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Concomitant administration of sitagliptin and rutin improve the adverse hepatic alterations in streptozotocin-induced diabetes mellitus in albino rats, an overlook on the role of alpha smooth muscle actin

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Abstract

Background: Diabetes mellitus (DM), one of the commonest worldwide metabolic conditions, recognized to persuade oxidant/antioxidant discrepancies. Sitagliptin is an oral anti-hyperglycemic remedy that blocks dipeptidyl peptidase 4 (DPP4). Rutin is a polyphenolic natural flavonoid which owns antioxidant and anti-proliferative activity. The aim of the present work is to elucidate the concomitant effect of Sitagliptin and rutin on the deleterious alterations in the liver of experimentally induced diabetes in rats.

Materials and methods: 50 adult male albino rats, weighing 170-200 g were used. Rats were randomly divided into 5 groups (n=10). Group 1 (control group), the other 4 groups (Groups II, III, IV and V) received a single i.p. injection of STZ, 65 mgKg-1 body weight to induce diabetes; group II (diabetic), group III (diabetic and rutin administered), group IV (diabetic and sitagliptin administered), and group V (diabetic with sitagliptin and rutin concomitantly administered). H&E, masson trichrome, PAS, immune-histochemical; α-smooth muscle actin (α-SMA), histomorphometric analysis, liver enzymes and oxidatants / anti-oxidatants; malondialdehyde (MDA)/ glutathione (GSH) and were done.

Results: Distorted hepatic architecture, dilatation, congestion of sinusoids and central veins as well as cytoplasmic vacuolations were remarkable changes in the diabetic group. There was extravasation of blood, diffuse fibrous tissue formation, increase in the mean

values of liver enzymes, oxidative markers and α-SMA expression in the same group. The aforementioned changes were ameliorated in groups III and IV. Concomitant administration of sitagliptin and rutin resulted in marked enhancement of these hepatic alterations.

Conclusions: Combination of sitagliptin and rutin has an ameliorating effect on the hepatic deterioration induced by diabetes, which is better than either sitagliptin or rutin alone.

Key words: **streptozotocin, liver, diabetes, α-SMA, sitagliptin, rutin**

INTRODUCTION

Diabetes mellitus is a metabolic syndrome categorized by elevated blood sugar levels and typical symptoms; polydipsia, polyuria and polyphagia [1]. Abnormal function of chief body organs comprising the liver can be a consequence of the upsurge of blood glucose levels [2]. The pivotal role of reactive oxygen species in the progress and exacerbation of DM impediments has been discussed for several epochs [3, 4]. Lipid peroxidation disturbs all lipid-encompassing structures in cells, resulting in cytopathological consequences [5].

Rutin is a flavonoid compound that exists in various plants and possesses several pharmacological functions; blood glucose drop, insulin release regulator, dyslipidemic modifier. Moreover, it owns anti-inflammatory, anti-tumor as well as reactive oxygen species (ROS) attenuation possessions [6, 7, 8, 9].

Many studies have revealed that rutin has a robust therapeutic influence on liver injury triggered by different reasons for instance; biliary obstruction and high fatty diet. Nevertheless, the mechanism of rutin in DM induced liver injury was not very distinct [10, 11]. Some researchers have exposed that the safety of rutin in diabetic liver can be attributed to its anti-inflammatory properties; impeding lipogenesis [12].

Sitagliptin is an antidiabetic prescription taken orally that blocks dipeptidyl peptidase 4 (DPP4). Suppression of DPP4 advances insulin sensitivity and hence, lessens blood glucose concentrations [13]. Sitagliptin definitely has been permitted by the FDA, Health Canada, as well as the European Commission as a solitary cure for the treatment

of diabetes and it can be efficiently mutually administered with either metformin or glitazone (14, 15).

The objective of this study is to elucidate the properties of Sitagliptin and rutin amalgamation on the pathological alterations of the liver of experimentally induced diabetes in rats.

MATERIAL AND METHODS

Chemicals

Sterptozocin: (STZ) (Trade name Zanosar) was purchased from Sigma chemical company, St. Louis Missouri, USA, in the form of 1 g vials. The drug was dissolved in 0.1 M sodium citrate (pH adjusted to 4.5).

Sitagliptin: in the form of Januvia 100 tablet. Each tablet was ground and dissolved in 10 mL solution of 0.5% carboxymethyl cellulose (CMC), and afterwards shaken to obtain a suspension form (10 mg/mL).

Rutin: was purchased from Sigma Chemical Co., St Louis, USA in the form of powder and dissolved in saline.

Experimental animals

50 adult male Sprague-dawley albino rats, weighing 170-200 g were used. They were retained in the animal house of Kasr Al-Aini Faculty of Medicine, Cairo University. The rats had free access to standard rat chow and water. They were maintained according to the standard guidelines of Institutional Animal Care and Use Committee, subsequent to Institutional Review Board approval. Rats were permitted to accustom for 2 weeks prior to the experiment.

Rats were randomly divided into 5 groups (n=10).

Group 1 (control) received an intraperitoneal (i.p.) injection of 0.1 mol/L sodium citrate buffer (pH 4.5).

The other 4 groups (Groups II, III, IV, V) received a single i.p. injection of Sterptozocin (STZ), 65 mg/Kg^{-1} body weight [16], freshly dissolved in 0.1 M citrate buffer (pH 4.5). Diabetes mellitus was verified by measuring blood glucose levels (after

overnight fast) with the use of glucose oxidase reagent strips (Lif3 scan, Milpitas, CA, USA). Rats with blood sugar level >250 mg/dl were used as the diabetic group. In order to monitor blood glucose levels, blood glucose was tested every week for 4 weeks.

