International Journal of Latest Research in Science and Technology **ISSN (Online):2278-5299**
Volume 7, Issue 1: Page No. 15 – 21 , January-February 2018
https://www.mnkpublication.com/journal/ijlrst/index.php

IMPROVEMENT OF IMPAIRED GLUCOSE TOLERANCE BY THE INCREASE IN SECRETION OF PANCREATIC GLP-1 INDUCED BY SILYMARIN IN RATS.

Claudia Soto[1*], Imelda González[2], Katia López[3]
[1]Research profesor, Sistemas Biológicos, Universidad Autónoma Metropolitana Xochimilco, Ciudad de México, México
[2,3]Student, Sistemas Biológicos, Universidad Autónoma Metropolitana Xochimilco, Ciudad de México, México
[1]uamx.casoto@gmail.com,[2]ime.glez@gmail.com,[3]qfblopez09@gmail.com

Abstract *– **Impaired glucose tolerance (IGT) is a pre-diabetic state of hyperglycemia associated with insulin resistance and glucose dysregulation. IGT may precede type 2 diabetes mellitus (DM2) by many years. IGT is a risk factor for mortality and can have damage to end organs, such as eyes, kidneys, blood vessels and heart. Relevant complications include nephropathy and chronic kidney disease, neuropathy, retinopathy and macrovascular disease. In IGT, β-cells decrease, and dysfunction is present. Studies have reported abnormal β-cell function (up to 80% decreased) insulin secretion in prediabetic individuals. Subjects with IGT have muscle insulin resistance plus severely impaired insulin secretion. At the pancreatic level, GLP-1 induces the differentiation, neogenesis, and proliferation of β-cells and plays a role in the long-term maintenance of these cell masses by inhibiting apoptosis . We reported that Silymarin increased pancreatic GLP-1 secreting cells in a pancreatectomy model. We treated IGT rats with Silymarin daily (200 mg/kg, p.o) for 9 weeks. The results showed that, Silymarin treatment increased pancreatic GLP-1R gene expression, tissue immunoreactivity for GLP-1, the level of GLP-1 peptide, and the proliferation of GLP-1 secreting cells and decreased IGT to control values. These results suggest that Silymarin improves IGT and may prevent the development of DM2.***

Keywords - *Pancreatic GLP-1, pancreatic GLP-1 receptor, impaired glucose tolerance.*

I. INTRODUCTION

Impaired glucose tolerance (IGT) is a pre-diabetic state of hyperglycemia associated with insulin resistance and glucose dysregulation. IGT may precede type 2 diabetes mellitus by many years. IGT is also a risk factor for mortality [1]. According to the criteria of the World Health Organization and the American Diabetes Association, impaired glucose tolerance is defined as follows: two-hour glucose levels of 140 to 199 mg/dL (7.8 to 11.0 mmol/l) on the 75-g oral glucose tolerance test (OGTT). A patient is considered as having IGT when he/she has an intermediately increased glucose level after 2 hours but less than the level that would qualify for type 2 diabetes mellitus. The fasting glucose may be either normal, < 6.1 mmol/L or mildly elevated [2], [3].

This prediabetic state can have concomitant damage to end organs, such as eyes, kidneys, blood vessels and heart. Particularly relevant complications include nephropathy and chronic kidney disease, as well as neuropathy, retinopathy and macrovascular disease [4].

In this prediabetic phase, β-cell dysfunction is already present. Studies using different measures of β-cell function have reported abnormal (up to 80% decreased) insulin secretion in prediabetic individuals [5]-[7]. Subjects with IGT who predominantly have muscle insulin resistance plus severely impaired insulin secretion may respond to agents that improve insulin resistance, such as agents that increase hepatic and peripheral insulin sensitivity, as well as insulin secretagogues, such as the GLP-1 analog [8].

