International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 4, 2016

Original Article

ANTIOXIDANT THERAPEUTIC ACTIONS OF MEDICINAL PHYTOCHEMICALS, SILYMARIN, AND SILIBININ, ON STREPTOZOTOCIN DIABETIC RATS: FIRST NOVEL COMPARATIVE ASSESSMENT OF STRUCTURAL RECOVERIES OF HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES ON ISLETS OF LANGERHANS, BETA CELLS, MITOCHONDRIA AND NUCLEUS

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Received: 15 Dec 2015 Revised and Accepted: 11 Feb 2016

ABSTRACT

Objective: We studied correlation between antioxidant properties of Silymarin and Silibinin and the restoration and recovery of normal structure of islets of Langerhans β-cells and mitochondria in Streptozotocin-diabetic rats.

Methods: Rats rendered diabetic and one week after diabetes induction, rats received orally Silymarin or Silibinin (100 mg/Kg). Biochemical parameters: glucose, HbA1c, lipid profile, hepatic SOD, GSH and MDA were determined. Furthermore, changes in islets of Langerhans, β-cells as well as mitochondria were recorded using electron microscope.

Results: 1) Silymarin and Silibinin treatment(s) of STZ-diabetic rats can correct and reverse the imbalance between ROS and antioxidant defense by restoring and augmentation of its capacity by significantly increasing SOD, GSH and modulating lipid peroxidation (by significantly decreasing MDA). 2) We provided evidence, using EM technique, to prove that hypoglycemic/antidiabetic/therapeutic actions of both Silymarin and Silibinin (by improving significantly both glucose and insulin levels), may be due to their ability to stimulate β-cells to secrete insulin through restoring antioxidant endogenous properties and hence, recovery of intact insulin secretory granules as well as restoration of normal structure of pancreatic endocrine cell islets of Langerhans. 3) We provided first novel comparative assessment of histological and ultrastructural changes on islets of Langerhans and β-cells as well as complete mitochondrial recovery by oral Silymarin and Silibinin treatment (s) in diabetic rats.

Conclusion: Both agents show Hypoglycemic, hpolipidemic and antioxidant properties, exhibit structural recovery of mitochondria, intact insulin secreting granules and nucleus, they act as anti-mitochondrial loss and/or dysfunction in diabetes.

Keywords: Silymarin, Silibinin, Diabetes, Antidiabetic agents, Antioxidants, Electron Microscope, Mitochondrial and β-cells Recovery

INTRODUCTION

Diabetes mellitus represents the most common endocrine disorder. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism [1]. In addition to hyperglycemia, hyperlipidemia are involved as well in diabetes complications [2], which leads to premature atherosclerosis. Thus, the ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile [3]. Moreover, oxidative stress (OS) induces tissue damage, as well as inactivates proteins, which are considered to be transitional mechanisms for the complications of diabetes [4], including coronary heart disease, nephropathy, and retinopathy [5, 6]. Thus, hypoglycemic compounds with antioxidant properties could be valuable as antidiabetic agents [7].

Some hypoglycemic drugs have inadequate efficiency and different adverse effects [8, 9]. Recently, scientific interests have focused on the use of antioxidants such as flavonoids to reduce the harmful effects of OS in diabetes mellitus [10]. They have been shown to exhibit diverse biological activities such as anti-cancer, antioxidant, anti-inflammatory, antimicrobial, anti-allergic, antimalarial properties [11-13]. Among these flavonoids, Silymarin, selected for the present study, is obtained from the fruits of *Silybum marianum L. Gaertner* [14, 15]. Silymarin composed of at least seven flavonolignans (Silibinin, silychristin, silydianin, isosilybin A, isosilybin B and isosilychristin) in addition to the flavonoid, taxifolin [16]. Silibinin is the most abundant compounds in Silymarin [17]. Silymarin and Silibinin are believed to act as antioxidant, anti-inflammatory and anti-fibrotic agents [18]. Several studies have shown that Silymarin has hypoglycemic properties [19], which supported its use as a diabetic compound.[20, 21] Nevertheless, the antihyperglycemic mechanism of Silymarin and Silibinin is not entirely understood [22].

The presented study was carried out to investigate the correlation between Silymarin and its main component, Silibinin, actions as antidiabetic agents and their antioxidant effects on islets of Langerhans using electron microscope technique on intoxicated rats with STZ as a diabetes mellitus model. Moreover, carrying out the first assessment of the amoriliation of the mitochondrial status after the administration of Silymarin and Silibinin.

MATERIALS AND METHODS

Chemicals

Silymarin and Silibinin were purchased from Sigma Chemical Co. (St. Louis, USA). Streptozotocin (STZ), thiobarbituric (TBA), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), NADH, NADPH were purchased from Sigma chemical Company (St. Louis, MO, USA). All other chemicals were of the best available analytical grade.

