The effect of Sitagliptin (Januvia) on the liver of adult Albino rats in cases of experimental diabetes mellitus(Microscopic and laboratory studies)

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Abstract

Introduction: The present study was carried out to evaluate the effect of Sitagliptin (Januvia) on the liver of experimentally induced diabetes in albino rats.

Material and Methods: Fifteen adult male albino rats were used and divided into three equal groups. The first group was considered as a control group. In the second group experimental induction of diabetes was performed by intraperitoneal injection of alloxan and left as a diabetic control for three weeks. The third group was consisted of rats of experimentally induced diabetes and treated by a daily dose of Sitagliptin (Januvia) as oral anti diabetic therapy for three weeks.

Two main parameters were performed; the first was microscopic and histochemical studies on the liver tissue while the second was laboratory evaluation of some liver functions.

Results: The hepatic tissue was affected by the experimental induction of diabetes in the form of cellular infiltration, hepatic cell cords disarrangement and vascular congestion after three weeks of induction.

The fibrous elements as well as mucopolysaccharides contents were greatly reduced. Histochemical changes in the liver enzymes showed mild decrease. Liver function tests showed mild changes.

Diabetic changes were gradually returned back to its normal state after the use of daily oral dose of Sitagliptin.

Conclusions: The antidiabetic drug (Sitagliptin) could be considered a good therapy in limiting the risk of diabetes Mellitus on liver tissue.

Keywords: Albino rats, Alloxan , Sitagliptin, structural and laboratory findings.

Review of Literatures

The liver has a wide range of functions, including detoxification, protein synthesis and production of biochemicals necessary for digestion , glycogen storage, decomposition of red blood cells and hormone production, (**Maton et al., 1993**). Hepatocytes make up 70 to 80 % of the total mass of the liver.

On year 2000 at least 171 million people worldwide suffer from diabetes mellitus, or 2.8% of the population**,** type II diabetes is by far the most common, affecting 90 to 95% of the U.S. diabetes population, (**Lambert , 2002 and Wild et al, 2004).**

Diabetes mellitus, often simply referred to as diabetes which is a group of metabolic syndrome characterized by high blood sugar level and classical symptoms of polyuria ,polydipsia and polyphagia .   There are three main types of diabetes**,** Type I diabetes, results from the body's failure to produce insulin (*insulin-dependent* diabetes mellitus, **IDDM** or *juvenile* diabetes). Type II diabetes, results from insulin resistance, a condition in which cells fail to use insulin properly (*non-insulin-dependent* diabetes mellitus, **NIDDM** or *adult-onset* diabetes)**. Gestational diabetes**; in pregnant women who have never had diabetes before and have a high blood glucose level during pregnancy. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery (**Rother, 2007)**.

Diabetic complications may be acute in the form of hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure and retinal damage, (**Rother, 2007)**.

Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type II diabetes. Many people destined to develop type II diabetes spend

many years in a state of pre-diabetes which has been termed "America's largest healthcare epidemic, **(Handelsman, 2009).**

Several methods have been used to induce diabetes mellitus in laboratory animals with variable success and many difficulties. Surgical removal of the pancreas is effective method; however, to induce diabetes at least 90-95% of the pancreas has to be removed, **(Akbarzadeh et al., 2007).**

Induction of experimental diabetes in the rat using streptozotocin is very convenient and simple to use, **(Ito, 1999).** Streptozotocin injection leads to the degeneration of the Langerhans islets beta cells, **(Ikebukuro et al., 2002 and Takeshita et al., 2006)**. Clinically, symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of 60mg/kg STZ, **(Elias et al., 1994).**

There are now four classes of hypoglycemic drugs, Sulfonylureas, Metformin,Thiazolidinediones and Alpha-glucosidase inhibitors. These drugs are approved for use only in patients with type II diabetes and are used in patients who have not responded to diet, weight reduction, and exercise.

Sitagliptin (Januvia) is an oral antidiabetic medicine that helps in controlling blood sugar levels. It acting through regulation of the insulin level in the blood.

Sitagliptin is used in cases of type II diabetes. It is sometimes used in combination with other anti diabetic medications, but is not for treating type I diabetes**, (Herman et al., 2006).**

Material and Methods

I. Experimental animals:
The present work was carried out on 15 mature male albino rats weighting 100± 20 *gms.*

The experimental animals were randomly divided into three groups (5/cage). The first group was considered as a control group while the rest of all animals were subjected to the induction of experimental diabetes mellitus by single intraperitoneal injection of freshly prepared alloxan in a dose

120 mg/kg dissolved in saline solution, according to **(Malaisse, 1982).**

Half of the induced diabetic rats were left as a control diabetic group (second group) while the rest of animals represent the third group which were treated by the sitagliptin in a dose of 0.14 mg /100 gm body weight, **(Paget, 1964)** for 21 days after the induction of the diabetes mellitus.

II. Preparation of microscopic sections:
Liver was obtained and fixed in neutral buffered formol saline, **(Clayden, 1971)** for 5 days, dehydrated ,cleared and embedded in a hard grade of paraffin for 4 hours.

Paraffin blocks were prepared and cut at 6 micrometers thickness using a rotary microtome.

Different staining techniques were used in this study including Heamtoxylin and Eosin stain, **(Clayden, 1971).**

The distribution of collagenous fibers was performed by the use of Mallory trichrome stain, **(Tarkhan 1968)**, while periodic acid-schiff reaction was used for the distribution of mucopolysaccharide contents in the liver tissues.

III. Histochemical techniques:
Frozen sections were cut at 15 micrometers thickness for demonstration of the different enzymatic activity.

Modified Gomori method for **Alkaline phosphatase enzyme** was used in this study**, (Pearse, 1977)**. Nitroblue tetrazolium method, **(Nachlas et al., 1957)** was used for localizing the activity of **Succinic deydrogenase enzyme** in the hepatic tissue.

IV. Biochemical and laboratory data:
Collection of rat's serum:
At the end of the experiment, animals were decapitated and blood samples were collected from the retro-orbital plexus. The samples were collected in clean dry graduated centrifuge tubes and left for 20 minutes to clot, then centrifuged at 5000 rpm, for 15 minutes.

Using pasture pipette about half of the supernatant serum was transferred into clean dry serology tubes for subsequent tests. Serum was separated and kept at -20ºC until analysis.

1. Assessment of serum glucose level: Serum glucose was estimated according to the enzymatic colorimetric method described by **Trinder, (1984)**.

2. Determination of serum Insulin level: This method is carried out according to **Reeves, (1983).**

3. <u>Alanine aminotransferase (ALAT):</u> It was determined according to **Breuer, (1996)**.
4. <u>Gamma-Glutamyltransferase (GGT):</u> It was determined by kinetic method according to **Szasz and Persijn, (1974).**

V. Statistical analysis: The obtained results were tabulated and statistically analyzed, *Snedecor et al.* (*1980*)

Results

I. Microscopic changes:

a. Control liver :

Sections in control liver stained by Hx&E stain shows normal hepatocytes arranged in radiating cords from the central vein to the periphery of hepatic lobule. The cords are separated by hepatic sinusoids.