Group II (diabetic): diabetic rats received no treatment during the course of the study. **Group III (diabetic + rutin):** diabetic rat received rutin at a dose of 10 mg/ kg/ day dissolved in saline orally for 4 weeks [11].

Group IV (diabetic + sitagliptin): Diabetic rats received oral Sitagliptin at a dose of 100 mg/kg/day sitagliptin via gastric gavage for 4 weeks (17).

Group V (diabetic + rutin+ sitagliptin): Diabetic group receiving oral Sitagliptin at a dose of 100 mg/kg/day concomitantly with rutin at a dose of 10 mg/ kg/ day orally via gastric gavage for 4 weeks.

All animals were clinically monitored and weighed on a weekly basis. After 4 weeks a blood sample was withdrawn from the tail vein consuming fine heparinized capillary tube for assessing liver function. Formerly, the rats of each group were sacrificed utilizing an over dose of intraperitoneal phenobarbital sodium (40mg – kg). The rats were dissected. The liver of each animal was excised and prepared for light microscopic study.

Liver specimens were fixed in formalin 10% dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of five micrometers thickness were cut and mounted on glass slides. Other sections were mounted on +ve charged slides for immunohistochemistry.

These sections were subjected to the following:

I. Light microscopic study

- H&E stain to study the changes in histo-pathological architecture.
- Masson's trichrome stain to demonstrate collagen fibers.

II. Histo-chemical study

- Periodic acid Schiff reaction (PAS) to demonstrate the glycogen. The paraffin sections were dewaxed, rehydrated and then oxidized in 1% of periodic acid (5 min).

Formerly, they were wash away with distilled water, pickled with Schiff's reagent for 15 min, rinsed in tap water for 5–10 min, counterstained in haematoxylin, discerned in 1% acid-alcohol, cleansed in tap water, dehydrated in ascending degrees of alcohol, cleared in xylene, and mounted in Canada balsam. Glycogen and other reactive carbohydrates appeared magenta.

III. Immune-histochemichal staining

- Alpha smooth muscle actin (α-SMA) to evaluate the fibrosis processes (18). The segments from each paraffin block were incubated with primary antibody α-SMA antibody ((ab7817) 1:100). Next, the sections were incubated with Goat anti-Rabbit IgG H&L (HRP) (ab205718) for 20 min at 37°C. Each phase was tailed by satisfactorily wash with PBS.

IV. Morphometric study: using Leica image analysis computer system (software Qwin 500, switzerland), the following parameters were assessed:

- **Area % of collagen** fibers in Masson's Trichrome
- **Mean optical density of PAS reaction in PAS**
- **Area % of immune reaction of α SMA**

Stained sections were inspected by magnification x 400 and measured within a field of standard measuring frame. This was completed in 10 non overlapping microscopic fields of each specimen and their mean values were acquired.

V. Biochemical study

- **Liver function assay**

Retro-orbital blood samples were extracted from each rat for valuation of liver enzymes. Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) enzymes levels were assessed using specific kits pro-vided by Bio-diagnostic Company (Bio-diagnostic eka@lycos.com and info@bio-diagnostic.com). These measurements were done in the Biochemistry Department, Faculty of Medicine, Cairo University.

- **Assays of oxidative/antioxidative markers**

We measured MDA using a commercial kit (**Biodiagnostic, Cairo, Egypt),** according to the manufacturer's instructions. Concisely, roughly 10-20 mg liver tissue was homogenized in 1 ml PBS, pH 7.0, utilizing a micropestle in a micro tube. At that point, 20% (w/v) trichloroacetic acid was supplemented to the homogenate to precipitate the protein, and centrifuged at 12,000 x g for 15 min., afterwards, 0.8% thiobarbituric acid solution was added to the liver homogenate to precipitate the protein. After boiling for 10 min in a water bath, the absorbance was measured at 405 nm using a spectrophotometer. The concentration of MDA was calculated per milli-gram protein using a standard curve. The standard curve was prepared as follows. We dissolved 25 µl 1,1,3,3 tetraethoxypropane (TEP) in 100 ml water to attain a 1 mM stock solution. We organized a working standard by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid and incubation for 2 h at room temperature. The resultant MDA 20 nmol/ ml standard was diluted with 1% sulfuric acid to yield the final concentrations of 10.5, 2.5, 1.25 and 0.625 nmol/ml to prepare a standard curve for estimating total MDA. Then, 0.250 ml standard were mixed with 25 µl DNPH solution and incubated for 10 min. A 20 µl volume of the reaction mixture was injected directly onto HPLC system (Pilz et al., 2000).

GSH the antioxidant stress marker was measured using a commercial kit to detect glutathione (**Biodiagnostic, Cairo, Egypt**), according to the producer's commands. The measurement was based on reduction of 5, 5 dithiobis-(2 nitrobenzoic acid), with reduced glutathione to create yellow compound. The reduced chromogen was directly proportional to GSH levels, and the ultimate reaction product was assayed spectrophotometrically by quantifying its absorbance at 405 nm.

Statistical analysis

The statistical package for the social science (SPSS) was used for data analysis. The data obtained from image analyzer were summarized as means and standard deviations and compared using one-way analysis of variance (ANOVA). P values C< 0.05 was considered statistically significant, while $P < 0.01$ was considered statistically

highly significant.