Experimental animals

Adult male albino rats with an average body weight of 100 to 120 g were used in all experiments and purchased from Theodore Bilharz Research Institute, Egypt. Procedures involving animals and their care were conducted in conformity with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals, Faculty committee approval number as follows 599-20th June 2013

Induction of experimental diabetes

The rats were fasted for 12 h before intraperitoneal injection with freshly prepared solution of STZ (60 mg STZ/kg body weight) [23, 24]. Rats with serum glucose higher than 250 mg dL^{-1} were selected as diabetics for the following experiments. The day on which

hyperglycemia had been confirmed was designated as day 0. The rats were randomly allocated and similarly grouped into five groups (ten in each):

Group 1: Control rats, received 1.0 ml physiological saline solution.

Group 2: Silymarin-treated control rats, received 100 mg Silymarin/kg body weight.

Group 3: Diabetic control, received 1.0 ml physiological saline solution.

Group 4: Silymarin-treated diabetic rats, received 100 mg Silymarin/kg body weight.

Group 5: Silymarin-treated diabetic rats, received 75 mg Silymarin/kg body weight.

Group 6: Silibinin-treated diabetic rats, received 75 mg Silibinin/kg body weight.

The daily oral administration of Silymarin and Silibinin dissolved in distilled water was performed at 24 h interval, using metal cannula attached to a 1 ml syringe.

Preparation of tissue homogenates

An accurately weighed piece of liver tissue from each rat was homogenized in ice-cold 0.9 % saline solution using a homogenizer motor to yield 5 % (w/v) liver homogenate. The homogenate was centrifuged at 5000 rpm for 30 min at 4 °C to remove cell debris and nuclei.

Biochemical analysis

Fasting serum glucose concentrations were estimated according to *Trinder* [23] using a commercial kit (SPINREACT, Spain]. Hepatic reduced glutathione (GSH) content was estimated according to *Beutler et al.* [24]. Glycosylated hemoglobin level was estimated according to *Hanas et al.* [25] using a commercial kit (Biosystem S. A., Spain). Hepatic lipid peroxidation was estimated through measuring the malondialdehyde (MDA) production according to the method of *Draper and Hadley* [26]. Hepatic superoxide dismutase (SOD) activity was determined using the method of *Nishikimi* [27]. In addition, lipid profile including: serum total cholesterol [28], triglyceride [29], were spectrophotometrically measured using commercially available kits (SPINREACT, Spain) and the serum HDL-cholesterol level was estimated according to the method as described by *Grove* [30]. The VLDL-cholesterol and LDL-cholesterol levels were estimated by computation, according to the methods and formula described by *Friedewald* [31].

Statistical analysis

Data analysis was performed using the statistical package for the social sciences software, release 15.0 for Windows (SPSS version 15.0, Chicago: SPSS Inc). All results were expressed as mean±SD. A P-value<0.05 was considered statistically significant. Statistical analysis was performed by analysis of variance (ANOVA) with LSD and Dunnett's test for PostHoc.

Histological stain

The rats' pancreas was dissected out from each group and were kept in 10% neutral formalin and processed to be paraffin blocks. Sections of five µm thick sections were prepared and stained with hematoxylin and eosin for light microscopic examination [32].

Ultrastructural studies

The pancreas tissues were fixed in 2.5% glutaraldehyde buffered in 0.1 M cacodylate buffer (pH 7.2) at 4 °C for 2 h, then post-fixed in 1% cold osmium tetraoxide in 0.1M cacodylate at pH 7.2 for 3 h. Ultrathin sections were obtained from specimens embedded in Lowicryl K4M resin after dehydration through graded ethanol series, substitution, and polymerization at–20 °C. Ultrathin sections were obtained using an Ultracut S microtome (Leica, Vienna, Austria). Sections were mounted on 400 mesh collodion-carbon-coated nickel grid (33] and examined with a Joel Electron Microscope (JAPAN) operating at 60 kV.

RESULTS

Biochemical effect of silymarin

Fasting blood glucose levels in all groups after four weeks of treatment have been demonstrated in table 1. There was no significant change in the mean serum level of glucose in control treated with Silymarin (100 mg/kg body weight) when compared with normal control group (P>0.05). A highly significant increase in fasting blood glucose level is observed in diabetic control rats compared with normal control rats. In contrast, a highly significant decrease in the blood glucose level is observed in STZ diabetic rats-treated with *Silymarin* (100 mg/kg body weight) as compared to the diabetic control group.

Table 1: Effect of four weeks treatment with Silymarin on fasting blood glucose and Glycosylated hemoglobin A1c of normal and STZ-diabetic rats

Parameter	Normal control	Control treated with Silymarin (100 mg/Kg/4 w)	Diabetic Control	Diabetic treated with Silymarin (100 mg/Kg/4 w)
Fasting blood glucose, mg/dl	100.08±13.94	86.53±10.69^{NS}	252.88±30.27*	92.41±10.691**
Glycosylated hemoglobin, %	5.73±1.55	5.43±0.38^{NS}	8.24±2.51*	5.1±0.71**

The results are expressed as mean±SD for five rats in each group., *Highly significant compared to normal control groups., **Highly significant compared to diabetic control groups., ^{NS}not significant.

