±1.8	41±14.5	6±1.7
P value	<0.0001	0.31	<0.0001	<0.0001

Table (4): shows the frequency distribution of histopathological changes in the liver in diabetic treated groups compared to diabetic control group

Group	No affection (%)	Mild affection (%)	Moderate affection (%)	Severe affection (%)
Control Diabetic(±SD)	51±15.6	2±1.8	41±14.5	6±1.7
Rosiglitazone group(±SD) P value	38±1.3 0.04	3±1.9 0.71	50±7.6 0.12	9±2.9 0.048
Gliclazide group(±SD) P value	41±7.5 0.12	2±0.8 0.84	50±8.2 0.14	7±8.5 0.13
Rosiglitazone & Gliclazide group(±SD) P value	40±12.1 0.11	1±0.8 0.45	53±10.7 0.048	6±3.5 0.86

Table (5): shows some histological and immunohistochemical parameters in diabetic group compared to control group

Group	Mean optical density of PAS +ve reaction ± ST	Mean optical density of α-1 antitrypsin reaction± ST	Mean color area percentage of collagen. ± ST
Control	0.30±0.05	0.28±0.04	0.09±0.03
Diabetic	0.37±0.04	0.32±0.04	0.14±0.06
P value	<0.0001	0.010	0.015

Table (6): shows some histological and immunohistochemical parameters in diabetic treated groups compared to diabetic non-treated group

Group	Mean optical density of PAS +ve reaction ± ST	Mean optical density of α-1 antitrypsin reaction± ST	Mean color area percentage of collagen. ± ST
Control Diabetic	0.37±0.04	0.32±0.04	0.14±0.06
Rosi group P value	0.38±0.06 0.004	0.27±0.04 <0.0001	0.14±0.05 0.82
Gliclazide group P value	0.36±0.06 0.41	0.29±0.06 0.039	0.16±0.06 0.1
Rosi&Glic group P value	0.35±0.06 0.08	0.28±0.04 <0.0001	0.15±0.06 0.2

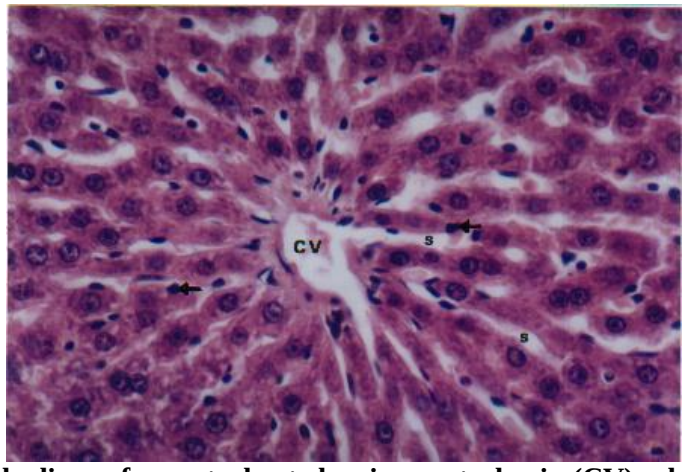

Fig 1- Section in the liver of a control rat showing central vein (CV), plates of hepatocytes separated by hepatic sinusoids (S) in which Kupffer cells (arrows) are associated in the lining. (H&E X 400).

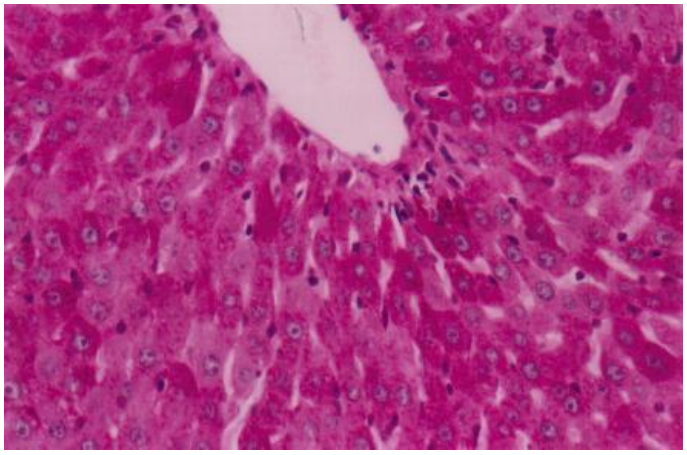

Fig 2- Section in the liver of a control rat showing PAS positive glycogen granules in the cytoplasm of hepatocytes. (PAS X 400).