Hepatocyte contains single or sometimes double central nuclei. Region of portal tract shows the normal tract contents of hepatic artery, portal vein and bile duct. (Fig. 1,2).

The normal distribution of the collagenous fibers around the region of portal tract were demonstrated in (Fig. 3) by Mallory stain technique.

While the normal hepatocyte content of mucopolysacharride were seen in (Fig. 4).

The activity of alkaline phosphatese enzyme in the control group seen in the (Fig. 13), while the activity of succinic dehydrogenase enzyme can be seen in (Fig. 16). The obtained histochemical changes were confirmed quantitatively by the use of image anaylsis system and statistically analyzed

b. Diabetic group:

Induction of diabetes by alloxan greatly affect the normal structure of the different liver elements in the form of dilated congested central veins and hepatic cell cords disarrangement. (Fig. 5,6)

The distribution of collagenous fibers in diabetic group showed increase in the collagen fibers around the region of portal tract (Fig.7).

The mucopolysacharrides contents in diabetic group show marked decrease in its distribution within the hepatocytes (Fig. 8).

The activity of alkaline phosphatese and succinic dehydrogenase enzymes in diabetic group were decreased (Fig. 14,17). The obtained histochemical changes were confirmed quantitatively by the use of image anaylsis system and statistically analyzed.

c. Effect of sitagliptin (Januvia):

After oral intake of sitagliptin, the liver structure showed variable microscopic changes in the form of dilated congested central veins and mild cellular infiltrations at the region of portal tract (Fig9,10).

The distributions of collagenous fibers were slightly decreased but do not return to control group (Fig. 11), while the mucopolysacharrides contents in the hepatocyte are more or less similar to those in the control group (Fig. 12).

The activity of both alkaline phosphatese and succinic dehydrogenase enzymes in this group returned back to near its normal when they were compared to the control group (Fig.15 and 18). The obtained histochemical changes were confirmed quantitatively by the use of image anaylsis system and statistically analyzed.
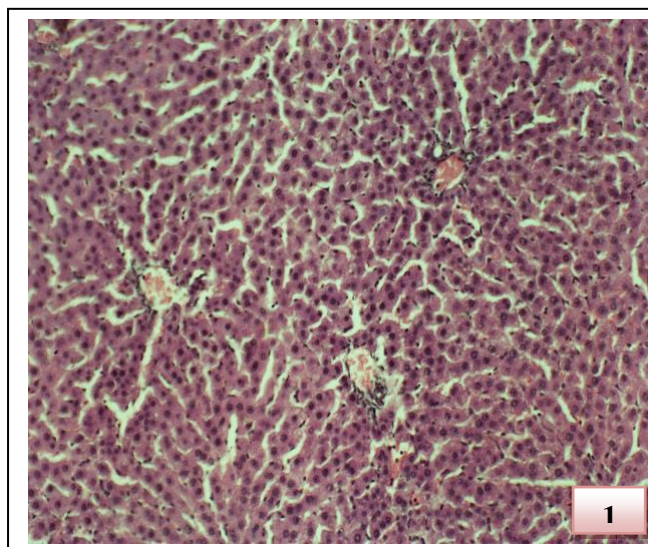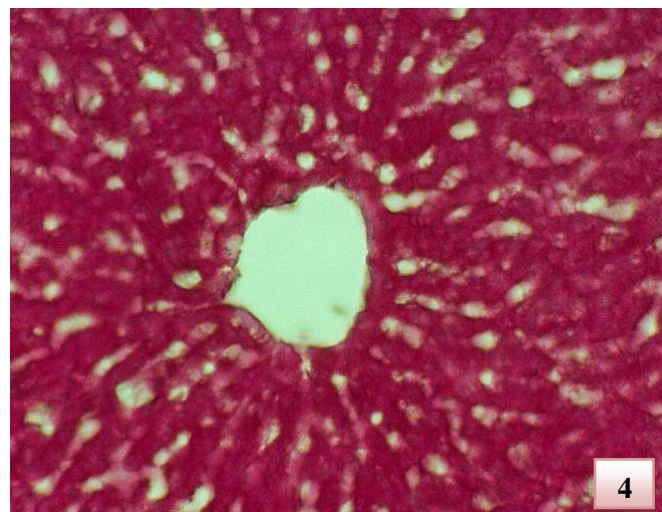
(Fig. 1) Photomicrograph of a section in the control liver of albino rat showing the normal structure of the liver cells , Cells are arranged in cords radiating from the central vein (Hx. & E. X 200)
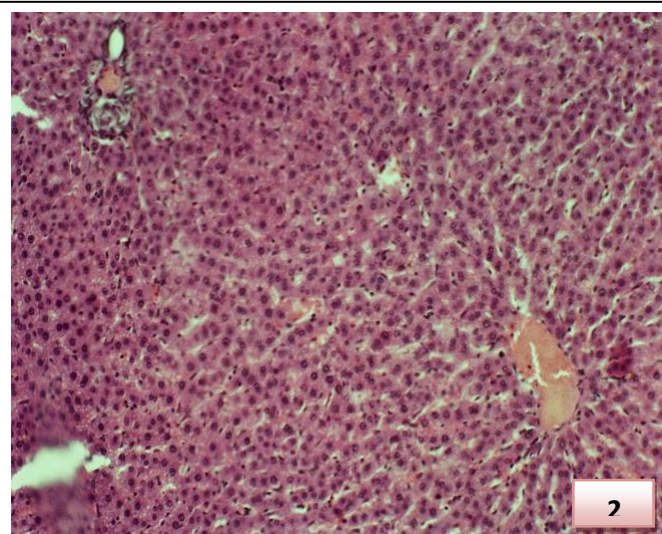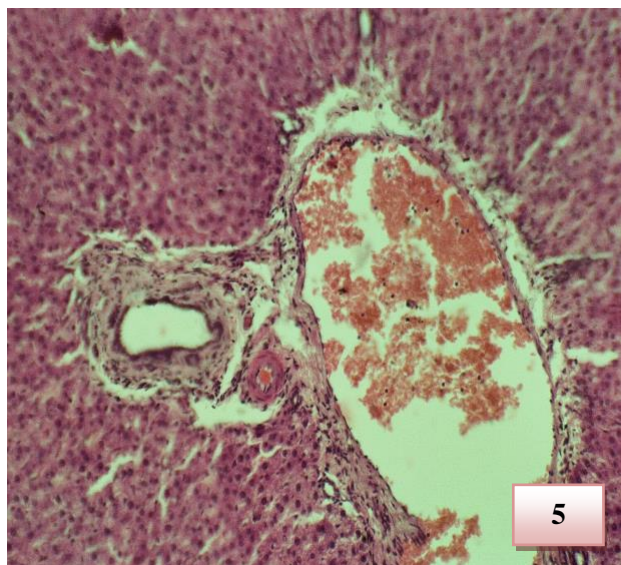