Glucagon-like-peptide-1 (GLP-1) is a glucose-dependent insulinotropic polypeptidic hormone with important roles in maintaining normal glucose regulation [9] by an increase in blood insulin in response to the ingestion of carbohydrates, lipids and proteins [10]. This increase accounts for approximately 80% of the total insulin release [11]. GLP-1 is formed in gut endocrine L-cells [12] and human and animal α-pancreatic cells [8]. At the pancreatic level, GLP-1 induces the differentiation, neogenesis, and proliferation of β-cells and plays a role in the long-term maintenance of these cell masses by inhibiting apoptosis, both in human and animal models [13] [14]. Additionally, GLP-1 inhibits glucagon secretion [15] and reduces gut movement and secretion [16], as well as induces satiety, reduces food intake and decreases body weight [17]. For the mentioned effects, therapies based on this peptide have been used for the management of type 2 diabetes mellitus (DM2) [10], [18], and in some studies, this treatment has been applied to DM1 [19]. GLP-1 exerts its biological action by stimulating the G-protein-coupled receptor (GLP-1R) located on pancreatic β-cells [20], [21]. Some authors have reported that the GLP-1 response in the OGTT test is reduced in impaired glucose tolerance [22].

We recently reported that Silymarin induces GLP-1 secretion and an increase in its receptor gene expression in pancreatic tissues in partially observed pancreatectomized rats.

Publication History
Manuscript Received : 29 January 2018
Manuscript Accepted : 29 January 2018
Revision Received : 27 February 2018
Manuscript Published : 5 March 2018

Silymarin is a standardized extract obtained from Silybum marianum, comprising seven flavolignans. This compound has been widely used for hepatic disorders of different etiologies and demonstrated hepatoprotective effects. In addition, other actions of this compound include antioxidant, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulatory and liver-regenerating effects [23]. At the pancreatic level, we reported that Silymarin increased the pancreatic expression of the GLP-1 receptor (GLP-1R), the immunoreactivity of this peptide and its pancreatic level. Additionally, Silymarin increased the number of GLP-1-secreting cells [24].

II. METHODS

All reagents used were analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO., USA), Promega (Madison, WI), Qiagen (Germany), or from local suppliers (Merck and J.T. Baker, Mexico).

The experiments in the present study were performed according to the guidelines stated in "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) and Mexican regulations "Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio" (published in December, 1999). The studies were approved by the Ethical Committee of the Biological Sciences Division Council of our University.

A. Experimental Animals

Male Wistar rats (220-250 g of b. w.) were obtained from the animal facility, fed with Purina standard chow, and maintained at 20-22° C with 12-hr. light /dark cycles. Four experimental groups were formed. 1) Initial control group. No treatment was administered to these animals, n=6. 2) Control group. These animals received purified water as a drinking solution for 9.2 months, n=6. 3) Saccharose-treated group. Rats received 30% saccharose solution in purified water, as a drinking solution, ad libitum for 9.2 months, n=5. 4) Saccharose + Silymarin-treated group. These animals received 30% saccharose solution in purified water for 8 months. At this time, the animals presented glucose intolerance and a daily dose of Silymarin (200 mg/kg) was administered (p. o). for nine weeks (total time of treatment, 9.2 months).

B. Glucose and Weight Monitoring

Each month, the animals were fasted for 7 hours. Blood glucose was measured with an automatic glucometer (Abbot Diabetes Care Ltd., U.K.) in the tail vein and their weight was registered.

C. Glucose Tolerance Test

Animals from each group were fasted for 7 hours. One dose of glucose (3 g/kg b.w.) was intraperitoneally administered. Blood glucose was measured in the tail vein with an automatic glucometer (Abbott Diabetes Care Ltd., U.K.) at 0, 15, 30, 60 and 120 min after glucose administration [25].

D. Insulin Sensitivity Test

One dose of insulin (1 U/kg) was intraperitoneally administered to each animal. Glucose was measured in the tail vein at 0, 15, 30, 60 and 120 min after insulin administration.

E. Tissue and Blood Collection

For sacrifice, animals were anaesthetized with a dose of sodium pentobarbital (50 mg/ Kg b.w., i.p.). Blood was collected by cardiac puncture. The pancreas was dissected through an incision in the abdominal cavity.