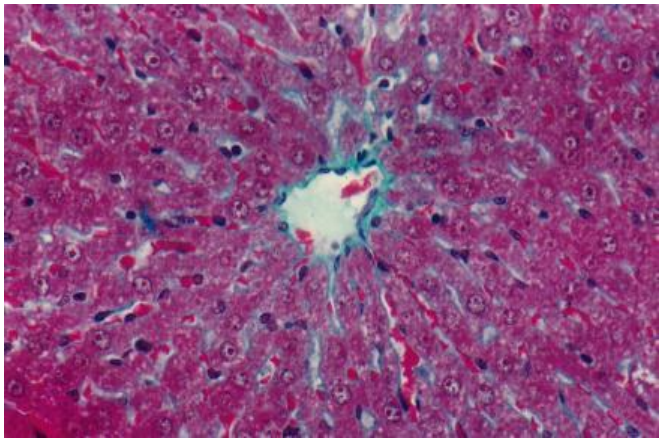

Fig 3- Section in the liver of a control rat showing some greenish stained collagen fibers in the area around the central vein and scanty fibers between hepatocytes. (Masson's trichrome X 400).

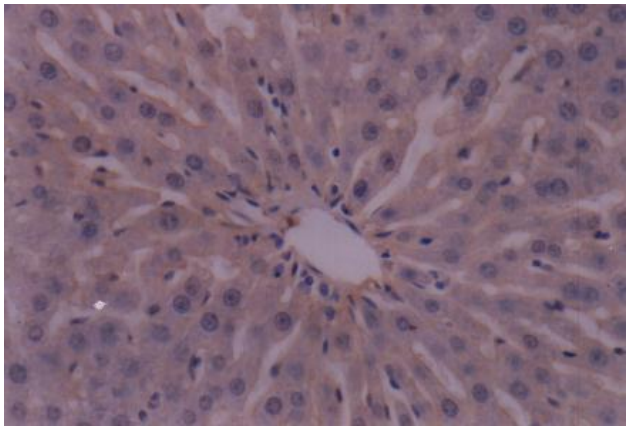

Fig 4- Section in the liver of a control rat showing weak staining of alpha-1 antitrypsin reaction. (Alph-1 antitrypsin immunostaining X 400).

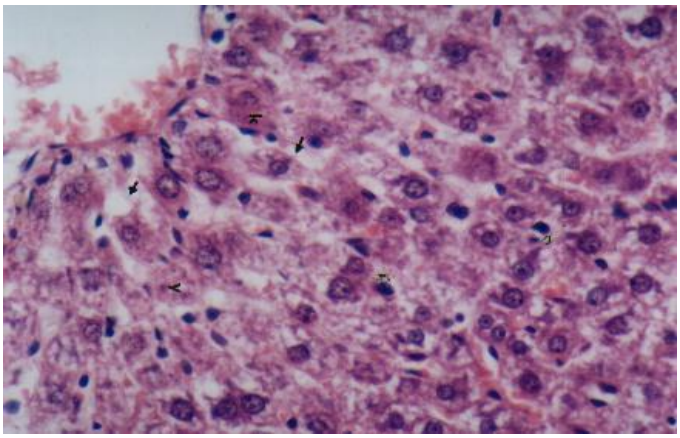

Fig 5- Section in the liver of a diabetic control rat showing degenerative and necrotic changes in hepatocytes in the form of pyknotic (P), karyorrhetic (K) or karyolytic (Y) nuclei. Other hepatocytes show vacuolated cytoplasm (arrows). (H&E X 400).