(Fig. 2) Photomicrograph of a section in the control liver of albino rat showing the normal structure of the liver cells , the region of the portal tract is seen in the upper left of the figure. (Hx. & E. X 200)
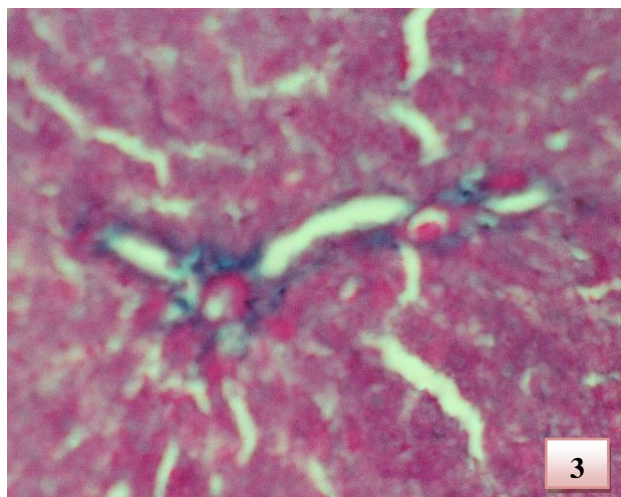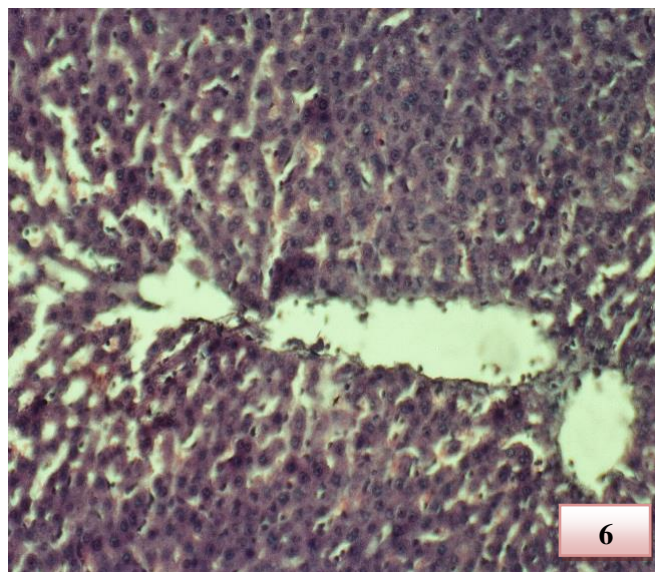

(Fig. 3) Photomicrograph of a section in the control liver of albino rat showing the normal distribution of the collagenous fiberes around the region of portal tract (Trichrome stain X 250)
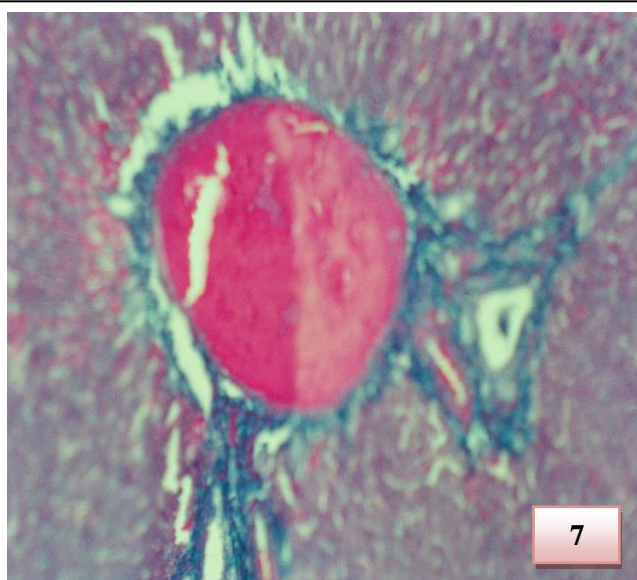

(Fig. 4) Photomicrograph of a section in the control liver of albino rat showing the normal distribution of PAS postive material in the liver cells (PAS technique X 250)
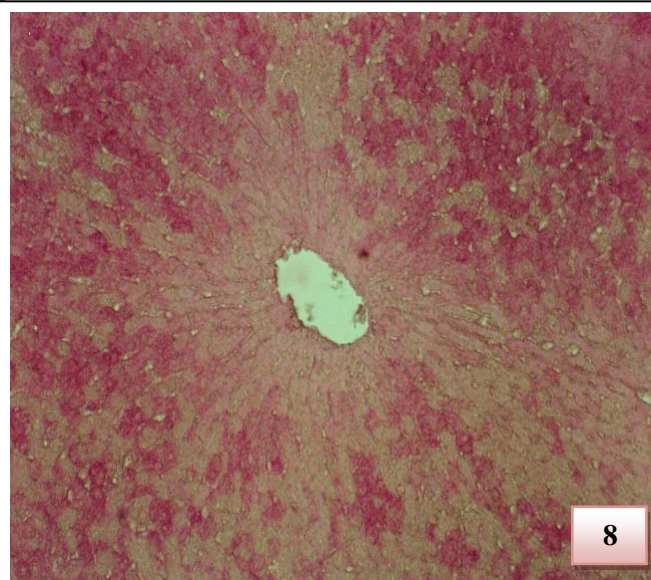
(Fig.5) Photomicrograph of a section in liver of induced diabetes of albino rat showing dilated congested vein (Hx.E. stain X 250)

(Fig.6) Photomicrograph of a section in liver of induced diabetes of albino rat showing hepatic cell cords disarrangement   (Hx.E. stain X 250)

(Fig.7) Photomicrograph of a section in the liver of induced diabetes of albino rat showing the distribution of the collagenous fiberes around the region of portal tract (Trichrome stain X 250)