In the STZ-diabetic untreated group, glycosylated hemoglobin (HbA1C) levels were significantly increased when compared to the normal control group. While the diabetic rats treated with Silymarin (100 mg/kg) showed a significant decrease in glycosylated hemoglobin level when compared to the diabetic control group (table 1). In addition, there was no significant change in the mean value of blood HbA1C level in control treated with Silymarin (100 mg/kg body weight) when compared with the normal control group.

Table 2: Effect of four weeks treatment with Silymarin on superoxide dismutase (SOD) activity, reduced glutathione (GSH) and malondialdehyde (MDA) contents in the liver of normal and STZ-diabetic rats

Parameter	Normal control	Control treated with Silymarin (100 mg/Kg/4 w)	Diabetic Control	Diabetic treated with Silymarin (100 mg/Kg/4 w)
SOD, U/g wet tissue	243.16±50.07	247.0±28.62^{NS}	182.44±17.22*	248.0±40.38**
GSH, mmole/g wet tissue	4.625±0.607	3.932±0.722^{NS}	2.37±0.455*	3.617±0.355**
MDA, nmole/g wet tissue	1.13±0.113	0.984±0.142^{NS}	1.47±0.248*	0.842±0.161**

The results are expressed as mean±SD for five rats in each group., *Highly significant compared to normal control groups., **Highly significant compared to diabetic control groups., ^{NS}Not significant.

Table 2 represents reduced glutathione (GSH) levels; malondialhyde (MDA) contents and Superoxide dismutase (SOD) activity in the liver of different groups. No significant changes were observed in the contents of hepatic GSH, SOD and MDA of the normal group treated with Silymarin (100 mg/kg body weight) when compared with normal control group. However, the hepatic contents of SOD, GSH in STZ-diabetic group were significantly depleted. Moreover, the hepatic content of MDA of STZ-diabetic group was significantly increased compared to healthy control rats. Furthermore, a significant increase in the content of hepatic GSH, SOD and a significant decrease in the level of MDA were found in diabetic rats group treated with Silymarin (100 mg/kg) when compared with the diabetic-control group.

The serum lipid profiles, including: triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, and VLDL-cholesterol contents as well as the atherogenic index of the different experimental rat groups were shown in table 3. A highly significant decrease found in total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol contents, atherogenic index but a significant increase in HDL-cholesterol content was observed in sera of STZ-diabetic rats treated with Silymarin (100 mg/kg body weight) when compared to STZ-diabetic control group. On the other hand, a significant decrease was observed in the level of total cholesterol, triglycerides, LDL-cholesterol, and VLDL-cholesterol contents in addition to atherogenic index of normal rats treated with Silymarin (100 mg/kg body weight) when compared to the normal control group.

Table 3: Effect of four weeks treatment with Silymarin on total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol contents in the serum of normal and STZ-diabetic rats

Parameter	Normal control	Control treated with silymarin (100 mg/Kg/4 w)	Diabetic Control	Diabetic treated with silymarin (100 mg/Kg/4 w)
Cholesterol, mg/dl serum	48.05±5.92	37.02±4.93*	66.66±8.13*	56.90±6.71**
Triglycerides, mg/dl serum	31.90±3.6	21.02±3.94*	40.90±5.8*	31.16±6.35**
HDL-Cholesterol, mg/dl serum	5.5±0.115	6.56±0.529*	4.35±0.773*	6.35±761**
LDL-Cholesterol, mg/dl serum	36.17±6.2	26.26±4.9*	54.14±8.2*	44.31±7.2**
VLDL-Cholesterol, mg/dl serum	6.65±0.52	4.20±0.78*	8.18±1.16*	6.23±1.26**
Atherogenic index	7.9±0.933	4.7±1.02*	14.8±4.1*	8.18±1.4**

The results are expressed as mean±SD for five rats in each group., *Highly significant compared to normal control groups., **Highly significant compared to diabetic control groups.

Histopathological examination

Histopathological examination of the pancreas of normal control rats showed a normal lobular architecture of the pancreas. The pancreas had abundant islet of Langerhans interspersed between the pancreatic exocrine acini. The islets appeared lightly stained than the surrounding acinar cells, with intact interlobular connective tissue and interlobular duct. The islet boundaries are clear, and the profiles of the islet cells are obviously visible. Each islet contained lightly stained polygonal cells arranged in cords separated by a network of blood capillaries. The overall architecture was found to be normal and healthy as illustrated in fig. (1a).

In contrast to control rats, pancreatic islets of rats injected with STZ (60 mg/kg), after four weeks they were atrophied and/or damaged. Islet of Langerhans cell's size was found to be shrunk having severe architectural disarray or damage, which was observed accompanied by the absence of islets cells. Typical cellular integrity was totally lost. Other cells of some islets showed cytoplasmic vacuolation. The cell disarrangement and architectural damage seemed to be due to the action of STZ as shown in fig. (1c).

Diabetic groups treated with Silymarin (100 mg/kg) for four weeks showed almost similar prominent recovery in the previous morphological changes in most of the rats, the border between exocrine and endocrine portions became more distinct. Many islets exhibited an increase in the cellular density along with a reduction in the inflammatory cells infiltration inside the islet as displayed in fig. (1d).