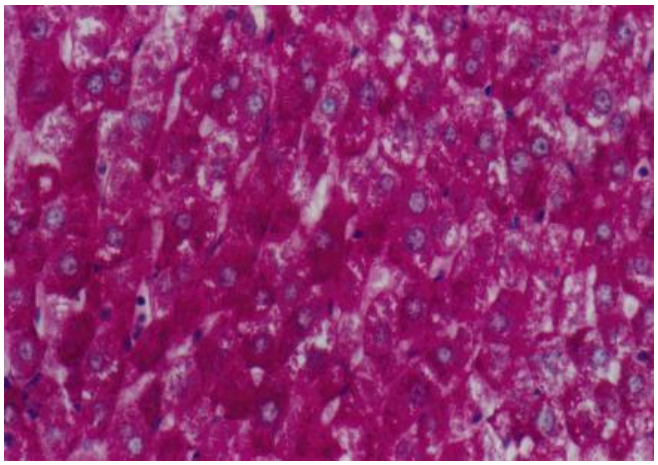

Fig 6- Section in the liver of a diabetic control rat showing increased PAS positive glycogen granules in hepatocyte cytoplasm. (PAS X 400).

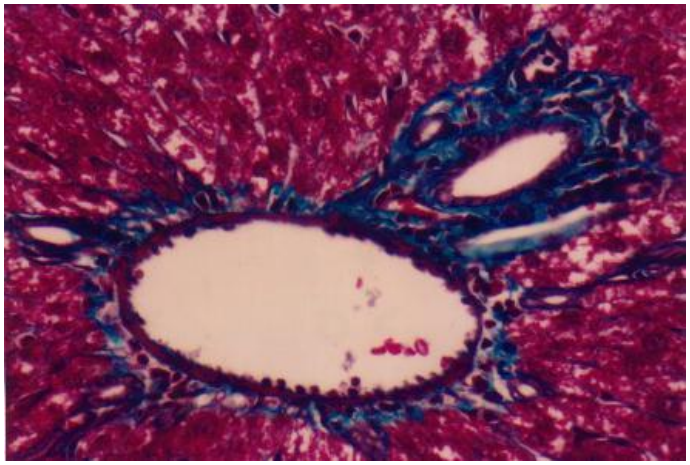

Fig 7- Section in the liver of a diabetic control rat showing an increase in the amount of collagen fibers in the portal tract and in some sites between hepatocytes. (Masson's trichrome X 400).

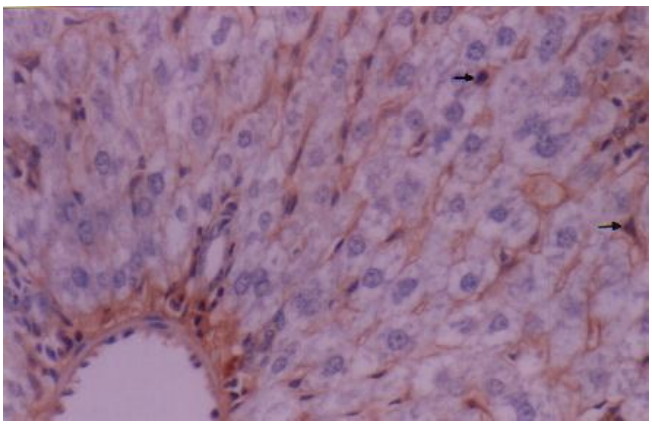

Fig 8- Section in the liver of a diabetic control rat showing increased expression of alpha-1 antitrypsin reaction in the area around the central vein, the lining of blood sinusoids and Kupffer cells (arrows) (Alph-1 antitrypsin immunostaining X 400).

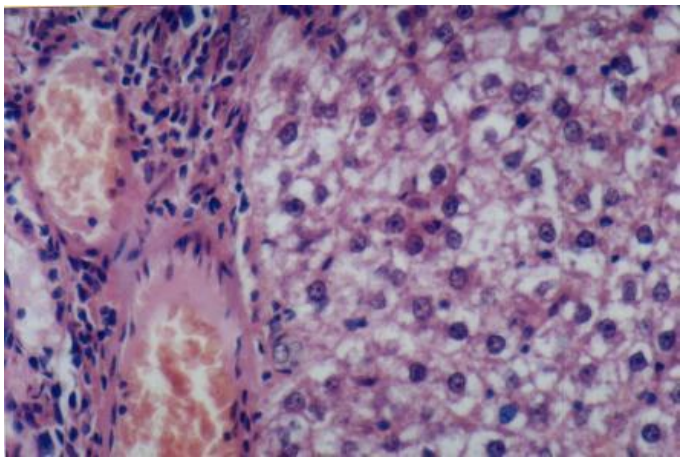

Fig 9- Section in the liver of a rat from group III (rosiglitazone group). It shows marked degenerative and necrotic changes with vacuolization of most of hepatocytes Vascular congestion and mononuclear cellular infiltration are also seen. (H&E X 400).