RESULTS

I. Light microscopic study

Hematoxylin and Eosin results. Liver sections obtained from rats of the control group showed classic hepatic lobules consisting of intersecting plates of liver cells (hepatocyte) radiating outwards from a central vein to the periphery of the lobules. Narrow blood sinusoids were seen intervening between cords of hepatocyte (Fig. 1A). The portal area at the periphery of the lobules was seen formed of the bile ductule, a branch of portal vein with thin wall and wide lumen and branch of hepatic artery which appeared narrower in lumen and thicker in wall (Fig.1 B). The diabetic group (group II) revealed distortion of the parenchymal architecture. The hepatocytes exhibited marked pathologic affection where the cytoplasm displayed marked degree of cytoplasmic vacuolations. The central vein was dilated and congested. There was marked congestion of portal vein with mononuclear cell infiltration in the portal area (Fig.1;C,D,E).The liver in H&E sections in the diabetic and rutin treated group (group III) revealed dilatation of central vein with vacuolated areas of degenerated hepatocytes (Fig. 1F). The diabetic and Sitagliptin treated group (group IV) exposed dilatation of central vein with small vacuolated areas between the hepatocytes. (Fig. 1G). **Concomitant administration of rutin and Sitagliptin (group V)** resulted in an apparently normal hepatic architecture as that of the control group apart from mild affections. Most hepatocytes were apparently normal with eosinophilic cytoplasm and rounded nuclei. There was mild dilatation of central vein (Fig. 1H).

Masson's Trichrome Results. Histological examination of sections in the liver from rats of group I showed minimal amount of collagen fibers in the form of thin layer of collagen fibers around the central vein and hepatic sinusoids (Fig. 2A). **Group II** exhibited an increase in the amount of dense collagen fibers around the portal tract and the blood sinusoids (Fig. 2B) and in between hepatocyte (Fig. 2B). **Meanwhile, group III and IV** displayed moderate increase in the amount of dense collagen fibers around the portal tract and the blood sinusoids (Fig. 2C and 2D). **On the other hand, group V**

showed mild increase in the amount of collagen fibers around the central vein and in the portal tract (Fig. 2E).

Histo-chemical study

PAS reaction results. In the control group the hepatocytic cytoplasm contained considerable amounts of glycogen and displayed strong positive PAS reaction in the form of small red granules filling the cytoplasm **(Fig.3A)**.**While,** group III exposed very faint weak positive PAS reaction **(**Fig.3B)**.** Meanwhile, group IV displayed weak positive and group IV showed moderate positive PAS reaction (Fig. 3D).However, group V revealed strong positive PAS reaction in the cytoplasm of hepatocyte (Fig. 3E).

Immune-histochemical results

Examination of the liver sections of group I revealed negative immunoreactivity of α-SMA in the hepatocyte. There was localized immunoreactivity around the central vein **(**Fig.4A). Group II and III showed strong positive immune-reactivity (Fig.4B and C). **Group IV** exposed moderate positive PAS reaction moderate positive immunoreactivity. (Fig. 4D). Nevertheless, **Group V** showed minimal positive immune-reactivity (Fig. 4E).

Morphometric results

- **Statistical study of the mean area percentage of collagen fibers:** The mean area percentage of the collagen fibers of group II, III and IV showed highly significant increase in its value compared with the corresponding control group. However, the mean value of the above mentioned parameter of group V was non- significant compared with the corresponding control group. The mean value of the area percentage of the collagen fibers of group II, III and IV increased significantly in comparison with group V (Tables 1 and Fig. 2F).
- **Statistical study of the mean optical density of PAS reaction:** a significant increase in the optical density was found in group II and III in comparison with the corresponding control group. On the other hand, a significant decrease in the optical density was found in group IV and V in comparison with the group II (Table 1 and Fig. 3 F).

- **Statistical study of the area % of immune reaction of α SMA:** The mean area % of α–SMA immune-positive cells showed no significant difference among the control group and group V. While diabetic group (group II) resulted in significant increase) in the mean area % of the α–SMA immunoreactivity as compared to groups I & V. (Table 1, Fig. 4 G).

Biochemical assay

Liver enzymes. Biochemical assay of the liver enzymes of the diabetic group revealed a marked increase in AST mean value which was statistically significant compared to the mean values of group I and groups IV, V. Meanwhile, treatment with sitagliptin and rutin in group V displayed a decrement in AST mean value which was statistically non-significant relevant to the corresponding values in the control group. Group II (diabetic group) showed marked increases in ALT mean value, which was statistically significant compared with the mean values of the control group and sitagliptin and rutin treated groups. Meanwhile, treatment with sitagliptin and rutin in group V showed reduction in ALT mean value which was insignificant in relation with the control group.

The diabetic group showed a marked increase in ALP mean value which was statistically significant compared to the mean value of the control, and sitagliptin and rutin treated groups. On the other hand, treatment with sitagliptin and rutin in group III showed a reduction in ALP mean value which was statistically significant compared with diabetic group and non-significant compared with the value in the control group (Table 2).

Assay of oxidant and antioxidant markers

Malondialdehyde (MDA). The liver homogenates of diabetic group demonstrated a marked upsurge in MDA mean value which was statistically significant compared with the mean value of the control group .Meanwhile, treatment with sitagliptin and rutin showed a reduction in MDA mean value compared with the same values in group II, and was statistically non-significant if compared with the values in the control group (Table 3).

Glutathione (GSH). The liver homogenates of group II demonstrated a marked decrease in GSH mean value which was statistically significant compared with the mean value of the control group. On the other hand, treatment with sitagliptin and rutin demonstrated an increase in the mean value, which was statistically non-significant compared with the value of the control group (Table 3).