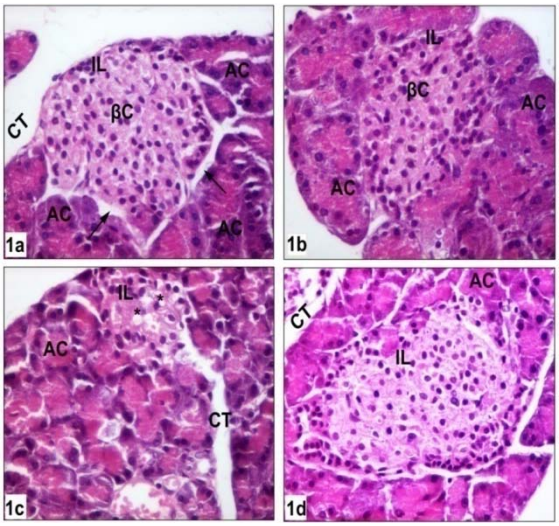

Fig. 1: Photomicrograph of thick H & E stained paraffin section from the pancreas of rat

(a) Control pancreas showing normal structure of (IL) islets of Langerhans and (βC) β-cells, (AC) acinar cell, (arrows) borders between the endocrine and exocrine region are distinct and (CT) interlobular connective tissue. (b) Normal rat treated with *Silymarin* (100 mg/kg/4 weeks) showing normal pancreatic architecture of (IL) islets of Langerhans and (βC) β-cells, (AC) acinar cell. (c) STZ treated rat showing degeneration and shrinkage of islets of Langerhans illustrated in islet amyloidosis with atrophied Islet's β-cells (arrowhead), (*) cytoplasmic vacuoles, (CT) interlobular connective tissue, (AC) acinar cells. (d) Diabetic rat treated with *Silymarin* (100 mg/kg/4weeks) displaying almost intact structure of the pancreas and restoration of pancreatic endocrine cells (IL) islets of Langerhans, (AC) acinar cell, and (CT) interlobular connective tissue. All photos X 400.

Investigation of Islets of Langerhans's structure revealed great improvement and appearance of healthy Islets construction compared to control group. Also, examination of the pancreas of diabetic groups treated with Silibinin (75 mg/kg) for two weeks illustrated that the architecture of cells observed in good health almost like that of the previous groups treated with Silymarin (75 mg/kg) for two weeks. The acinar cell arrangements were found to be nearly like normalized sufficiently compared to the previous control group as shown in figs. (2d, e).

These results suggested that both Silymarin and Silibinin with both doses effectively repaired the damaged pancreatic islets in the STZ-induced diabetic rats, however; the seniority as well as superiority-was with Silibinin (75 mg/kg) dose, which revealed best results in two weeks only.

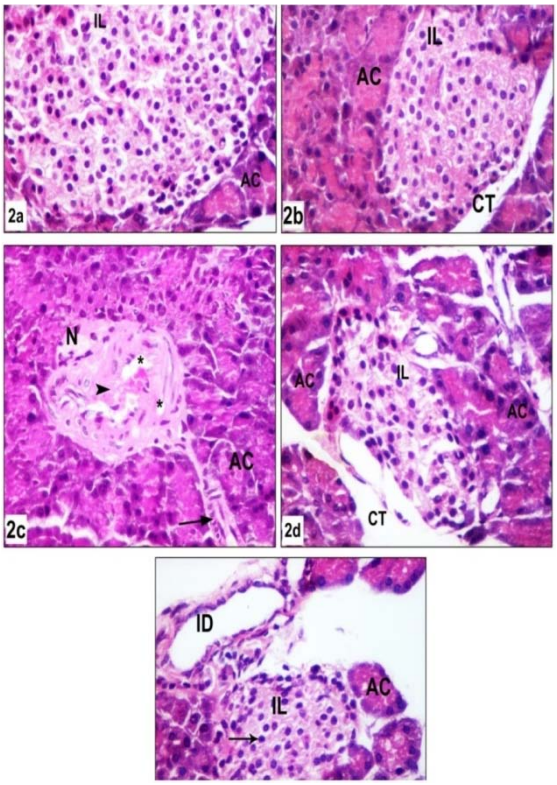

Fig. 2: Photomicrograph of thick H & E stained paraffin section from the pancreas of rat

(a) Control pancreas showing normal structure of (IL) islets of Langerhans and (AC) acinar cell. (b) Normal rat treated *Silymarin* (75 mg/kg/2weeks) showing normal pancreatic architecture, of (IL) islets of Langerhans, (CT) interlobular connective tissue and (AC) acinar cell. (c) STZ treated rat showing degeneration and shrinkage of Islets of Langerhans illustrated in Islet amyloidosis with atrophied Islets β-cells (arrowhead), (*) cytoplasmic vacuoles, (N) necrotic area and (AC) acinar cells. (d) Diabetic rat treated with *Silymarin* (75 mg/kg/2 weeks) rat displaying almost intact structure of the pancreas and restoration of pancreatic endocrine cells (IL) islets of Langerhans, (AC) acinar cell, and (CT) interlobular connective tissue. (e) Diabetic rat treated with *Silibinin* (75 mg/kg/2weeks) illustrated recovery of major structure of Islet β-cells, (arrow) β-cells, (AC) acinar cells, (ID) interlobular duct and (IL) islets of Langerhans. All photos X 400