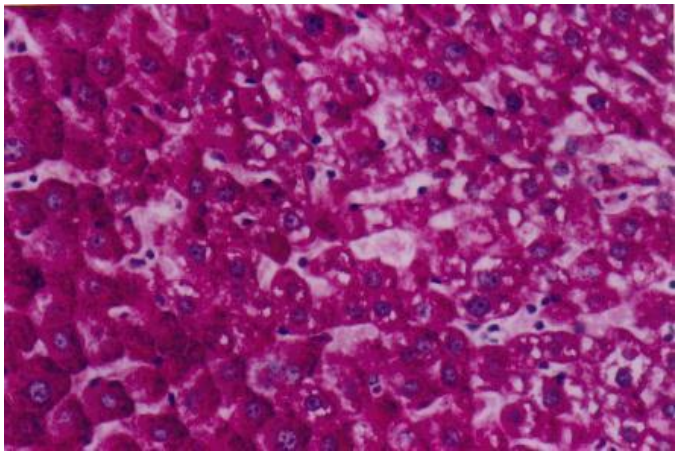

Fig 10- Section in the liver of a rat from group III showing increased PAS positive glycogen granules in hepatocyte cytoplasm. (PAS X 400).

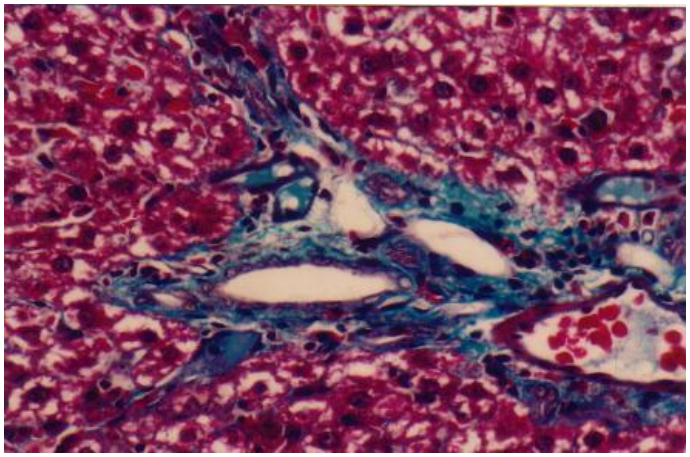

Fig 11- Section in the liver of a rat from group III showing an increase in the amount of collagen fibers in the portal tract. (Masson's trichrome X 400).

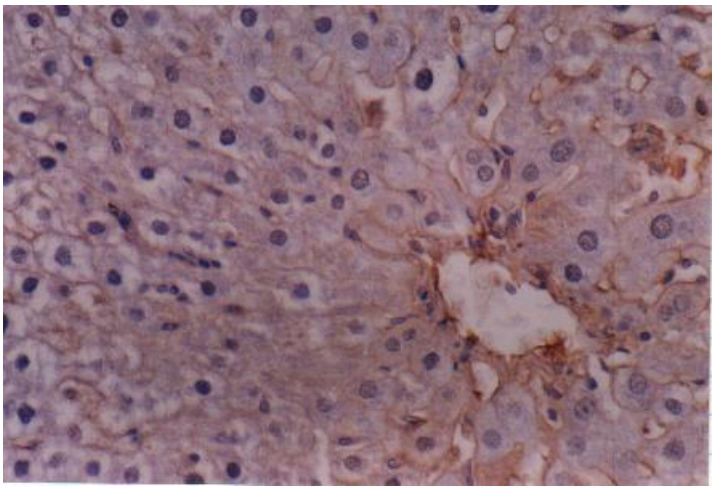

Fig 12- Section in the liver of a rat from group III showing decreased expression of alpha-1 antitrypsin reaction in blood sinusoids and Kupffer cells. (Alph-1 antitrypsin immunostaining X 400).