DISCUSSION

In the present study, manifestations of the pathological effects of diabetes on the liver were observed. These histopathological effects include distorted hepatic architecture, dilatation and congestion of central veins, hepatocytic degeneration in the form of cytoplasmic vacuolation. These alterations in the liver were attributed to cellular necrosis and inflammation, which might be a result of amplified mitochondrial oxidative stress. The later stress could be a consequence of triglycerides metabolism and the establishment of free radicals in peroxisomes [19, 20]. The cytoplasmic vacoulation of hepatocytes is due to deprivation of the ATP energy stocks; prerequisite to sustain ionic and fluid homeostasis (21). The aforementioned mechanism results in reduced activity of the energy-dependent sodium pump plasma membrane. The failure of this active transportation system grounds sodium to cross the threshold and accumulate within the cells and potassium to blowout followed by gain of water, triggering cellular swelling [22]. In addition, high deliberations of ROS caused by suppressed oxidative phosphorylation predictably contribute to depletion of ATP [23].

Histomorphometric studies exhibited diminished PAS reaction (glycogen content) in the hepatocytes of the STZ-treated animals. Glycogen dislodgment in the cytoplasm of the hepatocytes might be due to the accumulation of lipid droplets [24].

In the present study, fibrosis is an obvious manifestation in the diabetic group in the form of increased collagen fibers around central vein and portal area and increased expression of alpha smooth muscle actin. Beta-oxidation of fatty might occur due to inadequate insulin, and this leads to accumulation of hydrogen peroxide in tissues [25, 26, and 27]. The existence of collagen in the presinusoidal spaces may distress blood supply to liver cells and reduce metabolic exchange, probably leading to hepatocellular dysfunction and necrosis [28]. The deposition of collagen in the liver can be accredited to

to hepatic stellate cells (HSCs) that cause pathogenesis by liver damage-dependent activation. Activated HSCs discriminate into myofibroblasts (MFBs) [29]. MFBs exhibit a synthesis profile that lead to increase in their deposition in the extracellular matrix (ECM). This procedure helps them to proliferate, alters their morphology, and increases contractility by activating fiber formation of-smooth muscle actin (SMA). Then, this contributes to the constriction of sinusoidal blood flow and increases collagen fibers synthesis and release.

In the existing research, tests for liver function were conducted to observe the effects of STZ-induced diabetes on the liver at 4 weeks after STZ treatment. Compared with control rats, levels of AST, ALT, and ALP were increased in diabetic rats. AST and ALT are both enzymes that are established principally in liver mitochondria [13]. If there is liver impairment the enzymes are released into the bloodstream after death of the liver cells [30]. Peculiarly, great levels of AST and ALT are pivotal indicators of hepatic injury [5].

The present work revealed increment in MDA and decreased GSH in liver homogenates of diabetic rats. Recent studies have shown that the cause of DM advance and its complications is lipid peroxidase which leads to ROS formation (31). An increase in ROS generation and a decrease in antioxidant system activity result in an imbalance that leads to oxidative hassle [32]. The high blood sugar levels in diabetes lead to oxidative stress and fade the capacity of endogenous antioxidants. This is due to the production of many reducing sugars over both the glycolytic and polyol pathways [32].

In experimentally diabetic treated rats with oral intake of rutin (group III) or sitagliptin (group IV), microscopic examination of the liver disclosed variable microscopic changes in the form of dilated congested central veins and cellular infiltrations at the region of portal tract and some vacuolated areas. The allocations of collagenous fibers were slightly decreased than the control group.

In the current study co-administration of Sitagliptin and rutin in group V significantly improved the histological picture of the liver and the severity of liver damage was less as compared with the group treated with either Sitagliptin or rutin alone.

Sitagliptin is an oral anti-diabetes drug known as an inhibitor of DPP-4 used to treat type II diabetes mellitus [33]. DPP-4 inhibition was proposed to reduce hepatic

lipogenesis by several mechanisms; down-regulating the gene expression of sterol regulatory factor binding protein-1c (SREBP-1c), constraining fatty acid synthase, dropping the serum levels of VLDL and LDL cholesterol , subsequently decreased hepatic lipid accumulation and steatosis [10]. In addition, DPP-4 inhibition enhanced glycemic regulation; moreover, it modifies cholesterol synthesis, lipoproteins [34] **and** liver function enzymes (ALT, AST & ALP). Hence, all the previous mechanisms lead to amelioration of hepatic histo-pathological features in clinical trials of patients with type two diabetes [35].

Previous studies have publicized that rutin has a significant outcome on blood glucose control, and also has a very significant effect on the safety of liver cells [24, 29, 36]. DM related hepatic cellular damage is meticulously interrelated to burdened free radicals, therefore rutin's antioxidant activity can shield the liver cells [7, 11]

CONCLUSION AND RECOMMENDATIONS

Concomitant administration of sitagliptin and rutin has an outstanding ameliorating role on diabetes-induced hepatic histolo-pathological and biochemical alterations. This is better than either rutin or sitagliptin alone. Therefore, the use of both sitagliptin and rutin give outstanding results in liver protection against diabetic changes, it is recommended furtherly to use rutin on higher doses to test its effect on diabetic induced and other hepatic injuries.

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Table 1. Area % of collagen fibers, Optical density , and area % of α–SMA immune-reaction in different experimental groups

Groups	Parameters		
	Area % of collagen fibers	**Optical density**	**Area % of α–SMA**
Group I (Control)	1.89 ± 0.16	0.812 ± 0.065	1.36± 0.12
Group II (Diabetics)	15.74[a] ± 1.17	0.113 [a] ± 0.011	7.45 [a] ± 0.47
Group III (Rutin)	10.99[a,b] ± 1.06	0.339 [a,b] ± 0.016	5.86 [a,b] ± 0.43
Group IV (Sitagliptin)	7.19 [a,b]± 0.49	0.565 [a,b] ± 0.031	3.44 [a,b] ± 0.21
Group V (Rutin+ Sitagliptin)	3.19[b] ± 0.21	0.765 [b] ± 0.041	1.44 [b] ± 0.11

Data is shown as mean ± SEM, n = 10, Multiple comparisons were made using ANOVA one-way test followed by Tukey-Kramer as a post-hoc test. A: Certainly different from control $p < 0.05$. B: Significantly different from diabetic $p < 0.05$.