(Fig.8)Photomicrograph of a section in the liver of induced diabetes of albino rat showing the distribution of PAS postive material in the liver cells (PAS technique X 250)
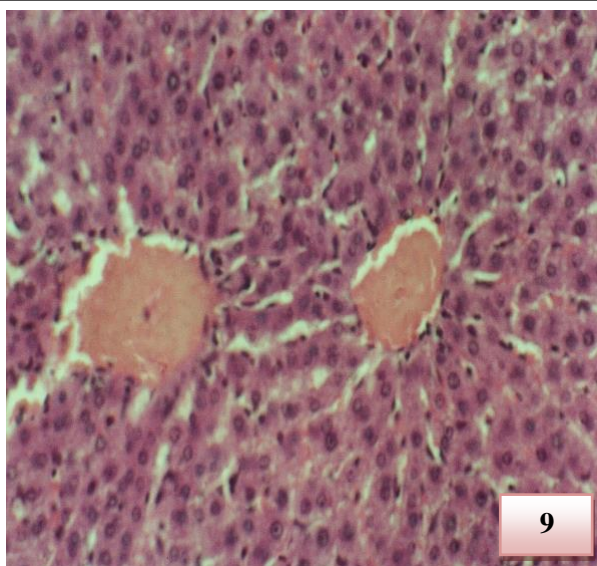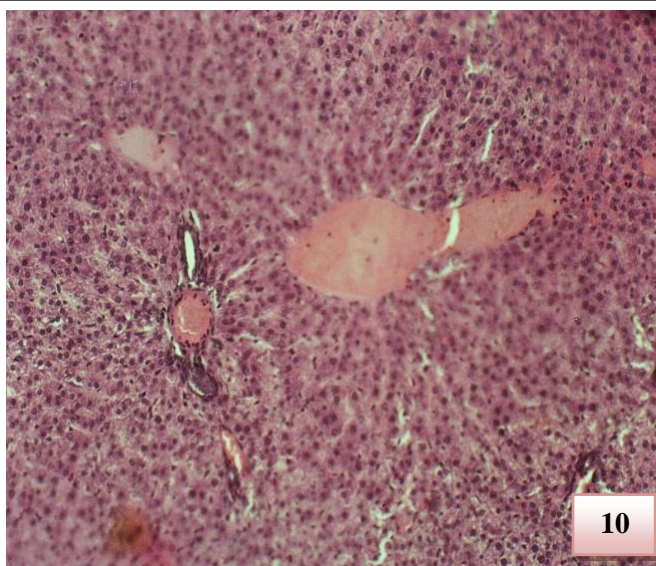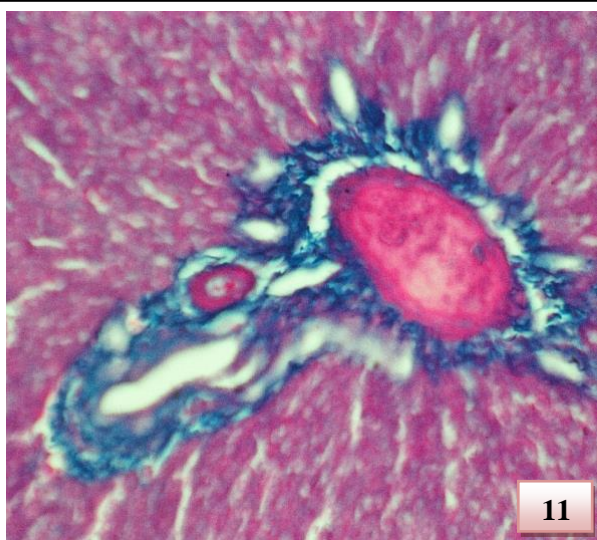
(Fig.9) Photomicrograph of a section in induced diabetes and treated by sitagliptin in the liver showing two dilated congested central veins (Hx.E. stain X 250)

(Fig.10) Photomicrograph of a section in induced diabetes and treated by sitagliptin in the liver showing) dilated congested vein and mild cellular infiltration at the region of portal tract. (Hx.E. stain X 200)

(Fig. 11) Photomicrograph of a section in induced diabetes and treated by sitagliptin in the liver showing the distribution of the collagenous fiberes around the region of portal tract (Trichrome stain X 250)
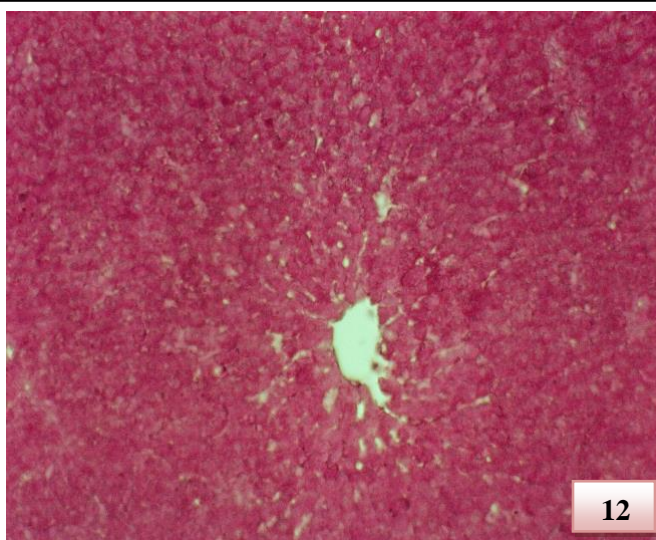
(Fig. 12) Photomicrograph of a section in induced diabetes and treated by sitagliptin in the liver showing the distribution of PAS postive material in the liver cells (PAS technique X 250)
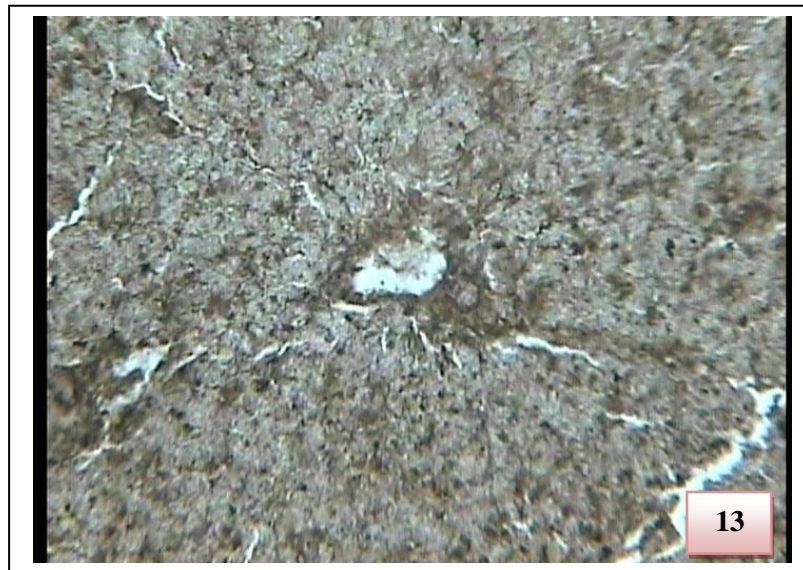
(Fig,13)Photomicrograph of a section in control liver showing
alkaline phosphatase enzyme (Modified Gomori stain x 200)

(Fig.14) Photomicrograph of a section in induced
diabetes in the liver showing alkaline phosphatase
enzyme activity. (Modified Gomori stain   x 200)
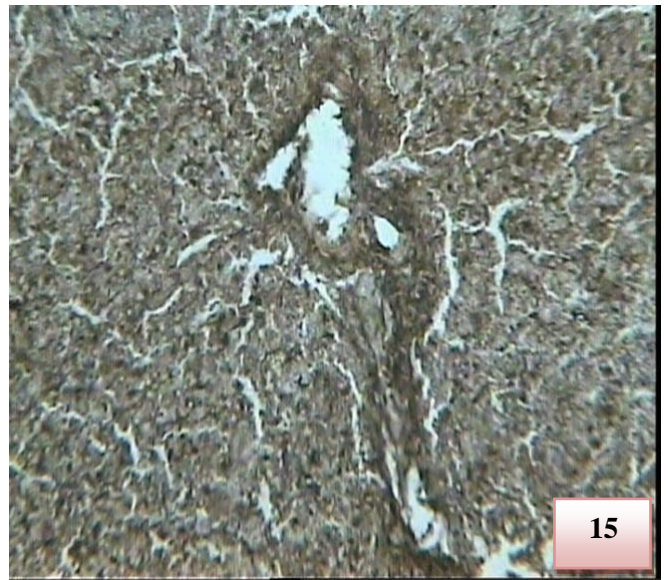
(Fig.15)Photomicrograph of a section in induced diabetes
and treated by sitagliptin in the liver showing alkaline
phosphatase enzyme activity. (Modified Gomori stain x 200)