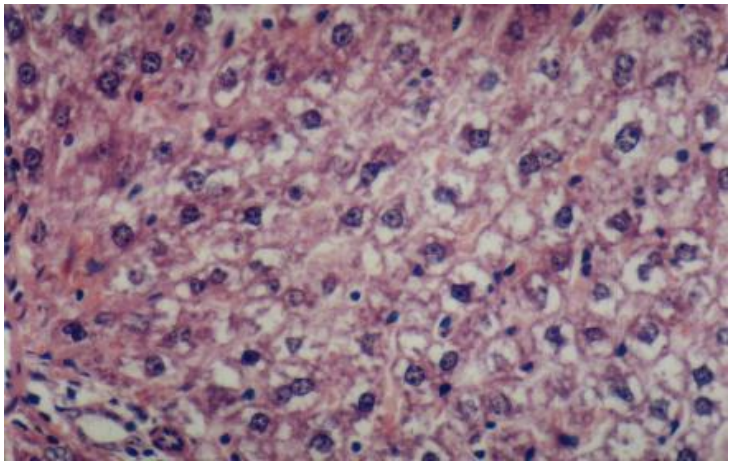

Fig 13- Section in the liver of a rat from group IV (gliclazide group) showing less degenerative and necrotic changes in hepaotcytes. (H&E X 400).

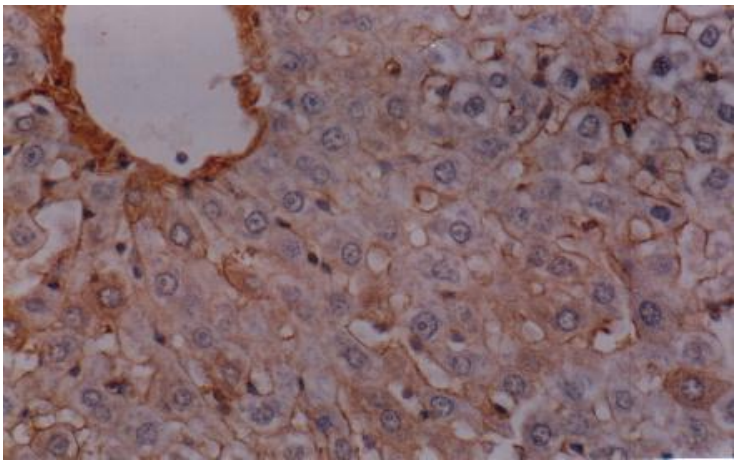

Fig 14- Section in the liver of a rat from group IV showing mild increase in alpha-1 antitrypsin immunostaining.
(Alph-1 antitrypsin immunostaining X 400).

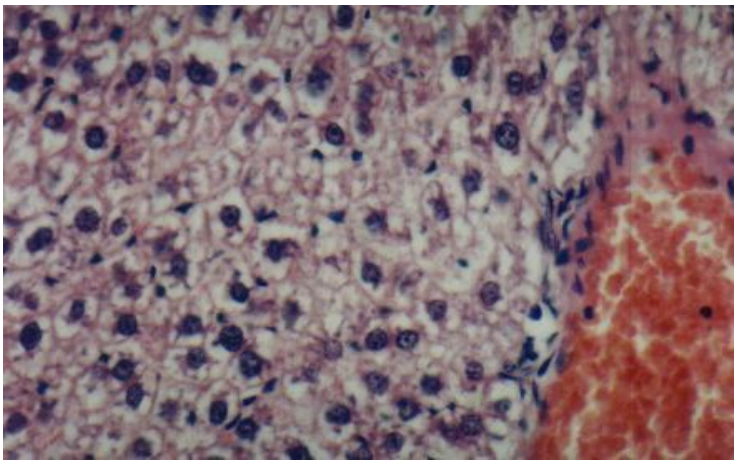

Fig 15- Section in the liver of a rat from group V (combination group) showing degenerative and necrotic changes in hepaotcytes as well as vascular congestion.
(H&E X 400).

