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Protective Effects of Niacin (Vitamin B3) on Pancreatic Functions, Hyperglycemia, Inflammatory Markers and Oxidative Stress in Alloxan-Induced Diabetic Rats

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Abstract

Numerous studies and experiments have revealed that the initiation and progression of type 2 diabetes mellitus is majorly triggered by oxidative stress. Niacin, generally referred to as vitamin B3, is a critical vitamin that is considered a significant food additive as it eliminates free radical damage. This investigation aimed to test the extent to which a diabetic environment is affected by antioxidants with nutraceutical potential. Four distinct experimental treatment groups were established for the 32 albino rats after the intraperitoneal infusion of Alloxan (120 mg/kg Bwt) to induce diabetes. These groups include the standard control, niacin (15 mg/kg Bwt), diabetic control, and diabetic niacin-treated groups. Blood and pancreatic tissue samples were collected from animals euthanized after four weeks to evaluate various biochemical and histological alterations. The expressions of insulin in β cell islets were also assessed using immunohistochemistry analysis. In diabetic rats, several physiological changes were observed, including Increased values of hyperglycemia, tumour necrosis factor-α (TNF-α), pancreatic malondialdehyde and interleukin-1β and 6 (IL-1β and IL-6). Additionally, there were decreased levels of plasma insulin and pancreatic antioxidants, specifically reduced concentrations of superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT). Furthermore, limited positive insulin immunoreactivity was linked to histological alterations in the pancreatic islets in the diabetic group compared to the standard control one. The activities and structure of β cells were significantly restored and recovered after niacin therapy. There was a discernible drop in oxidative stress markers as fasting blood glucose levels decreased. Dietary niacin supplementation could relieve diabetes mellitus symptoms owing to its hypoglycemic, antioxidant, and anti-inflammatory properties.

Keywords: β Cell function, Diabetes mellitus, Inflammation, Oxidative stress, Pancreas.

Introduction

The islets of Langerhans within pancreas produce insulin, somatostatin, pancreatic polypeptide, and glucagon in response to stimuli [1]. Diabetes mellitus (DM) is a collection of metabolic disorders distinguished by persistently elevated glucose concentration in the blood because of pancreatic dysfunction. Errors in the insulin mechanism of action, secretion, or both can result in hyperglycemia [2]. The most common metabolic illness worldwide is diabetes. Multiple pathogen-related processes are linked to the acceleration of diabetes, ranging from abnormalities that cause the action of insulin to stop the immune system from destroying the β-cells in the pancreas, which results in insulin shortage [3]. Chronic hyperglycemia causes malfunctions in many organs, mainly kidneys, eyes, heart, nerves, and blood vessels [1].

Diabetes mellitus is associated with difficulties from the imbalance of pro-oxidants and antioxidants, resulting in oxidative damage [2, 4]. Both type 1 and

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type 2 diabetes mellitus pathogenesis have been linked to reactive oxygen species (ROS), according to recent research [5]. ROS are essential for identifying healthy and pathological physiological states. Overproduction of reactive oxygen species (ROS) increases cell macromolecule oxidative stress, including DNA, proteins, and lipids, leading to cellular apoptosis [6, 7]. The mitochondria are the main source of ROS in diabetes; for complete physiological activity, insulin creates ROS. However, excessive ROS production raises insulin resistance and type 2 diabetes risk[8].

High blood sugar causes oxidative damage. Several promising in vivo studies show that oxidative stress causes organ dysfunction. [2, 3, 8, 9].

In all circumstances, oxidative stress (OS) is crucial to the pathophysiology of diabetes mellitus and its complications. Hyperglycemia and free fatty acid buildup deplete β-cell activity via producing ROS and reactive nitrogen species (RNS)[7].

Increased MDA (malondialdehyde) levels indicate cellular lipid oxidation and oxidative stress [3]. Higher levels of advanced oxidation protein products (AOPPs) and protein carbonyls in diabetics support ROS. This oxidative damage in diabetes worsens free radical damage[8]. Therefore, a shortage of cell pro-oxidants and antioxidants can accelerate diabetic problems [10]. Statins are available to treat diabetes. But long-term use of these drugs causes health problems[3]. Our current study aims to improve the system's antioxidant ability to combat the adverse effects of the condition.