(Fig.16)Photomicrograph of a section in control liver showing
     succinic dehydrogenaze enzyme activities.
     (Nachlas technique   x 200)

(Fig 17)Photomicrograph of a section in induced diabetes in liver showing succinic dehydrogenaze enzyme activities. (Nachlas technique x 200)

(Fig 18)Photomicrograph of a section in induced diabetes and treated by sitaglibtin in liver showing succinic dehydrogenaze enzyme activities (Nachlas technique x 200)

(Table 1) Changes in the mucopolysaccharide contents in the different groups of the study

	Control	Diabetic	Treated
1	1.12	0.36	1.02
2	1.13	0.4	1
3	1.16	0.45	1.02
4	1.11	0.4	0.94
5	1.12	0.42	1.12
mean	1.128	0.406	1.02
SD ±	0.019	0.033	0.065
SE±	0.009	0.015	0.029
Sig.		Sig.	Sig.

(Fig. 19)changes in mucopolysaccarides content in different groups of the study.
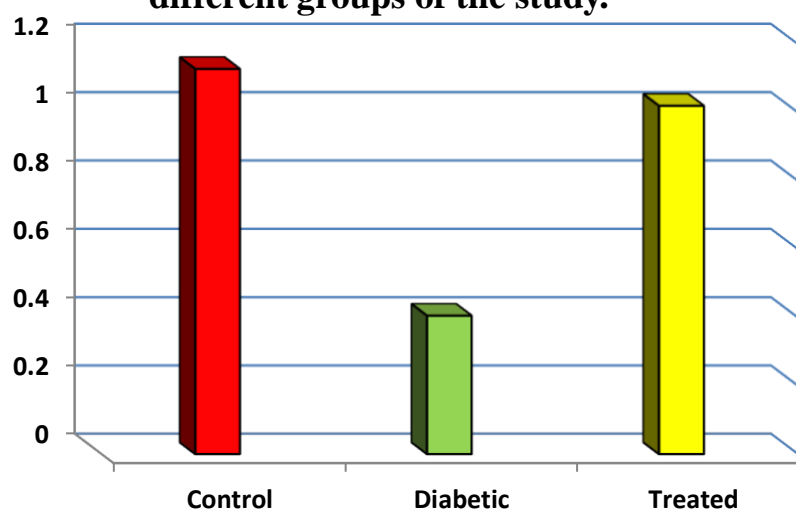
(Table 2) Changes in the alkaline phosphatase enzyme activity in the different groups of the study

	Control	Diabetic	Treated
1	1.52	0.5	1.02
2	1.49	0.49	1.03
3	1.4	0.5	0.91
4	1.41	0.51	1.05
5	1.39	0.49	1
Mean	1.442	0.498	1.002
SD ±	0.059	0.008	0.054
SE±	0.026	0.004	0.024
Sig.		Sig.	Sig.

(Fig. 20) changes in alkaline phosphatase enzyme activity in different groups of the study.
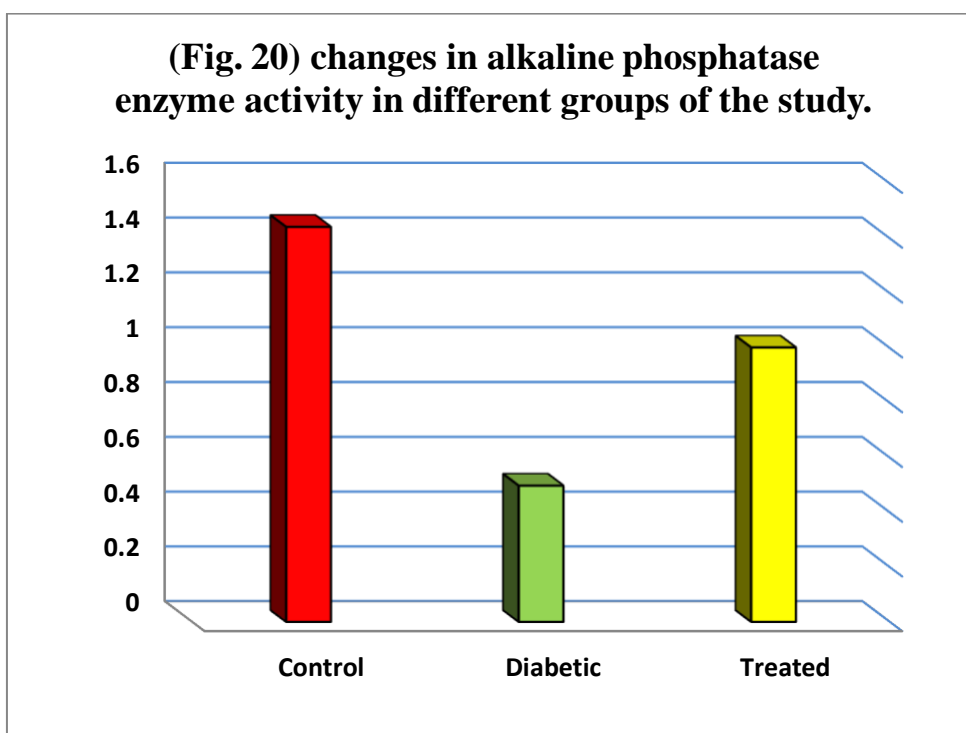
(Table 3) Changes in the succinic dehydrogenase enzyme activity in the different groups of the study

	Control	Diabetic	Treated
1	1.43	0.51	1.3
2	1.35	0.49	1.07
3	1.28	0.43	0.81
4	1.4	0.52	0.9
5	1.35	0.5	1.2
Mean	1.362	0.49	1.056
SD ±	0.057	0.035	0.203
SE±	0.026	0.016	0.091
Sig.		Sig.	Sig.