Ultrastructural studies using electron microscope

Examination of specimens obtained from the control animals showed the endocrine β-cell with dispersed chromatin in its nucleus and mitochondria, and granules have dark central core surrounded by an electron lucent halo, numerous cisternae of RER and well developed Golgi apparatus, and that were displayed in fig. (3a). β-cells were identified by the presence of a round core of the vesicles with the surrounding limiting membrane. The nucleus showed normal ultrastructural morphology with the chromatin adjacent to the nuclear membrane and the presence of a few clear matrixes (figs. 3a, b).

The cytoplasm of β-Cells Islets of Langerhans of control rats contains numerous electron-dense secretory granules surrounded by wide lucent halo, mitochondria, Golgi apparatus and euchromatic nucleus. As STZ is a specific drug for pancreatic β-cells destruction, STZ treated group showed remarkable damage of them without affecting α-cells (fig. 3c), which illustrated the great damage of almost all β-cells components including mitochondria, nucleus, empty secretory granules of insulin and dilated endoplasmic reticulum at the contrary were observed in α-cells of the same islets which were completely intact with no damage.

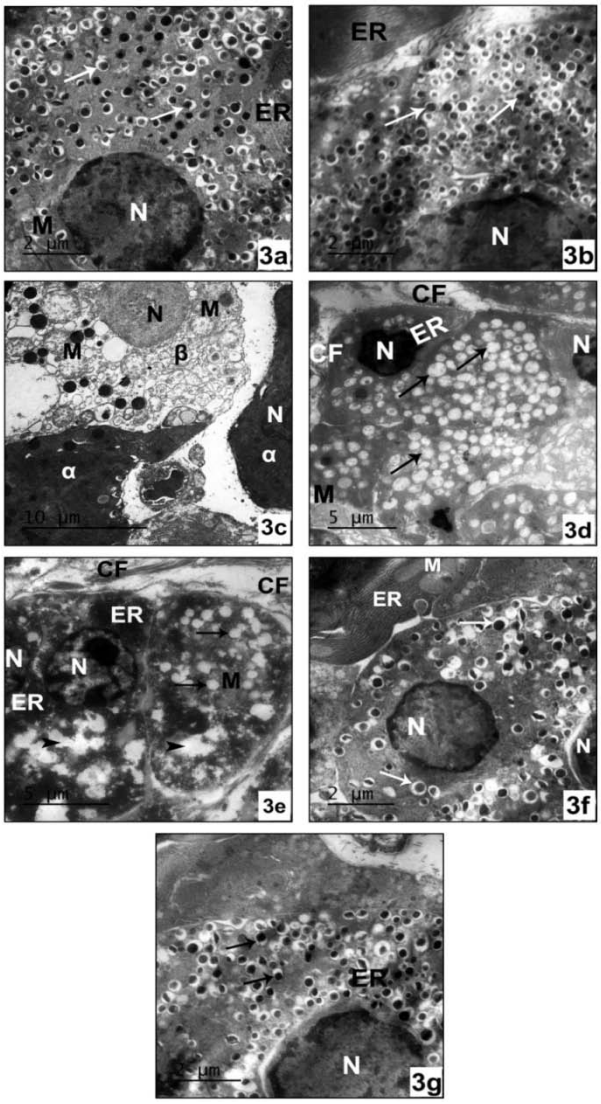

Fig. 3: Electron photomicrograph of pancreatic β-cell

(a, b) control group, *Silymarin* (75 mg/kg/2 weeks) treated groups respectively showing normal structural components; (N) nucleus, (M) mitochondria, (arrows) insulin secretory granules and (ER) endoplasmic reticulum.(c) STZ treated group displaying intact appearance of (α) α-cells, destruction of nearly all β-cell structures, (M) atrophied mitochondria, (N) damaged nucleus of β-cell and healthy one of α-cells, (d) STZ treated group showing (N) Pyknotic nucleus, (M) atrophied mitochondria, (arrows) empty secretory granules, (CF) collagen fibers and (ER) dilated endoplasmic reticulum. (e) STZ treated group illustrated (N) shrinking nucleus,

(M) atrophied mitochondria, (ER) dilated endoplasmic reticulum, (arrows) necrotic areas, (arrowheads) empty secretory granules, (CF) collagen fibers. (f, g) *Silymarin* (75 mg/kg/2weeks) and *Silibinin* (75 mg/kg/2weeks) treated groups respectively showing great amelioration in most β-cell structures illustrated in; (N) normal nucleus, (M) mitochondria, (arrows) intact insulin secretory granules and (ER)endoplasmic reticulum.