Nicotinamide and nicotinic acid are two water-soluble vitamins that constitute niacin (vitamin B3). Critical for oxidative reactions and energy metabolism regulation, nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD) are crucial coenzymes. These coenzymes control vital biological processes, such as DNA repair and cell death, and participate in non-redox signalling pathways [10]. One of the most important vitamins, niacin, is required for numerous essential physiological functions in the bodies of living things [11]. Niacin is possibly used to lower elevated blood fat levels. Niacin could also be synthesized from the amino acid tryptophan [12]. Because of its potent antioxidant properties and low toxicity, it is a good candidate for this usage [13]. Niacin has long been utilized as a cholesterol-lowering medication and as an antioxidant in managing problems linked to lipoprotein and plasma lipid metabolism. Since more than 50 years ago, the usage of niacin has been found to improve dyslipidemia and reduce the likelihood of cardiovascular complications associated with diabetes[12, 14]. Because blood lipid and lipoprotein levels vary, niacin reduces HDL and increases LDL.

Research has shown that niacin possesses the capacity to minimize the risk of heart attacks, atherosclerotic diseases, and high blood pressure associated with kidney disease [12]. Coenzymes associated with niacin, in addition to their antioxidant activity, NAD and NADP, play important functions as reduction/oxidation coenzymes in the metabolism of amino acids and energy, and in the detoxification of pharmaceuticals and other harmful chemicals [15].

One significant exogenous nutritional antioxidant is niacin. Two examples of redox enzymes that niacin coenzymes are nicotinamide mononucleotide (NMN) and nicotinamide adenine dinucleotide (NAD) [16]. Several research has investigated the impact of niacin on a variety of medical disorders, including anemia and hypertension [17], cardiovascular diseases (CVD) [11], liver disorders [6], and certain types of cancer (esophageal, skin, breast, and lung). However, the study of how niacin functions as an antioxidant and an anti-inflammatory has yet to be thoroughly studied, which is one way it may impact health. Numerous in vivo and in vitro studies have confirmed the potent antioxidant properties of nicotinamide (NA). Elevated oxidative stress was associated with inadequate niacin[17].

The objective of the current work was to determine whether niacin might safeguard pancreatic functioning in male rats with Alloxan-induced diabetes and to investigate its antioxidant and anti-inflammatory characteristics.

Material and methods

Ethics statement

The present protocol and guidelines for the care and use of laboratory animals were permitted by the Animal Research and Animal Care Review Committee of the Faculty of Veterinary Medicine at Alexandria University, Alexandria, Egypt (Committee permit number: AU-13-0611-2023-050). All methods were performed according to the relevant guidelines and regulations. The study was reported in accordance with ARRIVE guidelines[18].

Chemicals and reagents

We procured niacin and anhydrous Alloxan from Sigma-Aldrich (St. Louis, MO, USA). Upon its initial creation, anhydrous Alloxan existed as a pink, odorless powder that was dissolved in physiological salt (0.9 percent NaCl) until it achieved complete solubility. The Accu-chek Active glucose tester was acquired from Roche Diagnostics GmbH in Germany. Biodiagnostic, located in Tahrir, Cairo, Egypt, offers a range of diagnostic tests including, hemoglobin A1c (HbA1c) %, glucose, catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) concentration kits. Rat insulin enzyme-linked immunosorbent assay (ELISA) analysis is kit available from American Laboratory

Products Co., USA. The kit is identified by the catalog numbers 80-INSRTH-E01 and E10. Anogen, a Mississauga, Ontario, Canada-based Company, supplies ELISA assay kits for IL-6, TNF-α, and IL-1β, which are proinflammatory cytokines. All the chemicals used were analytical and did not require additional purification.

Experimental animals and protocols

Thirty-two mature male albino rats, weighing an average of 190±10 g and nine months old, appeared in good health. The rats were bought from Alexandria University in Egypt's Faculty of Agriculture. This experiment was conducted in the Physiology Department of the Faculty of Veterinary Medicine at Alexandria University. Rats were fed water and food pellets (Elfagr Company, Alexandria-Cairo Desert Road, Egypt) containing 23% crude protein, 3.35% crude fiber, 5% crude fat, and an energy content of at least 3000 Kcal/kg. Eight rats were kept in a wire box that was 80 cm long, 60 cm wide, and 40 cm high. Prior to the experimental procedures, by two weeks, the rats were allowed to acclimate to our laboratory conditions, in which there were equal periods of light and dark hours, a humidity level of 60±2%, and a temperature of 25±2℃.