Table 2. Mean values of liver enzymes in different experimental groups

Groups	AST Mean ± SD (IU/L)	ALT Mean ± SD (IU/L)	ALP Mean ± SD(IU/L)
Group I (Control)	89.24 ±4.1	36.18 ±2.3	71.22 ±6.2
Group II (Diabetics)	152.16 [a] ±8.2	74.14 [a] ±3.4	162.32 a ±8.4
Group III (Rutin)	125.99 [a,b] ± 1.06	59.39 [a,b] ± 0.016	115.86 [a,b] ± 0.43
Group IV (Sitagliptin)	112.15 [a,b] ± 0.49	51.55 [a,b] ± 0.031	89.44 a,b ± 0.21
Group V (Rutin+ Sitagliptin)	94.12 [b] ±4.1	40.18 [b] ±2.6	78.28 [b] ±9.2

Data is shown as mean ± SD, n = 10, Multiple comparisons were made using ANOVA one-way test followed by Tukey-Kramer as a post-hoc test. A: Certainly different from control $p < 0.05$. B: Significantly different from diabetic $p < 0.05$.

Table 3. Mean values of oxidative/ antioxidative markers in different experimental groups

Groups	MDA (nmol/g protein)	GSH (µmol/g protein)
Group I (Control)	18.62 ±2.4	0.28 ±0.02
Group II (Diabetics)	42.18 [a] ±3.6	0.12 [a] ±0.02
Group III (Rutin)	30.99 [a,b] ± 1.06	0.19 [a,b] ± 0.016
Group IV (Sitagliptin)	26,14 [a,b] ± 0.49	0.20 [a,b] ± 0.031
Group V (Rutin+ Sitagliptin)	20.16 [b] ±1.8	0.22 [b] ±0.01

Data is shown as mean ± SD, n = 10, Multiple comparisons were made using ANOVA one-way test followed by Tukey-Kramer as a post-hoc test. A: Certainly different from control $p < 0.05$. B: Significantly different from diabetic $p < 0.05$.

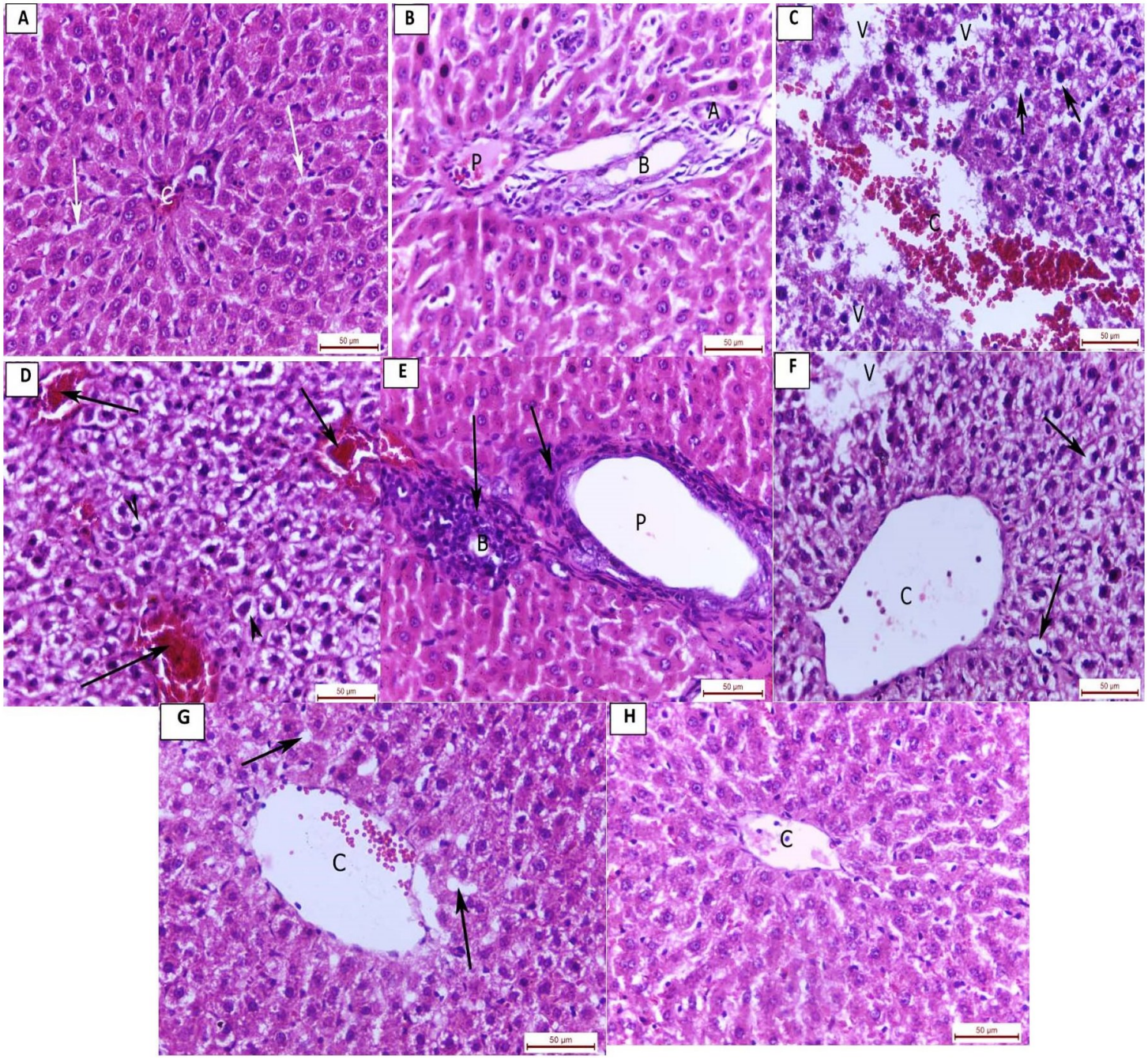
Figure 1. A photomicrograph of sections of liver **A, B** (control), **A:** hepatic lobules and central vein (C); **B**: the portal triad consisting of branch of portal vein (P), bile ductule (B) and branch of hepatic artery (A). C , D & E (diabetic) **C**: loss of hepatic architecture with marked dilatation of central vein (C), cytoplasmic vacuolations in the hepatocytes (arrows) and vacuoles (V) in between the hepatocyte. **D**: dilatation of blood sinusoids (arrows) and degeneration of hepatocyte (arrow head), **E**: marked congestion of portal vein (P) with mononuclear cell infiltration (arrows). **F** (diabetic and rutin treated): marked dilatation of central vein (C) and vacuolated areas (V). **G** (diabetic and Sitaglip**tin** treated): dilatation of central vein with minimal vacuolation (arrows).**H** (diabetic + rutin+ Sitagliptin treated): minimal dilatations of central vein. (H&E X400)