(Fig.21) changes in succinic dehydrogenase activity in different groups of the study
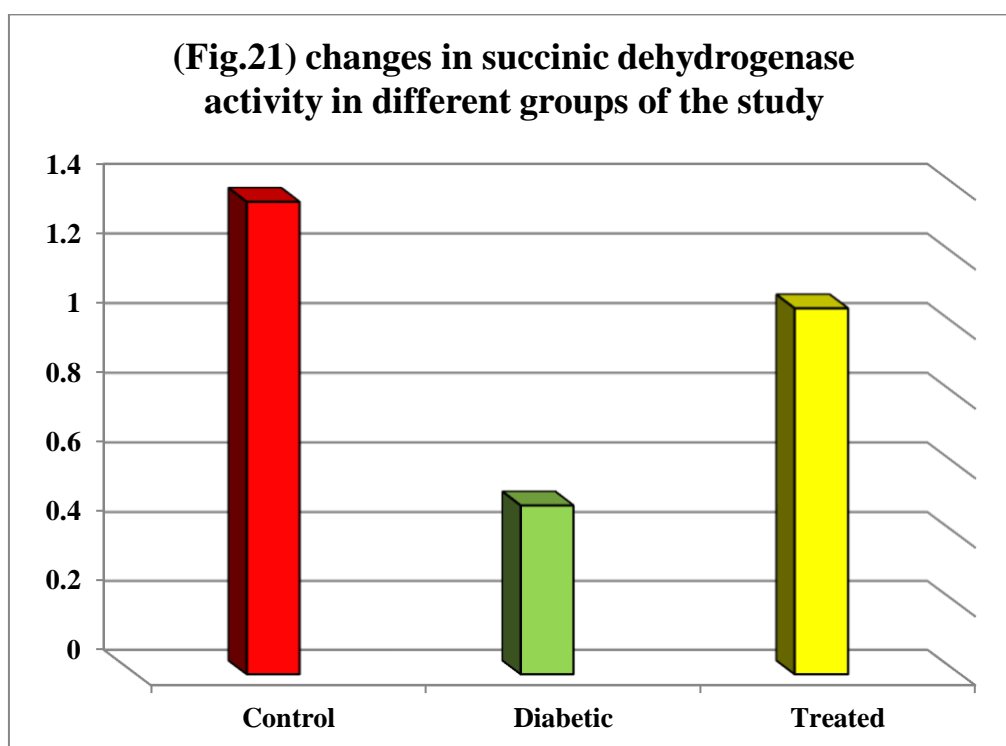
(Table 4) Changes in the blood glucose level in the different groups of the study

Glucose	Control		Diabetic		Treated diabetes with Januvia	
	Before	(After) Time of sampling	Before	(After) Time of sampling	Before	After Treatment
1	121	123	272	252	276	78
2	104	118	236	266	255	75
3	101	119	243	256	241	103
4	102	120	280	270	261	112
5	114	120	215	233	259	89
Mean	108.4	120	249.2	255.4	258.4	91.4
SD ±	8.73	1.87	26.70	14.48	12.56	15.92
SE ±	3.91	0.84	11.94	6.48	5.62	7.12
Age comp.	Control : diabetic		Control : treated		Diabetic : treated	
T Value	20.730		3.991		17.041	
P value	≥ 0.05		≥ 0.05		≥ 0.05	
Sig.	Sig.		Sig.		Sig.	

(Fig. 22) Changes in the blood glucose level in the different groups of the study

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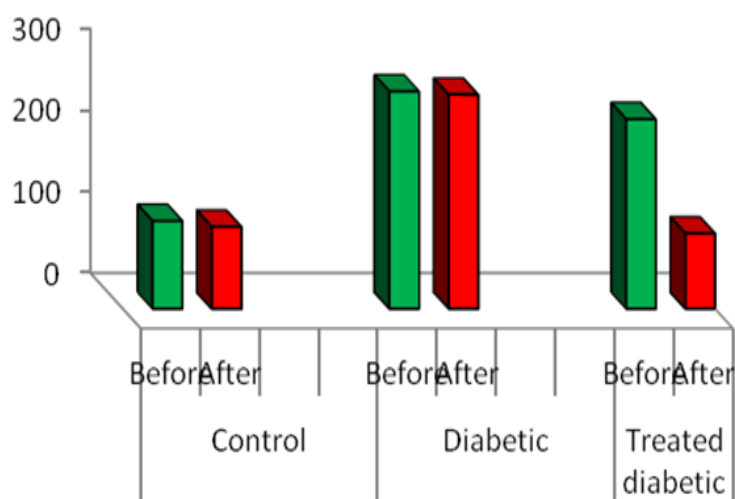
(Table 5) Changes in the serum insulin level in the different groups of the study

Insulin	Control	Diabetic	Treated diabetes with Januvia
1	37	2.8	2
2	32	2.4	2.1
3	30	4.1	1.6
4	29	4.5	2.4
5	25	3.5	2.2
Mean	30.6	3.46	2.06
SD ±	4.39	0.87	0.30
SE ±	1.96	0.39	0.13
Age comp.	Control : diabetic	Control : treated	Diabetic : treated
T Value	13.549	14.493	3.394
P value	≥ 0.05	≥ 0.05	≥ 0.05
Sig.	Sig.	Sig.	Sig.

(Fig.23) Changes in the serum insulin level in the different groups of the study
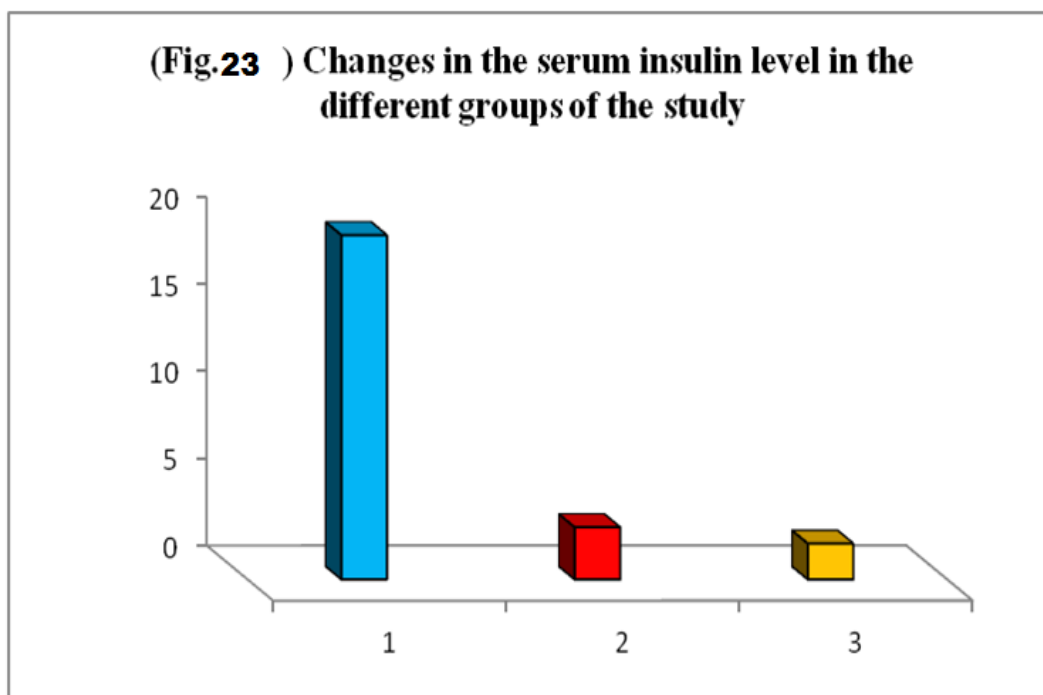
(Table 6) Changes in serum level of Alanine amino transferase in the different groups of the study

	Control	Diabetic	Treated diabetes with Januvia
1	58	79	81
2	49	67	66
3	44	57	90
4	49	64	88
5	65	72	89
Mean	53	67.8	82.8
SD ±	8.40	8.29	10.03
SE ±	3.75	3.71	4.49
Age comp.	C / D	C/ T	D / T
T Value	2.805	5.093	2.577
P value	≥ 0.05	≥ 0.05	≥ 0.05
Sig.	Sig.	Sig.	Sig.