Diabetes induction and niacin treatment

A diabetic model was established by injecting 120 mg/kg of Alloxan intraperitoneally in a single dosage [9]. After receiving an intraperitoneal injection of Alloxan, rats were given a 5% glucose solution to drink overnight to prevent the temporary hypoglycemia that the treatment created [9]. Following three days of Alloxan induction, peripheral blood was obtained from the tail vein. Concentrations of glucose were determined during the fast using the Accu-chek Active glucometer manufactured by Roche Diagnostics GmbH in Germany. It was considered diabetes if the fasting glucose level in the blood was ≥250 mg/dL [5]. Diabetic rats were randomly divided into two groups after diabetes development (groups III and IV). After 3 days of diabetes induction, the diabetic rats began receiving a course of niacin therapy (group IV). Niacin diluted in distilled water was given orally via an oral gavage tube at a dosage of 15 mg/kg/day for a successive 30 days [19].

Each of the four experimental animal groups consisted of eight rats. The categorizations were as follows:

a. Group I (Normal Control): A control group of healthy rats was used, and they were given a regular meal and normal water.

b. Group II (Niacin): Healthy, normal rats were administered niacin at a dosage of 15 mg/kg body weight, a normal meal, and water.

c. Group III (Diabetic control): consisted of diabetic rats provided with a regular meal and water.

d. Group IV (Niacin +Diabetic): the diabetic rats were administered niacin at a dosage of 15 mg / kg of body weight.

Blood and tissue sampling

After 24 hours of final treatment, two samples of cardiac blood were collected in tubes at room temperature using sodium fluoride while the rat was under ketamine/xylazine anesthesia (7.5 and 1.0 mg/kg intraperitoneal injection). The HbA1c% was assessed in one sample, while the other sample was centrifuged at 3000 ×g for fifteen minutes to isolate the plasma. The separated plasma was then examined for pro-inflammatory markers, insulin, and glucose. After collecting blood, the pancreas was promptly taken out, dried, visually inspected, weighed, and split into two equal parts. For histological and immunohistochemistry analysis, one portion was preserved in a 10% neutral buffered formalin solution. The other part was frozen at -80ºC to measure oxidative/antioxidant parameters.

Blood biochemical measurements

The glucose concentrations in plasma [20] and HbA1c% [21] were measured by spectrophotometry using the analytical kits given by Bio-diagnostic Co., Cairo, Egypt .In accordance with the manufacturer guidelines, plasma insulin levels were estimated by an ELISA kit for rats developed using American Laboratory Products Co., USA.

Homeostasis model assessment of β-cell function (HOMA-β-cell) index

HOMA β-cell index was calculated using Matthews' formula, which considers the values of insulin in plasma and fasting glucose. [22] .Using the formula: (20 × (Fasting plasma insulin (μU/ml)) / (Fasting plasma glucose (mmol/ml))) – 3.5, the HOMA β-cell Index is calculated. The fasting glucose and plasma insulin values were used to determine insulin sensitivity indices, including the fasting glucose/insulin ratio and insulin^{-1} [23].

Analysis of proinflammatory cytokines

Proinflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factorα (TNF-α)were measured in plasma by using ELISA kits that were received from Millipore, CA, USA, and following the manufacturer's guidelines [24]. An ELISA Plate Reader (Bio-Rad, Hercules, CA, USA) was employed to check all analyses.

Lipid peroxidation and antioxidants enzymatic activities

Malondialdehyde (MDA) levels were determined using homogenates of pancreatic tissues [25]. Super oxide dismutase(SOD) and catalase (CAT) enzyme

activity [26], and reduced glutathione (GSH) [27] were assessed using the diagnostic examination kits provided by Bio-diagnostic Co., Cairo, Egypt. According to [28], the protein concentration in pancreatic tissues was measured.