Figure 2. Photomicrographs of sections of liver **A** (control): minimal amount of collagen deposition (arrow) around the central vein. **B** (Diabetic): increased collagen fibers deposition (arrows) in the portal area. Note the marked engorged portal vein (P). **C** (diabetic and rutin treated): increased collagen fibers deposition (arrows). **D** (diabetic and Sitagliptin treated): moderate collagen fibers deposition (arrows). **E** (diabetic + rutin + Sitagliptin treated): minimal collagen fibers deposition (arrows) **F:** area Percentage of collagen content in liver following administration of Rutin and/or Sitagliptin to diabetic

Rats. *: Significantly different from control at $p < 0.05$. #: Noticeably different from diabetic $p < 0.05$. (Masson's trichrome X 400)

Figure 3. Photomicrographs of sections of liver **A (**control): strong positive PAS reaction of the hepatocyte around the central vein (C), **B (**Diabetic): weak positive PAS reaction of the hepatocyte. **C (**diabetic and rutin treated): moderate positive PAS reaction of the hepatocyte. **D (**diabetic and Sitagliptin treated): moderate positive PAS reaction of the hepatocyte. **E (**diabetic + rutin+ Sitagliptin treated): strong positive PAS reaction of the hepatocyte around the central vein (C). **F:** optical density in liver following administration of Rutin and/or Sitagliptin to diabetic Rat.*: Significantly different from control at $p < 0.05$. #: Noticeably different from diabetic $p < 0.05$ (PAS X400).

Figure 4. Photomicrographs of sections of liver **A (**control): localized positive immune-reactivity around central vein (arrows) and negative immune-reactivity in other parts, **B & C (**diabetic): strong positive immune-reactivity. **D (**diabetic and rutin treated group): positive immune-reactivity. **E (**diabetic and Sitagliptin treated): moderate positive immunoreactivity. **F (**diabetic + rutin+ Sitagliptin treated): weak positive immunoreactivity (arrows). **G:** area % of α–SMA in liver following administration of Rutin and/or Sitagliptin to diabetic Rats. *: Significantly different from control at $p < 0.05$. #: Noticeably different from diabetic $p < 0.05$ (α-SMA X 400).