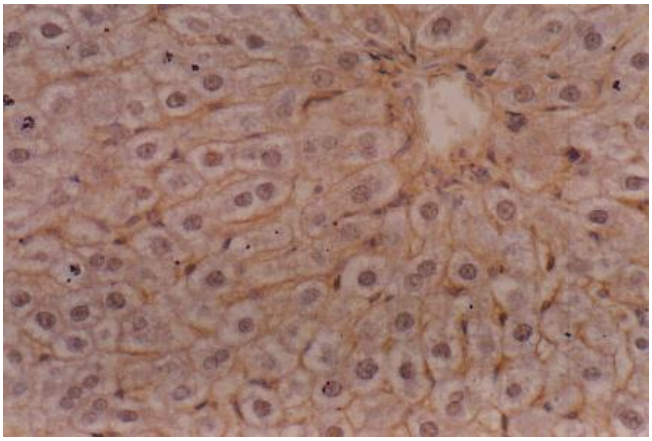

Fig 16- Section in the liver of a rat from group V showing alpha-1 antitrypsin immunoreactivity nearly similar to the control.
(Alph-1 antitrypsin immunostaining X 400).

Discussion

Insulin resistance seems to precede the clinical expression of many cardiovascular risks in diabetic patients. The treatment of diabetes mellitus requires reducing insulin resistance by exercising dietary adaptations and drugs. Thiazolidinediones (TZDs) are a new class of oral antidiabetic drugs that improve metabolic control in patients with type II diabetes through the improvement of insulin sensitivity (Haffiner and Miettnen, 2002). TZDs exert their effect by activation of gamma isoform of peroxisome proliferator- activated receptor (PPAR gamma), a nuclear receptor. This induced-activation alters the transcription of several genes involved in liver, glucose metabolism and energy balance (Reginato and Lazar, 2002). Troglitazone "one of TZDs" is not still in use due to its proved hepatotoxicity (Bailey, 2000).

So the present work was designed to study the effect of the newer TZD "rosiglitazone" & the older antidiabetic sulphonylurea "gliclazide" and combination of both on the liver of streptozotocin induced diabetic rats. The results of the present work revealed that when rosiglitazone was given orally daily, in a dose of 0.03 mg/ kg BW for 6 weeks, it produced a significant reduction in the fasting plasma glucose level ($P<0.05$). However this decrease became more significant ($P<0.01$) when gliclazide was given concomitantly, in a dose of 10 mg/ kg BW to rosiglitazone. This observation was in accordance with the findings of Scott and Donnelly (2001), who reported an improv-ement of blood glucose tolerance after combined therapy rather than monotherapy with gliclazide alone.

Combination of rosiglitazone and gliclazide in the present work also led to more significant ($P< 0.01$) decrease in TGs level, than when either drug was used alone. This finding was consistent with that obtained by Wolffenbuttel *et al*. (2000) who studied the effect of rosiglitazone and pioglitazone administration on metabolic risk factors and denoted that serum lipids were favorably affected. In this study it was also noticed that rosiglitazone alone was significantly effective to decrease TGs and cholesterol levels compared to the diabetic control group (Table 2). This was in contrast to results of Einhorn *et al*. (2002) who reported that glitazones should be licensed for combination use in non-insulin dependent diabetes mellitus with either metformin or a sulphonylurea for additional beneficial effect on serum lipid profile.

Serum Alanine Amine transferase (ALT) which is also called Serum Glutamic Pyruvic Transaminase (SGPT; Reitman, and Frankel, 1957), is an enzyme present mainly in liver cells, so it is considered as a specific biochemical marker for liver diseases. Rosiglitazone, when given alone resulted in rise in serum ALT, however this rise was non-significant compared to diabetic non-treated group. This observation was confirmed by histopathological results obtained from H&E stained sections

which revealed that although streptozotocin caused marked hepatocyte damage, the damage was further increased significantly in diabetic animals under rosiglitazone treatment. As seen in table 3 only 38% of hepatocytes were apparently normal whereas the rest were either mildly (3%), moderately (50%), or severely (9%) affected in diabetic rats received rosiglitazone. Hepatocellular injury was also reported in patients receiving rosiglitazone by both Al-Salman *et al*. (2000) and Forman *et al*. (2000). On the other hand, Berne (2001) denied this injury and concluded that rosiglitazone has no further bad impact on hepatocytes.