Histopathological examination

After collection from six rats per group pancreatic tissue of about 1 cm^3 was preserved for at least one day, in 10% buffered formalin. The fixed samples were dried in successive ethanol concentrations (70%, 80%, 90%, and 100%) for 60 minutes except for 100% which took 30 minutes. Samples were cleaned using xylene I and II. They were implanted with Paraplast I, II, and III for 1 hour per concentration. (Millipore Sigma, St. Louis). Using a Leica RM2125 microtome (Leica Microsystems, Wetzlar, Germany), 5-6 µm paraffin sections were cut and dried at 40°C in an incubator. A light microscope examined general histology, with H&E staining of the tissue sections. [29] describe H&E staining procedures.

Immunohistochemical staining and evaluation

Two-step IHC staining with horseradish peroxidase mouse (HRP; mouse; Agilent Technologies, Santa Clara, CA, USA) was done with Dako EN Vision + Single Reagent. Abd-Elhafeez et al.[30, 31] described staining. Briefly, 5-µ-thick paraffin-embedded sections were dewaxed, rehydrated, and washed three times with PBS (pH 7.4) for 5 minutes every time. We used a few drops of 3% hydrogen peroxide in methanol at room temperature for 20 min and intensive washing by running tap water for 10 min to block endogenous peroxidase activity. For antigen retrieval, slides were put in a 10_mm sodium citrate buffer (pH 6.0) and heated in a tap water bath to 95°C–98°C for 10 min, then at room temperature for 10 min. Next, the sections were incubated with the primary antibody (Insulin Rabbit monoclonal, Abclonal company, Cat Number, A19066 with dilution of 1:100). Following incubation, slides were washed three times with PBS (pH 7.4) for 5 min each and incubated with the secondary antibody for one hour at room temperature (Biotinylated goat Anti-Polyvalent, Anti-mouse Igg + Anti-Rabbit Igg. Ready to use, Catalog # TP-015-BN Thermo Fisher Scientific, UK). The slides were cleaned three times in PBS (pH 7.4) for 5 min and treated with 3,3'-diaminobenzidine (DAB) and substrate-chromogen for 5–10 min at room temperature to form a brown precipitate at the antigen location. Sections were counterstained with Harris hematoxylin for 30 seconds. Each slice was dehydrated in 90% and 100% ethanol for 5 min, cleaned in xylene, and then coated with DPX. We examined IHC staining with a Leitz Dialux 20 microscope and Canon Power Shot A95 digital camera. Negative control samples were obtained using the same method without main antibodies. Following [32] procedure, we were able to determine the percentage of the pancreatic islets of Langerhans' positive region for insulin secretion using ImageJ software (v1.46r, NIH, Bethesda, MD, USA).

Color segmentation according to CMEIAS (for negative images)

CMEIAS Segmentation, a free, updated computational approach, separates foreground objects from backgrounds in color pictures. The user chose "Negative image" from the "process" menu after opening the image in CMEIAS Color Segmentation[33, 34].

Statistical analysis

Statistical analysis was achieved by using GraphPad Prism 7 software. A one-way ANOVA was used to examine various parameters with Tukey's post hoc multiple range testing. Mean ± SE is used to express all the data for continuous variables. For the treatment, $P < 0.05$ was set to be of statistical significance.

Results

β-cell functions indices

Table 1 shows that the diabetes group had statistically significant ($P \leq 0.05$) enhancement in fasting glucose in plasma, HbA1c%, glucose/insulin ratio, and $insulin^{-1}$ relative to the non-diabetic control animals. The obtained data additionally demonstrated a significant reduction in the fasting insulin levels and HOMA β-cell index in diabetic control rats as relative to non-diabetic control animals. These indicators showed considerable improvement in the diabetic niacin-treated rats relative to the diabetic control group, with the glucose/insulin ratio, $insulin^{-1}$, HbA1c%, and fasting plasma glucose significantly ($P \leq 0.05$) lower in the niacin-treated diabetic animals relative to the diabetic group. Although fasting insulin and the HOMA β-cell index were significantly ($P \leq 0.05$) increased in diabetes + niacin-treated rats than in diabetic control ones.