Specimens obtained from diabetic group revealed endocrine β-cells of islet-containing vacuolated mitochondria, lost their cristae and increased in the halo spaces areas around β-granules (fig. 3d). Most nuclei show progressive aggregation of chromatin. The diabetogenic action of Streptozotocin has been attributed to the irreversible injure caused to the islet β-cells. Other endocrine β-cells of islets revealed the presence of many β-granules with increase electron lucent halo spaces surrounded the dense core. Vacuolated and damaged mitochondria dilated cisternae of rough endoplasmic reticulum (fig. 3e), some electron lucent vacuoles were also noticed, and the presence of autophagic vacuoles. Other β-cell showed nucleus with euchromatin predominant over the heterochromatin and decrease or even depletion of β-granules.

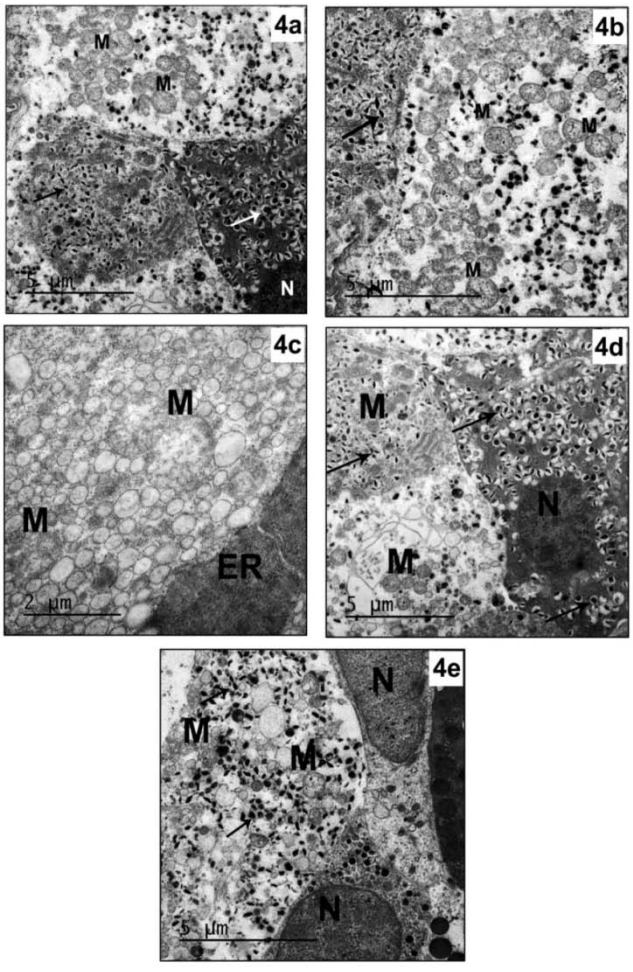

Fig. 4: Electron photomicrograph of pancreatic β-cell containing mitochondria

(a, b) the control group and *Silymarin* (75 mg/kg/2weeks) treated groups respectively showing a normal mitochondrial structure with double outer and inner membrane (M) mitochondria, (N) nucleus and (arrows) insulin secreting granules. (c) STZ treated group displaying (M) destructed mitochondria and (ER) endoplasmic reticulum. (d, f) *Silymarin* (75 mg/kg/2 weeks) and *Silibinin* (75 mg/kg/2 weeks) treated groups respectively illustrated recovered mitochondrial almost like healthy structure of control group, (M)mitochondria, (N) nucleus,(arrows) insulin secreting granules

Pancreatic islets showed advanced fibrosis, which originated in the surrounding connective capsule, with massive collagen fascicles that intruded among cells (fig. 3d and e) and blood capillaries, isolating and in some areas, destroying them. Necrotic β-cells were also detected (fig. 2e). There were a massive lipid droplets proliferation and pyknotic nuclei (fig. 2d). In contrast to the diabetic group, there was other diabetic group treated with Silymarin with, (75 mg/kg) and one treated with Silibinin (75 mg/kg) revealed ameliorative action compared to the diabetic group.

Endocrine β-cell apparently had retained their morphology and cellular organelles. Also, normal shaped appearance of mitochondria uniformly spread in the cytoplasm, and so were RER. There were marked decreased vacuolation in many islets. There was an obvious improvement in endocrine β-cells secretory granules and euchromatin containing nuclei. There were no collagen infiltrations or lipid droplet proliferation. Only very few cells had medium electron-dense cytoplasm. Notably, there were no cells with electron-dense cytoplasm or hyperchromatic nuclei. The synthesis of insulin was active, similar with that of the control group (fig. 3f, g).

Ultrastructural investigation of mitochondrial

Ultrastructurally, the cytoplasm showed a granular appearance. There were numerous rounded and elongated mitochondrial profiles. The mitochondrial matrix was electron–dense, and occasionally contained extremely long cristae and more than a few electron-dense granules. Mitochondria with normal size were round or oval in their typical construction; normal ultrastructure was also observed as showed in fig. (4a).