(Fig. 24) Changes in the serum level of ALAT in the different groups of the study
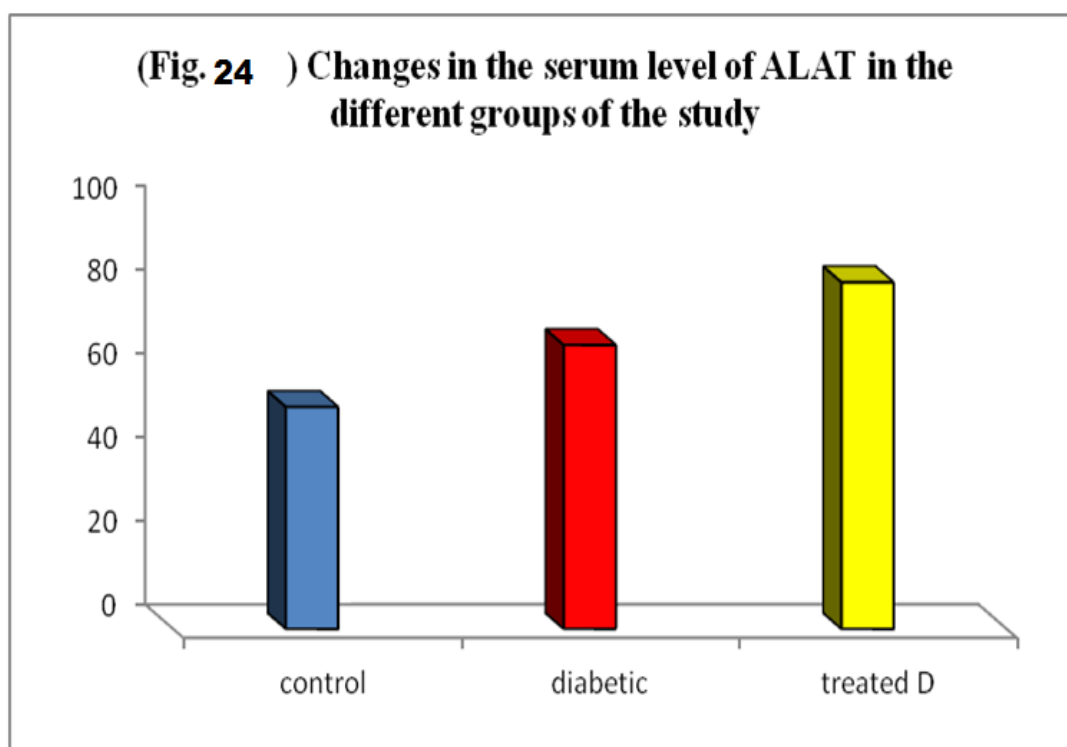
(Table 7) Changes in serum level of (GGT) in the different groups of the study

	Control	*Diabetic*	*Treated diabetes with Januvia*
1	1	2	1
2	2	3	2
3	4	2	1
4	1	4	3
5	1	3	1
Mean	**1.8**	**2.8**	**1.6**
SD ±	1.30	0.84	0.89
SE ±	0.58	0.37	0.40
Age comp.	C / D	C/ T	D / T
T Value	1.443	0.283	2.191
P value	≤ 0.05	≤ 0.05	≤ 0.05
Sig.	N Sig.	N Sig.	N Sig.

(Fig. 25) Changes in the serum level of GGT in the different groups of the study
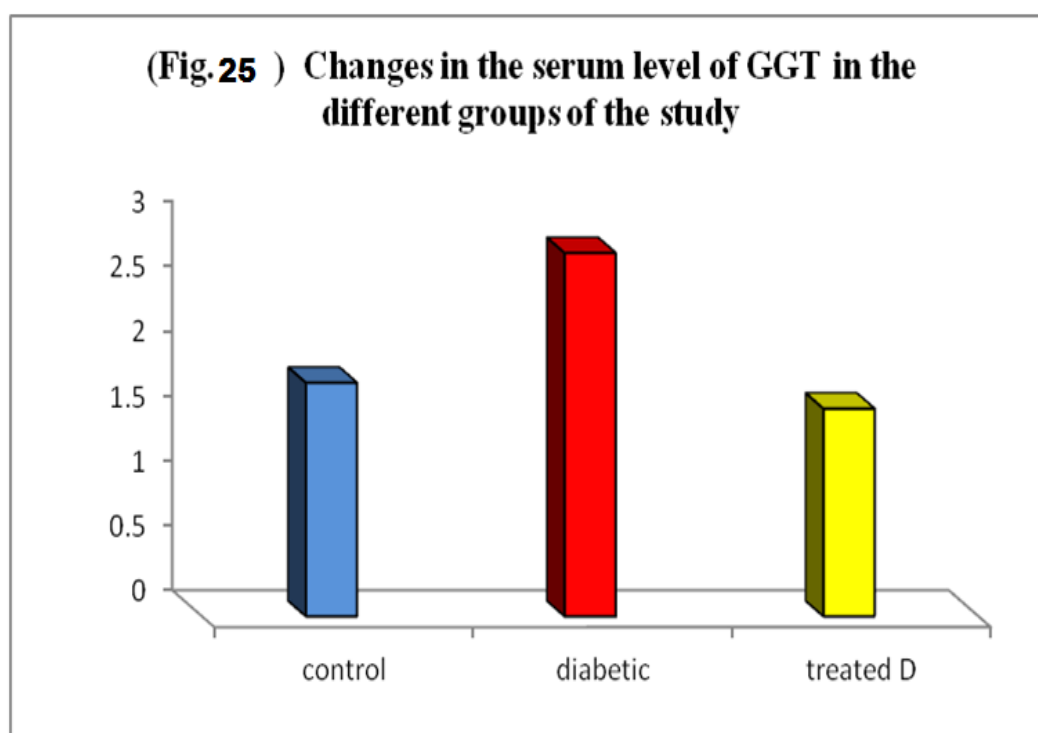
II.   Biochemical parameters:

a.   Blood glucose level:

There was change in the blood glucose level in the different groups of the study which appear to be normal in control group and increased with induction of diabetes and return to normal level under the effect of sitagliptin (Table 4, Fig22).

b.   Serum insulin level (u/ml):

There was change in the serum insulin level in the different groups of the study which appear normal in control group and decreased in both diabetic group and under the effect of sitagliptin.(Table 5,Fig. 23)

c.   Liver function tests:

1.   Alanine amino transferase (ALAT):

There was moderate increase in the serum level of alanine amino transferase in the diabetic group which become highly increased in diabetes treated by sitagliptin group (Table 6 , Fig 24).