F. Gene Expression Analyses

1) RNA isolation: RNA was isolated from a fragment of pancreatic tissue (50 mg) from each animal and prepared using the column method according to the manufacturer's protocol (PROMEGA, Madison, WI). Briefly, the tissue was homogenized in 100 µL of lysis buffer, and 350 µL of dilution buffer was added to 175 µL of lysate. The sample was heated at 70° C for 3 minutes and then centrifuged for 10 minutes. An 250-µL aliquot of 95% ethanol was added to and mixed with the cleared lysate. The lysate was transferred to a column and centrifuged for 1 minute. Then, 600 µL of wash solution was added and the column was centrifuged for another minute. Next, 50 µL of DNase was applied to the column, followed by incubation for 15 minutes at room temperature. Subsequently, 200 µL of DNase stop solution was added and centrifuged for 1 minute, followed by washing two times. To elute the RNA, 100 µL of nuclease-free water was added to the column membrane.

2) *RT-PCR:* Total RNA was spectrophotometrically (Nanodrop) quantified at 260/280 nm. The integrity of the RNA samples was evaluated on a 1% agarose gel. The cDNA from each sample was synthesized from 2 µg of total RNA in a total volume of 25 µL using oligo-(dT)20 primers and the PROMEGA kit (Madison, WI) at 37 °C for 60 min according to the manufacturer's protocol. The PCR mixture contained 0.5 µL (5 µM) of each primer, 2.5 µL of MgCl2, 0.3 µL of Go-Taq polymerase, 0.25 µL of dNTPs, 5.0 µL of buffer, 15.95 µL of nuclease-free water, and 1.0 µL of the cDNA sample. A parallel reaction was performed with ribosomal 18s primers as an internal control for the PCR analysis. Oligonucleotide primers were designed based on published sequences: 5' -CAG AAG TTG GTC GTG AGG CA-3' sequence for the sense (5') primer and the 5' -GCC TTT CAC CAG CCA AGC AA-3' for the anti-sense (3') primer for GLP-1R and, 5'- GTA ACC CGT TGA ACC CCA TT-3' for sense (5') and 5'- CAA TCC AAT CGG TAG TAG CG-3' for anti-sense (3') primers for ribosomal 18s mRNA amplification. Amplification was initiated for 5 min at 94 ºC, followed by 35 cycles at 95 ºC, 60 ºC and 72 ºC, for 1 min for each step, and the reaction products were sequence verified. The products were separated on a 2% agarose gel, followed by ethidium bromide staining and densitometry analysis using a Kodak EDAS 290 (Kodak, USA).

3) Sequentiation of PCR products: The Automated DNA sequencing of each PCR product was carried out on capillary-based electrophoresis sequencers. The unit utilizes an ABI Prism 310 (1-capillar) and an ABI Prism 3100 (16-capillary). Genetic Analyzers from Applied Biosystems, with Big Dye Terminator Cycle Sequencing chemistry.

G. Pancreatic Tissue GLP-1, Glucagon Double Immunoassay

To study the GLP-1 secretion in α-pancreatic cells, a double-glucagon immunoassay was performed for GLP-1 and glucagon. An anti-GLP-1 (1:100) mAb was utilized as the primary antibody and HRP-rabbit anti-goat IgG (1:75) was used as the secondary antibody. As a primary antibody, anti-glucagon (1:75) mAb was used for double immunostaining α-pancreatic cells, and HRP- goat anti-rabbit IgG (1:100) was used as the secondary antibody. Double-immunolabeled sections were analyzed with a DM-1000 Leica microscope. Cells with double immunolabel were counted in five fields of each pancreatic tissue sample. Analyses were performed in triplicate for each animal.