Silymarin treated group also illustrated usual intact structure alike that of the control group (fig. 4b). One of the most striking changes was the breakdown of the intramitochondrial limiting membrane with its cristae in STZ treated group. In addition, the mitochondrial matrices lost its electron density. Other mitochondria were found completely broken down, and its internal granular material discharged into the cytoplasm. The later tiny flocculent materials give the cytoplasm its granular appearance (fig. 4c). Mitochondria were considerably large, and degenerative changes were noticed within some of them. Some mitochondria were found to be swollen with occasionally absence and/or loss of cristae.

Examination of Silymarin (75 mg/kg) and Silibinin (75 mg/ kg) treated diabetic groups revealed great improvement of most mitochondrial constructions, less degenerative and normal appearance in general (figs. 4d, e).

DISCUSSION

Diabetes mellitus is a clinical disease characterized by improper hyperglycemia caused by a relative or absolute deficiency of insulin.[34] Streptozotocin (STZ) induced hyperglycemia has been described as a useful experimental model to study the effect of antidiabetic agents against diabetes mellitus [35, 36]. Silymarin is a mixture of flavonolignans from the fruits of milk thistle *Silybum marianum* [37, 38]. Silibinin is a major active constituent of Silymarin [17]. The expanded hypoglycemic effect of Silymarin till two weeks may be attributed to its long efficacy on glucose uptake by the liver cells. Our study showed that the blood glucose of Silymarin treated group was significantly decreased when compared to initial blood glucose level. Our results are consistent with *Jadhav et. al* and *El-Far et. al* who demonstrated that Silymarin showed a significant decrease in the fasting blood glucose level in STZ-treated groups [39, 40].

In the diabetic control group, glycosylated hemoglobin was significantly increased, but in the treatment group, it was significantly decreased this is in consistency with others findings [41]. In diabetes mellitus, the increased blood glucose binds with the free amino groups of the N-terminals of the b-chain of the hemoglobin molecules leads to increase of glycosylated hemoglobin. This is a continuous and irreversible process which is a marker of metabolic control [42].

The disorders of lipid metabolism, which occur in diabetes mellitus, are considered to be effective diabetic complications [43]. This has

been observed in our study in which, in the diabetic group, decreased levels of serum HDL-cholesterol content and increased concentrations of triglycerides, total cholesterol, and LDL-cholesterol when compared to normal control group are shown, which increases the atherogenic index. These results are in consistence with those of other investigators [44]. The increase of the markers of lipid profiles in STZ-diabetic rats may be referred to the increase of the lipolysis process accompanied by the decrease of the rate of lipogenesis leading to release free fatty acids into the blood circulation [45]. In addition, insulin deficiency will lead to a decreased lipoprotein lipase activity and increased free fatty acids metabolism from peripheral fat depots [46]. The treatment with Silymarin significantly reverted the influence of STZ i. e improved lipid profiles as compared to STZ-diabetic rats. These results are in consistent with our previous studies using stem cells, which provided novel evidence of the possible restoration and augmentation of antioxidant defense, hypoglycemic plants and other natural products [40, 47-49].

The impairment of glucose utilization leads to the increase of the production of oxygen free radicals, especially reactive oxygen species (ROS), and the reduction of antioxidant levels, which play an important role in biochemical disturbances in oxidative defense system, lipid peroxidation and leading to diabetic complications [7]. In fact, it was observed that STZ injection produced a significant decrease in hepatic superoxide dismutase (SOD) activity in comparison to the normal control group. The decreased SOD level in diabetic rats could be referred to its consumption in the conversion of superoxide anions into hydrogen peroxide to protect the cell from the harmful effect of superoxide anions. Additionally, the decrease of SOD activity may be due to increase of glycosylated SOD that leads to the inactivation of this enzyme [50]. Moreover, the administration of Silymarin (100 mg/Kg) restored the hepatic level of SOD. Our results indicated that, STZ injection produced a significant decrease in hepatic glutathione (GSH) content in comparison to the normal control group. It is well known that hyperglycemia indirectly causes GSH depletion, a sign of a direct reaction between GSH and free radicals generated by STZ, which leads to increase the oxidative stress [51].

We found that, STZ injection produced a significant increase in hepatic MDA content in comparison to the normal control group. This could be due to an increase in oxygen free radicals due to autoxidation of monosaccharides, which leads to the production of superoxide and hydroxyl radicals which react with polyunsaturated fatty acids in membrane and cause tissue damage [52]. Silymarin induced an increase in hepatic GSH content and decrease hepatic lipid peroxidation (MDA) and hence, improved glucose regulation because thiol groups are important in intracellular and membrane redox state [53].

Our results reinforced the previous recorded result which mentioned that diabetes caused extensive β-cell degranulation and decreased cellular density in pancreatic islets [54]. The pancreas sections of the diabetic rats, examined in our present study, showed alterations such as islets shrinkage, cellular swelling, β-cell vacuolation, and apoptosis, confirming previous findings [55]. Our diabetic animals which were treated with *Silymarin* or *Silibinin* showed an increase in the number of islets, lesser degree of shrinkage and necrosis of β-cells of the pancreas. This demonstrates that the decreased blood glucose is due to the released insulin from the regenerated pancreatic β-cells. Our data are consistent with the concept that *Silymarin* and *Silibinin* have the ability to lower blood glucose [40, 56-58].