A
C
50 µm
B
A
P
B
50 µm
C
V
V
C
V
50 µm
D
V
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E
P
B
50 µm
F
V
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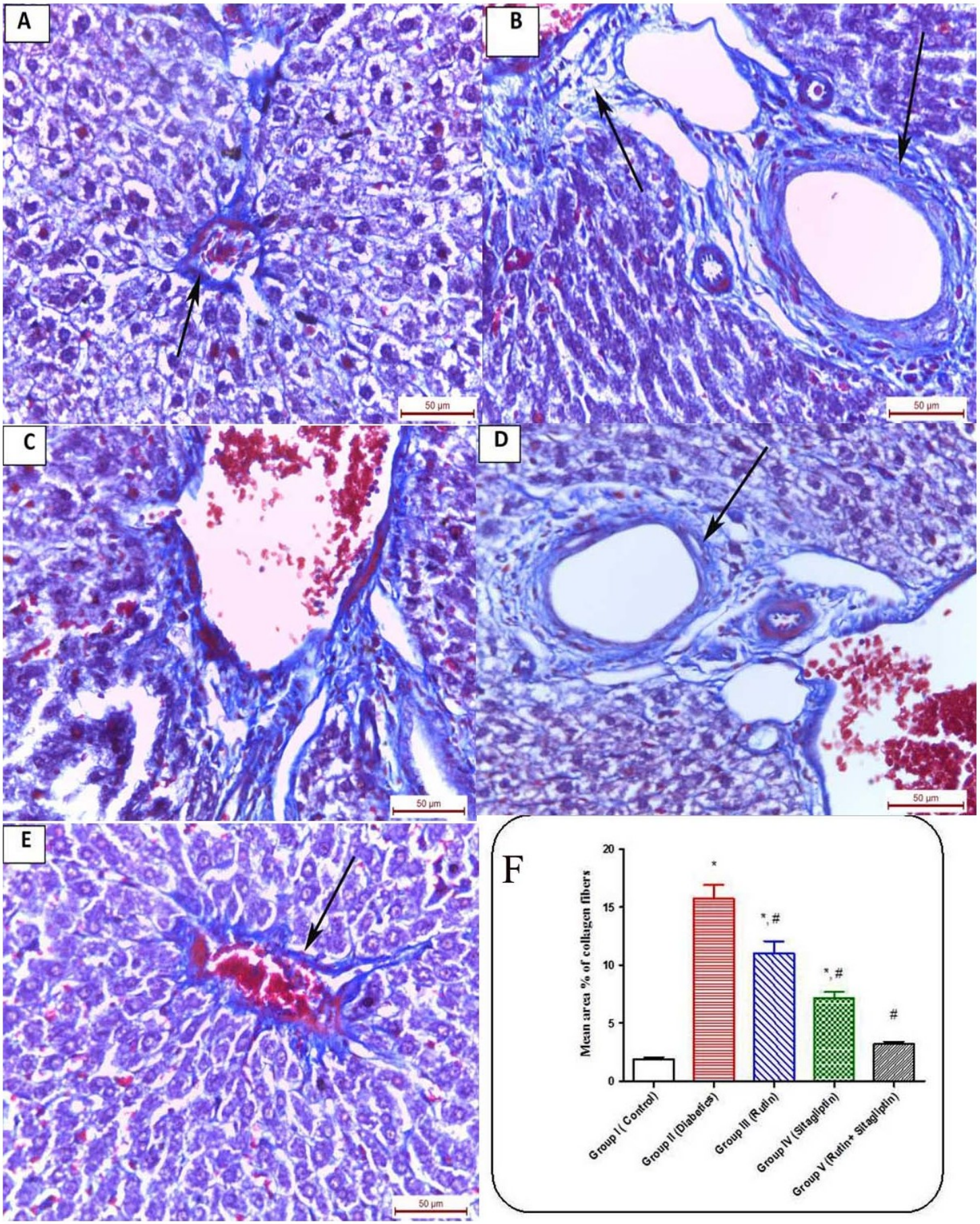
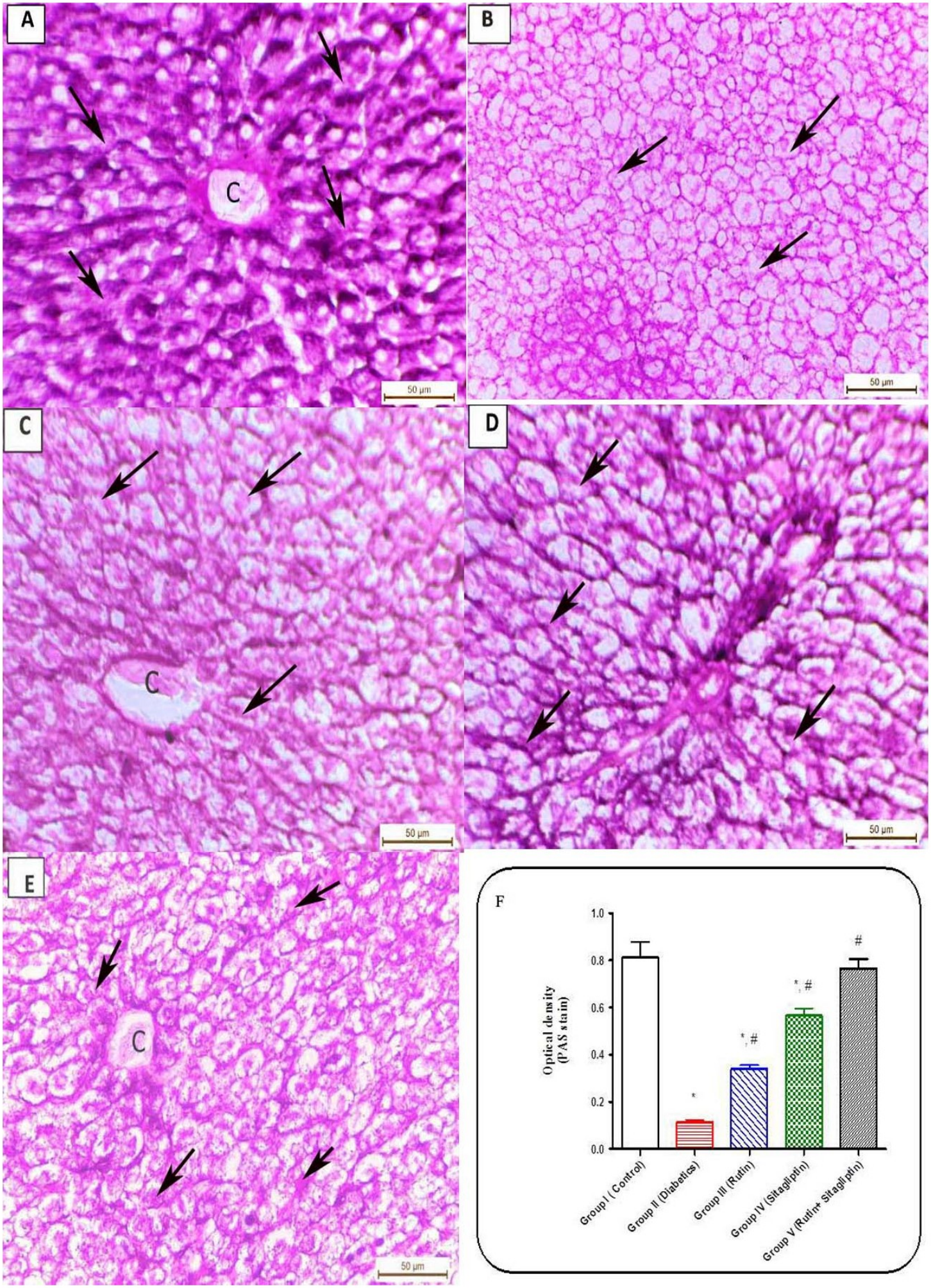
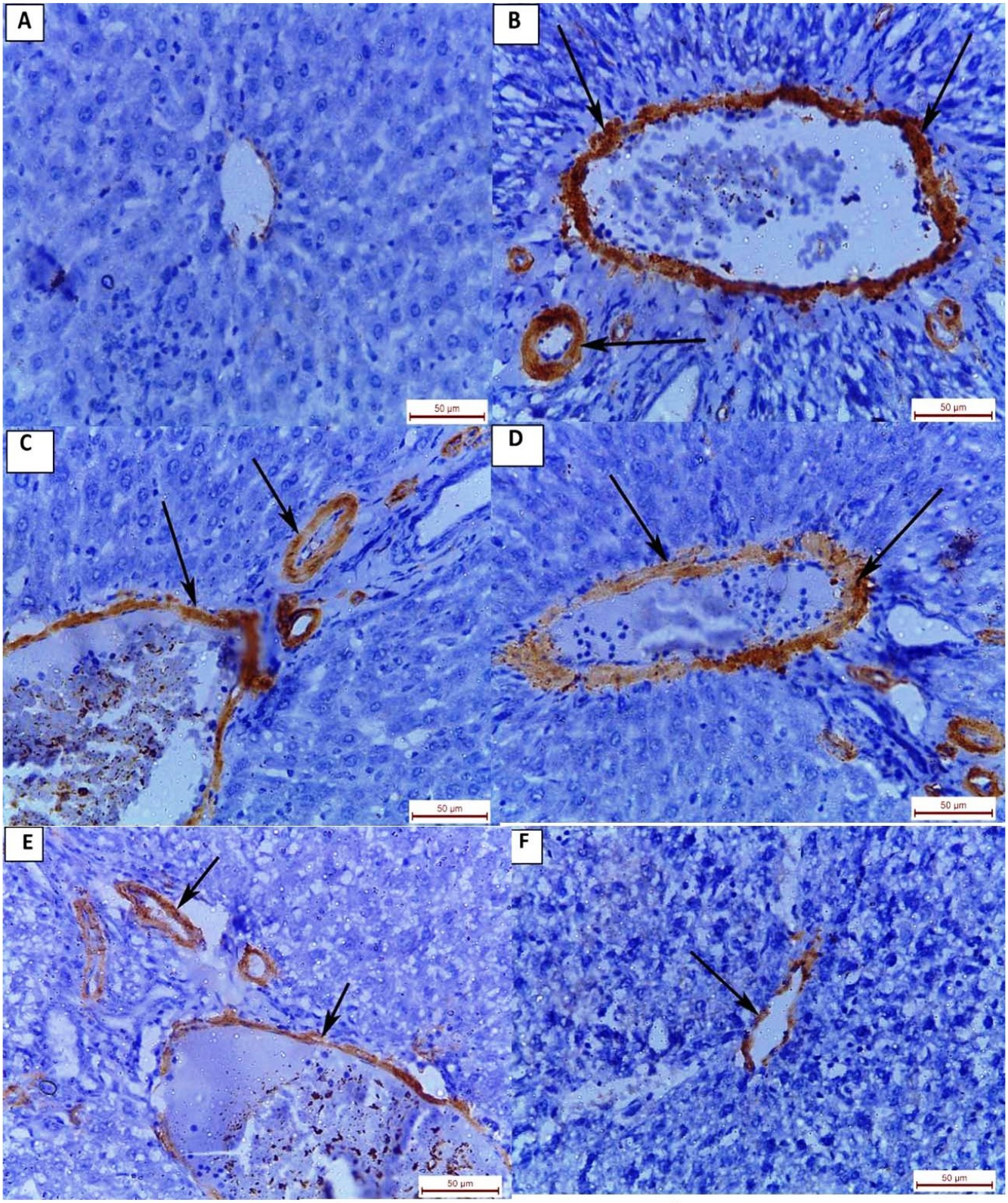
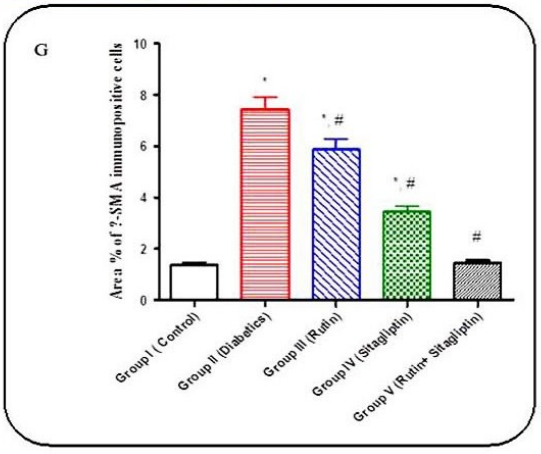

A
B
C
D
E
F
50 µm
Mean area % of collagen fibers
20
15
10
5
0
*
*, #
*, #
#
Group I (Control)
Group II (Diabetics)
Group III (Rutin)
Group IV (Sitagliptin)
Group V (Rutin+ Sitagliptin)


A
C
50 µm
B
50 µm
C
C
50 µm
D
50 µm
E
C
50 µm
F
Optical density (PAS stain)
1.0
0.8
0.6
0.4
0.2
0.0
*
*, #
*, #
#
Group I (Control)
Group II (Diabetics)
Group III (Rutin)
Group IV (Sitagliptin)
Group V (Rutin+ Sitagliptin)


A
B
C
D
E
F
50 µm


G
Area % of ?-SMA immunopositive cells
10
8
6
4
2
0
*
* #
* #
#
Group I (Control)
Group II (Diabetics)
Group III (Rutin)
Group IV (Sitagliptin)
Group V (Rutin+ Sitagliptin)