H. Western Blot Analysis

To determine the level of pancreatic GLP-1, a fragment (40 mg) of this tissue was homogenized in 10 mL of an ice-cold isolation solution (250 mM sucrose, 10 mM triethanolamine, 1 mg/mL leupeptin, and 1 mg/mL phenylmethylsulfonyl fluoride, pH 7.6) at 15,000 rpm with 3 strokes per 15 sec with a tissue homogenizer (IKA Works, Inc. Wilmington, NC). After homogenization, total protein concentrations were measured using the Bradford protein assay (Bio-Rad Laboratories Inc., Hercules, CA). The samples were stabilized by adding 1 volume of 2X Laemmli sample buffer to 1 volume of sample, and heated at 70℃ for 10 min. Then, 100 µg of protein from each sample was loaded into individual lanes and electrophoresed on 12.5% polyacrylamide-SDS minigels using a Mini-PROTEAN Tetra Cell electrophoresis apparatus (Bio-Rad Laboratories Inc.). The proteins were then electrophoretically transferred to PVDF membranes (Immobilon–P, Millipore, Billerica, MA, USA). After blocking with 5% skim milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, and 0.2% Tween-20, [pH 7.5]) for 1 hour, the membranes were probed overnight with anti-GLP-1 mAb, as a primary antibody, at 4◦C (1:500) (Zymed Labs, Inc., San Francisco) and subsequently incubated with a secondary horseradish peroxidase conjugated antibody (1:1000) (Zymed Labs, Inc., San Francisco). Antigen-antibody reactions were viewed using enhanced chemiluminescence substrate (Immobilon Western, Millipore, Billerica, MA, USA) and analyzed in Chemidoc XRS (Bio-Rad, Laboratories, Inc.) with the Quantity One 1-D Analysis v4.6.5 image processing software (Bio-Rad).

III.RESULTS

A. Serum Glucose Concentration and Body Weight

The parameters were measured monthly for the time of treatment, 9.2 months, in fasting animals of each group. In the control treatment groups, the values of serum glucose were higher in the second and fourth weeks than in the other weeks, but there were no changes in these values during the next time of the experiment. Saccharose treatment presented a decrease only at five weeks, but at the end of the treatment period, the value of this parameter was significantly increased.

The saccharose + silymarin treatment group did not present variations during the time course of the experiment, but in the last week, the glucose serum value was significantly increased. Nevertheless, this value was significantly lower than that in the group treated with saccharose (Table I). Body weight increased monthly in all groups. Compared with the control group, the increment was significantly higher in both groups treated with saccharose and saccharose + silymarin and there was no difference between these two groups (Table II).

TABLE I: SERUM GLUCOSE LEVELS (mM/L) IN EXPERIMENTAL GROUPS

TIME (Months)	GLUCOSE (mM)		
	Control	Saccharose	Saccharose + Silymarin
0	5.31 ± 0.34	3.99 ± 0.17	4.18 ± 0.16
1	4.84 ± 0.13	4.18 ± 0.41	4.40 ± 0.40
2	6.08 ± 0.38	3.77 ± 0.48	4.34 ± 0.24
3	4.77 ± 0.09	3.77 ± 0.41	4.18 ± 0.29
4	5.79 ± 0.09	4.12 ± 0.31	4.20 ± 0.31
5	4.90 ± 0.49	3.94 ± 0.33	4.32 ± 0.11
6	4.63 ± 0.3	4.53 ± 0.38	4.53 ± 0.46
7	4.32 ± 0.27	4.53 ± 0.37	4.58 ± 0.29
8	4.99 ± 0.17	4.57 ± 0.42	4.58 ± 0.12
9.2	5.10 ± 0.38	5.77 ± 0.14^{+}	5.02 ± 0.04*****

Serum glucose levels measured monthly for the time of treatment, 9.2 months, in fasting animals of each group. Mean values ± SEM, * $p < 0.05$ respect to the group treated only with saccharose at the same time. + $p < 0.05$ respect to the initial value in this group. n=6