2.   Gama glutamic transferase (GGT):

There was highly increase in serum level of (GGT) in diabetic group which return back to normal level under the effect of sitagliptin (Table7,Fig 25).

DISCUSSION

In this study, adult male albino rats were used and divided into three equal groups. The first group was considered as a control. In the second group experimental induction of diabetes was performed by intraperitoneal injection of alloxan and left as a diabetic control for three weeks . The third group was consisted of rats of experimentally induced diabetes and treated by a daily dose of Sitagliptin (Januvia) drug in a dose of 0.14 mg /100 gm body weight, **(Paget, 1964)** for three weeks.

In the present study , the microscopic examination of the rat's liver in the second group (induced diabetes) was greatly affecting the normal structure of the liver cellular elements in the form of dilated congested central veins , hepatic cell cords disarrangement , increase in the collagen fibers around the region of portal tract and decrease distribution of hepatic mucopolysacharrides.

Hramiak et al., 1997, support our results and stated that in newly diagnosed diabetes showed higher long-term risk of serious liver affections, including cirrhosis and liver cell failure.

In experimentally diabetic treated rats with oral intake of sitagliptin in group 3 microscopic examination of the liver showed variable microscopic changes in the form of dilated congested central veins and mild cellular infiltrations at the region of portal tract. The distributions of collagenous fibers were slightly decreased but do not return to its normal architecture as in the control group ,while the mucopolysacharrides contents in the hepatocytes were more or less similar to those in the control group .

Horn et al., (1995) reported that the presence of collagen in the presinusoidal spaces might affected the blood supply to liver cells and would be reduce the exchange of metabolism, perhaps causing hepatocellular dysfunction and necrosis**.**

Sitagliptin is a new class of oral anti-hyperglycemic drugs known as a DPP-4 inhibitor or incretin enhancer for the treatment of Type II diabetes mellitus**, (Amori et al, 2007)**.   Clinical studies have shown improvement in beta-cell function, suggesting a potential for preservation of beta-cells, **(Richter, et al.2008).**

Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues such as the liver lead to abnormal morphological changes in hepatocytes, **(Neuschwander-Tetri et al., 2003).**

In insulin resistance characteristic of type II, there is increase in the uptake and storage of triglycerides fats from the diet, abdominal fat and nearby muscles into compartments within the hepatocyte vesicles. This infiltration of fat is called steatosis. The results are fatty liver which is larger than normal, **(Standards of medical care in diabetes 2011).**

Elevated activities of serum aminotransferases are a common sign of liver diseases and are observed more frequently among people with diabetes than in general population, **(Arkkila *et al*., 2001).** Serum ALAT, AST and ALP levels were determined to evaluate the hepatic functions, **(Degirmenchi *et al*., 2002)**. The increase in aminotransferases levels may be due to the cellular damage in the liver caused by alloxan-induced diabetes. Although ALAT is also present in mitochondria and cytosol, the mitochondrial form is low in activity and is very unstable. The detailed mechanism by

which enzymes are released from the cytosol and mitochondria of hepatocytes is not completely known.

Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space, **Garella, (1997)**.Very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood, **Garella, (1997).**

Baxter and Schofield, (1980) reported an increase in AST and ALAT in diabetics. This alteration in hepatic function may be because of increase activity and mRNA levels of araginase as reported by **Salimuddin *et al.* (2008).**

Increase in the levels of ALP in diabetic rats was also reported by **Ramesh & Pugalendi,(2006).**

Insulin regulates the entry of glucose into tissues and promotes glycogen storage. Insulin is metabolized in the liver, where it promotes the production of glycogen, protein, cholesterol, and triglycerides and stimulates the formation of low density lipoproteins (LDL), which transport cholesterol into the arteries. In diabetes, excessive output of glucose by the liver contributes to elevated fasting blood sugars, **Ghishan ,(1996).**

Davidson et al., (2009) reported that sitagliptin enhance the body's own ability to control blood glucose by increasing the active levels of incretin hormones in the body.

Sitagliptin (DPP-4 inhibitors) control elevated blood glucose by triggering pancreatic insulin secretion, suppressing pancreatic glucagon secretion, and signaling the liver to reduce glucose production. This study open the door for further investigations of other recent oral antidiabetic therapy for better improvement of different diabetic complications.

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تأثير عقار السيتاجليبتين (الجينوفيا) على كبد الجرذان البيضاء البالغه في حالات مرض السكر التجريبي (دراسة ميكروسكوبية ومعملية)

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تم إجراء هذه الدراسة لتقييم تأثير عقار السيتاجليبيتين (الجينوفيا) علي كبد الجرذان البيضاء البالغة فى حالات مرض البول السكرى التجريبى.

تم استخدام 15 من ذكور الجرذان البيضاء البالغة حيث قسمت إلي 3 مجموعات متساوية, اعتبرت المجموعة الأولي مجموعه ضابطه أما المجموعة الثانية فقد تم إصابتها بمرض البول السكري بعد حقنها بعقار الألوكسان داخل الغشاء البريتوني وفى المجموعة الثالثة تم إستخدام عقار السيتاجليبتين (الجينوفيا) عن طريق الفم فى علاج مرض البول السكرى التجريبى بجرعة قدرها 0.14 مليجرام لكل 100 جرام من وزن الجرذان المستخدمة.
وقد إستمر العلاج بالعقار المستخدم لمدة 21 يوما من تاريخ إحداث المرض .

وتم تقسيم النتائج إلى إتجاهين مختلفين شمل الإتجاه الأول دراسة ميكروسكوبية لخلايا الكبد لتتبع التغييرات المحتمل حدوثها في المجموعات المختلفة أما الإتجاه الثاني فهو عبارة عن تحليل معملي لبعض وظائف الكبد بجانب تقييم كمى لنسبة السكر والأنسولين فى الدم والتي تظهر مدي كفاءة خلايا الكبد والبنكرياس في المجموعات المختلفة .

وقد دلت النتائج علي مدي تأثر خلايا الكبد بمرض البول السكري فى زيادة الترسيبات الخلوية وعدم إنتظام الترتيب الميكروسكوبى لخلايا الكبد وإحتقان في الأوعيه الدموية كما لوحظ إنخفاض واضح فى كمية الألياف الكولانجية ونسبة كل من مادة الميوكوبولي سكارايد وكذلك النشاط الإنزيمى فى هذه الخلايا.
ولقد كان لإستخدام عقار السيتاجليبيتين أثر واضح فى تحسن تلك التغييرات وعودتها إلى الصورة الطبيعية لخلايا الكبد. وقد دلت النتائج المعملية على مدى فاعلية العقار المستخدم فى تحسن الصورة المرضية وعودتها للحالة الطبيعية.

ويستنتج من هذه الدراسة أن عقار السيتاجليبيتين (الجينوفيا) له دور جيد في تحسين المضاعفات الناتجة عن تأثير مرض البول السكرى علي خلايا الكبد .