Our results showed lots of histological changes in pancreas of diabetic rats, which were greatly supported by the ultrastructural outcome. Streptozotocin administration resulted in significant morphological changes in diabetic rats with severe injury of pancreatic β-cells, such as decreasing the islets cell numbers, cell damage, and cell death. The thickened and hyalinized blood vessels cause insufficient oxygen to reach the tissues which resulted in degenerative changes and necrosis [59]. In addition, the observed vacuoles may be attributed to increased cellular damage. Our present results showed dramatic changes in nuclei of islets of diabetic pancreas, some appeared vesicular, and others pyknotic nuclei where the euchromatin predominates over the heterochromatin. These changes may be explained in view of other study which suggested that these changes to be owing to condensation and shrinkage of the nuclear material [60, 61].

the Golgi apparatus, Ribosomes of the rough endoplasmic reticulum, and the secretory granules are actively involved in the synthesis and the secretion of proinsulin and insulin [62]. All these vital intracellular structures were affected thus inhibiting the synthesis and release of insulin. Specifically, It has been previously reported that decreased glucose stimulated insulin release may be associated with reduced insulin content, lower insulin granule amount, diminished islet insulin mRNA [63, 64].

The present study also demonstrated the presence of huge amounts of collagen fibers inside β-cell of STZ-induced diabetic group, which was in agreement with Pribac,[65] Our present novel investigation showed the great significant ameliorative action of Silymarin or Silibinin on STZ-induced diabetic rats illustrated in healthy β-cells with numerous insulin granules in the cytoplasm, endoplasmic reticulum, mitochondria and the Golgi complex were active and had a normal structure.

The normalization of hyperglycemia profiles may in part due to effects of Silymarin and Silibinin on β-cell regeneration and antioxidant properties. This may be explained on view of *Adeyemi et al.*, who suggested possible regeneration of β-cells, which is probably due to the fact that the pancreas contains stable (quiescent) cells which have the ability of regeneration [66].

In the present study, pancreatic β-cell of STZ-induced diabetic rats showed noticeable mitochondrial damaged appearance and this was in agreement with [67] who illustrated that hyperglycemia provokes generation of superoxide radicals in the endothelial cells of mitochondria and promotes evil sequence of oxidative reactions involved in diabetic complications development. Our present study showed that Silymarin or Silibinin can normalize excessive mitochondrial ROS through highly significant SOD activities, we reported here using both of them.

According to [68], overlong dysfunction of β-cell induced by acute oxidative stress illustrated with permanent injury of mitochondrial constituents associated with generation of mitochondrial endogenous ROS. ROS production includes various actions of respiratory enzymes and aerobic O2 consumption that are acting under physiological conditions. It was evident that elevated blood glucose level was involved in overproduction of ROS by means of mitochondrial electron transport chain during respiration [69-76].

CONCLUSION

The improvement of glucose level, lipid profile, SOD activity, GSH content and decreased lipid peroxidation recorded after Silymarin and Silibinin treatment of STZ-diabetic rats might suggest a treating influence of Silymarin and Silibinin against STZ action that might be mediated through neutralization of oxygen free radicals produced by STZ. In addition, Silymarin and Silibinin stimulate insulin secretion from β-pancreatic cells of rats. Therefore, the hypoglycemic action of Silymarin and Silibinin may be due to its ability to stimulate β-cells to secrete insulin and its antioxidant properties. Further clinical investigations will be conducted prior utilization as a safe oral hypoglycemic agent. The histopathological and ultrastructural studies undertaken on the islets demonstrated the recovery of damaged islets and an improvement in the β-cells after treatment with Silymarin and Silibinin. It can thus be assumed that Silymarin and Silibinin have a therapeutic effect that alleviates diabetes mellitus. We summarize our conclusions as follows: 1) Silymarin and Silibinin treatment(s) of STZ-diabetic rats can correct and reverse the imbalance between ROS and antioxidant defense by restoring and augmentation of its capacity by significantly increasing SOD and GSH as well as modulating lipid peroxidation (by significantly decreasing MDA). 2) We provided evidence, using EM technique, to prove that hypoglycemic/antidiabetic/therapeutic actions of both Silymarin and Silibinin (by improving significantly both glucose and insulin levels), may be attributed to their stimulation of β-cells for

insulin secretion via restoring antioxidant endogenous properties and hence, recovery of intact insulin secretory granules as well as restoration of normal structure of pancreatic endocrine cell islets of Langerhans. 3) We provided first novel comparative assessment of histological and ultrastructural changes on islets of Langerhans and β-cells as well as complete mitochondrial recovery by oral Silymarin and Silibinin treatment (s) in STZ-diabetic rats. Both agents exhibit structural recovery of mitochondria, intact insulin secreting granules and nucleus. All became like ordinary structures when compared to controls.

CONFLICT OF INTERESTS

Declared none

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