TABLE II WEIGHT (g) IN EXPERIMENTAL GROUPS

TIME (Months)	WEIGHT (g)		
	Control	Saccharose	Saccharose + Silymarin
0	225.23 ± 2.39	251.50 ± 12.79	261.36 ± 18.91
1	357.56 ± 5.40	422.70 ± 27.11	437.16 ± 20.78
2	382.66 ± 5.07	510.60 ± 43.85	563.26 ± 33.16
3	391.63 ± 0.56	549.70 ± 28.95	585.30 ± 22.43
4	438.80 ± 7.85	608.33 ± 4.41	635.00 ± 27.83
5	473.16 ± 5.89	651.33 ± 18.97	663.33 ± 27.28
6	480.50 ± 5.75	754.00 ± 58.52	814.66 ± 37.73
7	502.83 ± 7.17	833.33 ± 65.92	864.00 ± 63.90
8	527.73 ± 8.80	937.66 ± 71.61	943.66 ± 62.17
9.2	530.50 ± 9.57	936.66 ± 81.63	952.33 ± 65.58

Weight measured monthly for the time of treatment, 9.2 months, in fasting animals of each group. Mean values ± S.E.M, n=6.

B. Glucose Tolerance Test

This test was performed for measuring insulin-stimulated glucose clearance in the three experimental groups of animals fasted for 7 hrs. a) Initial test (before of any treatment). Fifteen minutes after glucose administration we observed a sharp increase in serum glucose in the three experimental groups. After 120 min, the serum glucose level reached normal levels. There were no differences between the groups (Fig. 1a). b) Test before treatment with Silymarin (Fig. 1b). There was no difference between initial control group values with the control of the treatment group; in contrast, the saccharose treated group at each time was higher than the other groups. c) Final test (at the end of Silymarin administration). The serum glucose levels in the saccharose-treated group were higher than those in the other groups. Silymarin administration to the saccharose-treated group caused a decrease in the serum glucose levels and did not show differences from both control groups (Fig. 1c).

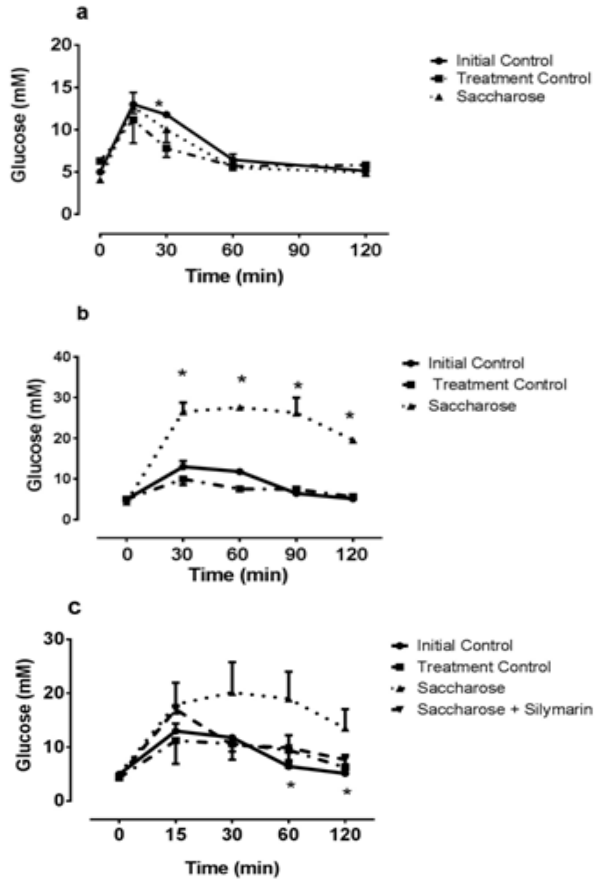

Fig. 1 Glucose tolerance test. Each point represents the mean ± S.E.M, n=6. (a) Initial test before of any treatment. (b) Treatment with saccharose before the Silymarin administration. (c) Silymarin administration to saccharose treated group. (a) * $p < 0.05$ respect to initial control. (b) * $p < 0.05$ with respect to initial and treatment control. (c) * $p < 0.05$ with respect to treatment control.

C. Insulin Sensitivity Test

a) Initial test. This group presented a decrease in the glucose serum levels for the time course of this test, and at the final time period, the glucose levels did not reach the initial values. Notably, there were some significant differences in the insulin sensitivity values between the experimental groups (Fig. 2a). b) Test before treatment with Silymarin (Fig. 2b). These groups, presented behaviors similar to those in the initial test. c) Final test. Both saccharose and saccharose + silymarin groups presented significantly increased insulin sensitivity with respect to the treatment control group, which decreased along the time course and reached control values (Fig. 2c).