The rise of ALT in case of giving gliclazide alone or in combination with rosiglitazone was less marked (Table 2). This was clearly evidenced by histopat-hological results which revealed that although there was some degree of hepatocyte damage in either cases, the damage was insignificant compared to diabetic control group. This may indicate better liver function in both cases compared with rosiglitazone.

Quantitative assessments using the image analyzer showed a significant increase in all measurements of diabetic non treated group compared to control (mean optical density of PAS positive reaction, mean optical density of alpha-1 antitrypsin and mean color area percentage of collagen). Giving each of the 2 drugs alone or in combination resulted in a non significant difference-when compared to diabetic control group-in both mean optical density of PAS positive reaction and mean color area percentage of collagen. However the mean optical density of alpha-1 antitrypsin reaction decreased significantly in all treated diabetic groups compared to the diabetic one.

Accumulation of glycogen in the liver of diabetic control rats in the present study was consistent with findings of Ugochukwu and Babady (2003). This accumulation in case of control diabetic and in diabetic treated rats may be related to changes in glucose metabolism due to the effect of diabetes and the drugs. Day (1999) reported that TZDs increase glucose uptake from adipose tissue, skeletal muscles and liver, as well as increase glycogenesis in skeletal muscles and decrease glycogenolysis in the liver.

The increased mean color area percentage of collagen in portal tracts revealed by the image analyzer was mainly in the liver tissues of diabetic control rats which indicated that the fibrotic changes (which are a consequence of liver cell injury) were mainly induced by streptozotocin toxicity rather than the antidiabetic drugs. Liver fibrosis was also observed by Das et al. (1996) in streptozotocin-induced diabetic rats.

Alpha-1 antitrypsin is a member of the serine proteinase inhibitor super family (Parfrey *et al*., 2003). It was reported as a useful immunohistochemical marker of histiocytes (monocytes/macrophages) and malignant tumours derived from them (Isaacson *et al*., 1981). Therefore, it was used in the present work to study the effect of diabetes as well as the antidiabetic drugs on liver histiocytes, beside the histological study of hepatocytes.

In the present work, alpha-1 antitrypsin expression increased significantly in the diabetic control group, compared to control, which showed nearly negative or very weak reaction. This was in accordance with results of Ray and Desmet (1978) who demonstrated negative alpha-1 antitrypsin immunoreactivity in normal human liver using both immunofluorescence and immunoperoxidase techniques. The increased expression of the antitrypsin in case of diabetic control group in lining of blood sinusoids including Kuppfer cell and in the area around the central vein would probably indicates increased function of hepatic sinusoidal macrophages as Kupffer cells. This might represents functional immune response although there was no apparent increase in Kuppfer cell number in H&E stained sections. Direct functional relation between Kupffer cells and hepatocytes proposed by Hoebe *et al*. (2001) might play a key role in the development of hepatic inflammatory response in reaction to toxic agents.

The decrease of optical density of alpha-1 antitrypsin immunoreactivity-to a level similar to that of control-in diabetic

treated groups may be related to improvement of some metabolic parameters due to the use of the antidiabetic drugs.

In conclusion, combination of both rosiglitazone and gliclazide resulted in better metabolic control of diabetes as well as better histopathological picture of the liver. However careful monitoring of liver function tests is recommended at least during the first year of therapy.

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مقارنة بين تأثير عقار الثيازوليدين ديون- روزيجليتازون- وكل من السلفونايل يوريا- جليكلازايد - أو كليهما على كبد الجرذان المصابة بمرض السكر عن طريق حقن استربتوزوتوسين