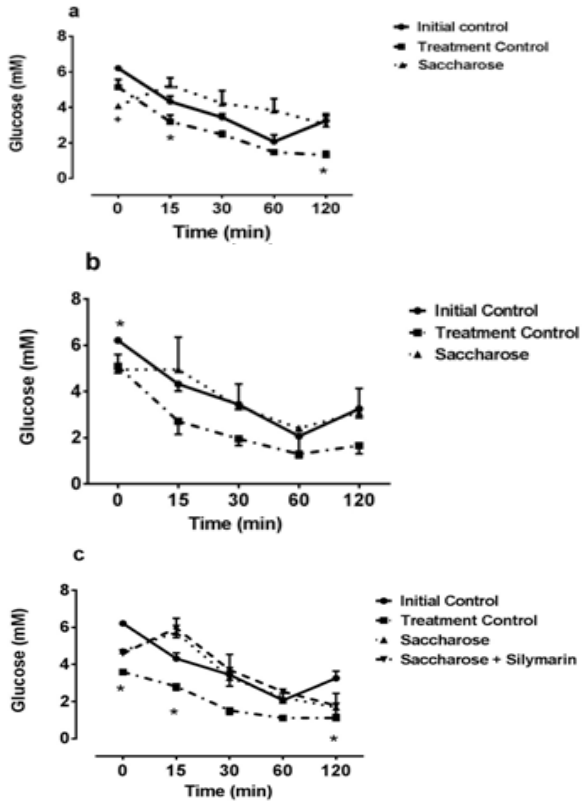

Fig. 2 Insulin sensitivity test. Each point represents the mean ± S.E.M, n=6. (a) Initial test before of any treatment. (b) Treatment with saccharose before the Silymarin administration. (c) Silymarin administration to saccharose treated group. (a) * $p < 0.05$ with respect to saccharose treated group, + $p < 0.05$ respect to initial control group. (b) * $p < 0.05$ with respect to saccharose treated group. (c) * $p < 0.05$ Treatment control with respect to saccharose treated group.

D. GLP-1R Pancreatic Gene Expression

Figure 3 shows that GLP-1R was significantly increased at 9.2 months (time of the treatment) with respect to that in the initial control group. Saccharose treatment significantly decreased at this time compared to that in the control treatment group. Silymarin administration to the saccharose group induced a significant increase of GLP-1R gene expression compared with the saccharose group and all experimental groups.

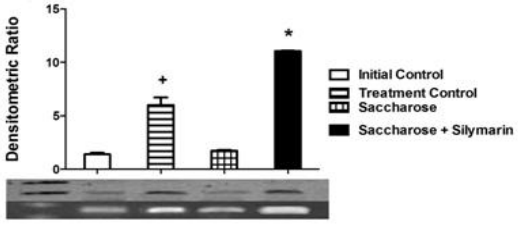

Fig. 3 GLP-1 receptor (GLP-1R) gene expression. Each bar represents the mean ± S.E.M, n=6. * $p < 0.05$ with respect to all other experimental groups. + $p < 0.05$ with respect to initial control and saccharose treated groups

E. GLP-1, Glucagon Double-Immunolabeled Pancreatic Tissue Cells

The number of double-immunolabeled cells did not present a difference between the animals treated with saccharose and the control groups (initial and treatment control). Silymarin administration to the saccharose-treated group induced a significant increase of GLP-1 secreting cells (Fig. 4). These results are shown in the panel of representative photomicrographs of the experimental groups (Fig. 5).

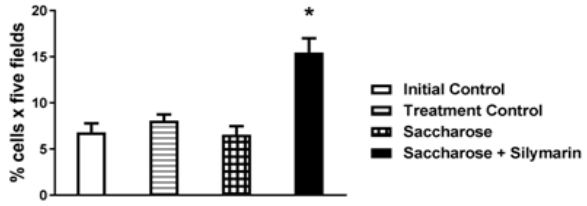

Fig. 4 Percentage of cells secreting GLP-1. Each bar represents the mean ± S.E.M. * $p < 0.05$ with respect to all other experimental groups.

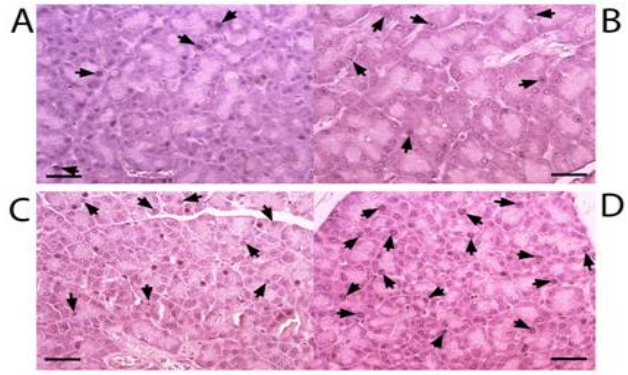

Fig. 5 Double immunolabelling for GLP-1 and glucagon. Representative photomicrographs of double immunolabelled pancreatic tissue (arrows) of each experimental group (x 400 of magnification). Scale bar = 25 μM). (A) initial control group, (B) saccharose treated group, (C) treatment control group, (D) saccharose treated group and administered with Silymarin

F. GLP-1 Western Blot Analysis

The results obtained from this analysis showed that the level of pancreatic GLP-1 in the control group (a) was higher in the initial control than that in the control time group (at the end of the treatments) (b). The saccharose-treated group showed a lower level of peptide (compared with all groups) (c). Silymarin administration to the saccharose-treated group (d) increased the level of GLP-1 compared with that in the group only treated with saccharose. This group showed a higher level of the peptide (Fig. 6).

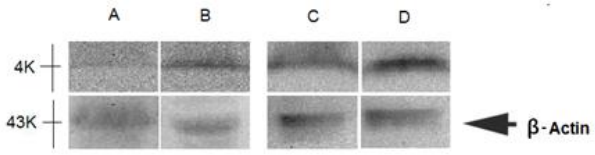

Fig. 6 Representative western blot analysis of GLP-1 and β actin in pancreatic tissue of each experimental group. (A) initial control group, (B) treatment control group, (C) saccharose treated group, (D) sacharose + Silymarin treated group.

IV. DISSCUSION

Impaired glucose tolerance is an important patho-physiological risk factor for the development of DM2. At this stage, there is significant damage to some tissues and tissue functions. β-cell dysfunction, with up to 80% decrement, and impaired insulin secretion have been reported. Studies have shown that when plasma glucose was 180-190 mg/ dL during an oral glucose tolerance test (OGTT), the α-cell function declined 75 to 80% [26]. Approximately 20-34% of the individuals with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) progress to T2D over five to six years, and individuals with IFG and IGT have an incidence of 38-65% if they have low insulin secretion and severe insulin resistance [27], [28]. β-cell decline and failure is responsible for the progression from normal glucose tolerance (NGT) to IGT and eventually T2D. Notably, the increase and function of these cells is preserved, indicating a high possibility of β-cell recovery [29].

Glucagon-like peptide-1 (GLP-1) is an incretin produced in gut epithelial L-cells by post-translational modification of the glucagon precursor, and this peptide is secreted in response to the presence of nutrients in the gut lumen and communicates this information to the pancreas where it acts as a potent insulin secretagogue [30]. The incretin effect could account for an estimated 60% of the insulin secretory response in healthy subjects [31]. GLP-1 is also secreted in pancreatic tissue from α-cells through PC1/3 convertase [32]. In IGT, β-cell dysfunction and thus impaired insulin secretion are dependent on incretins secretion, which involves both α and β cells [33].