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أجريت هذة الدراسة للمقارنة بين التأثيرات المحتملة لكل من:- روزيجليتازون –(وهو عقار جديد من أدوية السكر لة تأثير مماثل للجاما ب ب ا ر الاختيارى يستخدم عن طريق الفم في جرعة 03و مج لكل كجم من وزن الجسم) و جليكلازيد – (وهو عقار أيضا ضد مرض السكر من عائلة السلفونايل يوريا والذي يعطى بالفم في جرعة تساوى 10 مج لكل كجم من وزن الجسم). كل واحد منهم على حدة أو يعطو سويا لمدة 6 أسابيع على الكبد ومستوى السكر والدهون فى الدم فى الجرذان البيضاء والتى أصيبت بمرض السكر عن طريق حقنها بحقن استربتوزوتوسين.
قسمت ثلاثون من الجرذان البيضاء الى 5 مجموعات بكل مجموعة6 جرذان :-
المجموعة الاولى:وهى المجموعة الضابطة وقد حقنت بمحلول ملح عن طريق الفم يوميا لمدة 6 اسابيع .
المجموعة الثانية:- وهى مجموعة السكر الضابطة التى أصيبت بمرض السكر عن طريق حقنها باستربتوزوتوسين.
المجموعة الثالثة:- هى التى أخذت عقار روزيجليتازون .
المجموعة الرابعة:- هى التى أخذت عقار جليكلازايد.
المجموعة الخامسة:- هى التي أخذت العقارين معا.
أظهرت النتائج ان استربتوزوتوسين رفع كلا من مستوى السكر والكوليستيرول والترايجليسرايد فى دم الجرذان بمقدار يعتد بة إذا قورن بالمجموعة الضابطة.
بينما كان استخدام محفز الأنسولين روزيجليتازون مفردا أو متحدا مع جليكلازايد مؤديا الى إنقاص سكر الدم بمقدار يعتد بة إذا قورن بمجموعة السكر الضابطة .
وقد ادى جليكلازايد وحدة نفس التأثير وكان استخدام روزيجليتازون مفردا مؤديا الى نقص كل من الكوليستيرول و نوع من انزيمات الكبد وعند اتحادة مع جليكلازايد ادى الى نقص نوع اخر من انزيمات الكبد بمستوى يعتد بة اذا قورن بمجموعة السكر الضابطة.
بالنسبة للدراسة الباثولوجية النسيجية فقد استخدم نسيج الكبد لكل من الطرق النسيجية(صبغة الهيماتوكسيلين والايوسين – صبغة شيف الحامضية - وصبغة ماسون ثلاثية اللون) والهستوكيميائية المناعية لدراسة توزيع الفا -1- انتيتريبسين فى نسيج الكبد وقد اجرى تحليلا نوعيا وكميا لتقدير درجة اصابة الكبد .
وتم فحص شرائح الهيماتوكسيلين والايوسين عدديا على حسب خواص معينة لتقدير درجة تأثر خلايا الكبد هذا الى جانب القياسات الكمية (الكثافة الضوئية والنسبة المئوية لمساحة اللون) باستخدام جهاز تحليل الصور.

اظهرت النتائج ان استربتوزوتوسين سبب تاثرا شديدا فى 6% من خلاياالكبد وتأثرا طفيفا فى 2% وتأثرا متوسطا فى 41% .

وايضا سبب العقار ظهرات زيادة يعتد بها فى كل من حبيبات الجلايكوجين والمصبوغة بصبغة شيف الحامضية فى خلايا الكبد والياف الكولاجين فى البقعة البابية بالكبد.

اظهرت الصبغة الهستوكيميائية المناعية زيادة فى تفاعل الفا -1- انتيتريبسين فى كل من بطانة الجيوب الدموية المتعرجة متضمنة خلايا كوبفر وكذلك فى المنطقة حول الوريد المركزى.

بالنسبة للمجموعات الثالثة والرابعة والخامسة والتى كانت تحت تاثير عقاقير روزيجليتازون أو جليكلازايد أو كليهما فقد سببت اصابة فى خلايا الكبد مماثلة للتى حدثت فى مجموعة السكر الضابطة ولكن لا يعتد بدرجة الاصابة الا فى حالة المجموعة الثالثة.

وعندما قورنت هذة المجموعات الثلاثة بمجموعة السكر الضابطة لم توضح المقارنة أى فرق يعتد بة فى قياس كل من الكثافة الضوئية لتفاعل شيف الحامضى أو النسبة المئوية لمساحة لون الكولاجين ولكن متوسط الكثافة الضوئية للصبغة الهستوكيميائية المناعية قلت بدرجة يعتد بها.

يستخلص من هذا ان عقار روزيجليتازون بمفردة أو باستعمالة مع جليكلازايد فى الجرذان التى اصيبت بالسكر عن طريق حقن استربتوزوتوسين فانة يحدث تحسن فى الانضباط الايضى ولكن لابد من الاخذ فى الاعتبار احتمالية حدوث سمية الكبد .