In the present study, after four months of oral saccharose (30%) administration, as a drinking solution, the animals presented IGT, and treatment with silymarin improved this alteration of glucose tolerance to control values. Silymarin reverted the damage induced by saccharose. In addition to this effect, silymarin treatment produced a significant increase in the number of GLP-1-secreting cells and the gene expression of the GLP-1 receptor (GLP-1R), which is essential for the activity of this incretin. Faerch et al., (2015) showed a reduction in the content and response to GLP-1 in prediabetic individuals and suggested that higher GLP-1 responses were associated with better insulin sensitivity and β–cell function. This finding may be related to the findings of the present study showing glucose tolerance in animals treated with saccharose and after treatment with silymarin. Several studies have shown that the local production of GLP-1 by islets improves insulin secretion and may play a role in long-term β-cell adaptation via GLP-1 and glucagon secretion [8], [34], [35], [36]. Traub et al., (2017) studied this

local action (paracrine action) of GLP-1 in mice with prolonged α-cell ablation (GluDTR mice), and these animals showed impaired insulin secretion, reduced islet glucagon protein contents and decreased GLP-1 activity. The content of GLP-1 in the terminal ileum was similar to of the GluDTR and in control mice, confirming the specificity of α-cell participation. In GluDTR mice, glucose tolerance was impaired, fasting insulin secretion was decreased and insulin levels were reduced during the glucose tolerance test. In these mice, decreased glucagon expression and glucagon systemic levels and the impairment of β-cell function caused by the lack of α-cell-derived GLP-1 were observed. Moreover, these authors showed that α-cell-ablated islets presented a blunting insulin response to glucose, suggesting that α-cell-derived products are necessary for proper β-cell function. These authors support the contribution of the paracrine effect of GLP-1 with α-cell specific PC1/3 knockout mice (Pcsk1_/ _), showing reduced levels of active GLP-1, while glucagon levels were not reduced. These mice presented glucose intolerance and decreased insulin concentration. In young Pcsk1_/ _ mice, glucose tolerance was slightly improved compared to that in control animals, suggesting a compensation of duodenal GLP-1 production. However, this effect was lost upon aging. With these studies, the authors showed the importance of the paracrine action of α-cell derived glucagon peptides for normal glucose homeostasis and β-cell function. Indeed, α-cells are required for β-cell adaptation to aging and metabolic stress.

The present results demonstrated that silymarin treatment to saccharose administered animals produced an increase in the number of secreting GLP-1 cells, in the content of this incretin in the pancreatic tissue and in the genic expression of GLP-1R (in this animal model) at the end of treatment. These effects were not observed in the control treatment animals. Importantly, treatment with silymarin caused an improvement in animals with IGT to normal values in the glucose tolerance test. These results may consistent with those of Traub et al., (2017), who showed an increase in active pancreatic GLP-1, which may contribute to its paracrine action and induce improvements in the observed alterations by the decrease in the pancreatic secretion of this incretin as demonstrated by Traub et al., (2017), as observed in impaired insulin secretion, glucose-stimulated insulin secretion, impaired glucose tolerance by the decrease of insulin levels and impairment of β-cell proper function by the lack of pancreatic active GLP-1. The pancreatic increase of this incretin is essential for β-cell function and its actions in this tissue lead to the expansion of the β-cell mass by neogenesis and proliferation and a decrease of the apoptosis of these cells [37]. Studies with exendin-4 (GLP-1 analog) reported an enhancement of the pancreatic expression of PDX-1 transcription factor, which plays a critical role in pancreas development, differentiation, maintenance of the pancreatic cells and plays a key role in insulin gene expression [38], thereby stimulating cell neogenesis and increasing islet size [39]. This may be related with our findings of the pancreatic GLP-1 increase induced by Silymarin, which could stimulate the pancreatic expression of PDX-1 transcription factor.

V. CONCLUSIONS

The increase of pancreatic GLP-1 induced by Silymarin may contribute to the paracrine actions of this incretin and in this way induce an improvement of alterations observed in the impaired glucose tolerance and prevent the development of diabetes mellitus type 2. In addition, our studies contribute to support the proposition that in IGT a β-cell dysfunction and thus an impaired insulin secretion is dependent of incretins secretion and its action that which involves both α and β cells

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