Proinflammatory cytokines:

There was a substantial difference ($P \leq 0.05$) between the mean quantities of IL-1β, TNF-α, and IL-6 in control diabetic rats and normal control rats, as revealed in Table 2. The amounts of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) in the blood of normal rats that were given niacin did not differ significantly from those of the normal control rats. The mean value of IL-1β, TNF-α, and IL-6 in the blood of diabetic animals dropped greatly ($P \leq 0.05$) when they were given niacin.

Lipid peroxidation and antioxidants enzymatic activities

The results of the present study indicated that control diabetic animals exhibited significantly increased values of MDA than normal control rats

following the induction of diabetes. Additionally, the mean value of pancreatic GSH, SOD, and CAT values had substantial decreases ($P \leq 0.05$) (Table 3). The pancreatic oxidative/antioxidant indicators exhibited insignificant changes when niacin was administered to normal rats compared to normal control rats. In contrast to control diabetic rats, niacin administration led to a substantial increase ($P \leq 0.05$) in the mean value of pancreatic SOD, GSH, and CAT concentrations, as well as a considerable reduction in MDA values (table 3).

Histopathological findings

Pancreatic samples of both control and niacin-treated animals revealed normal pancreatic glands and islets of Langerhans, as observed in the histological examination presented in Figure 1 (a-b). Rats with diabetes induced by Alloxan exhibited a notable reduction in β-cells within the islets of Langerhans, which was associated with pancreatitis and infiltration of lymphocytic cells. This contrasted with the control group (Fig. 1c). Unlike diabetic animals, the pancreatic tissue of the diabetic rats treated with niacin showed less structural damages and better shape of β-cells in the islets of Langerhans (Fig. 1d).

Immunohistochemical evaluation of insulin

Figure 2 (a-d) displays the outcomes of the immunohistochemical examination of insulin antibodies in pancreatic samples. Both control and niacin-treated rat showed a significant presence of brown immunoreactivity in the interlobular ducts and islets of Langerhans (Fig.3 a, b). In contrast, islets of Langerhans in diabetic rats showed a limited response to insulin antibodies (Fig.3 c). In addition, niacin-treated diabetic rats exhibited increased insulin antibody immunostaining within β islets of Langerhans, compared to untreated diabetic rats (Fig. 3d). Negative control images are also shown in Figure 3.

Table 4 shows the positive area % of insulin in the various experimental groups.

Figure 4 shows the experiment design and conclusion.

Discussion

The use of antioxidants as a treatment has long been recognized as an alternative to traditional methods for managing diabetes mellitus. According to a report, the presence of active antioxidants could help battle insulin resistance, release insulin, and regenerate β cells in the pancreas [2, 35]. This study assessed niacin's hypoglycemic, anti-inflammatory, and antioxidant properties as a potential therapeutic agent for diabetes treatment. Hyperglycemia, along with insufficient insulin production, is regarded as a hallmark of type 2 diabetes mellitus[8], which the results of current experiment also support. The insulin sensitivity (IS) and β cell indices can be accurately determined from a single fasting blood sample. The significant decrease in the index of β cell functions and rise in IS indices in diabetic rats may be brought about by hyperglycemia, β cell malfunction, or decreased insulin synthesis [4].

In the present experiment, niacin administration enhanced HOMA β-cell indices and restored normal histology in type-2 diabetic rats' pancreatic tissues. As a result, the data suggest that niacin can scavenge free radicals, which could improve HOMA indices by lowering production and buildup of free radicals [17, 19]. According to the current research, niacin therapy significantly altered blood serum chemistry, including capacity for antioxidants. Reduced oxidative stress may facilitate tissue regeneration in the treatment groups' pancreas [6, 15] .

Oxidative stress is a substantial element in the progression of diabetes complications. Compared to other essential organs, the pancreas already has a low level of antioxidant defense. According to our research, the levels of the pancreatic antioxidants SOD, GSH, and CAT were reduced in diabetic rats, whereas levels of oxidative MDA were elevated. Our findings are comparable to [36] They found that Alloxan (120 mg/kg bw) intraperitoneal infusion significantly increased the level of malondialdehyde and markedly decreased the activity of catalase, glutathione, and superoxide dismutase. Furthermore, our results are consistent with [37]. They discovered that administering 125 mg/kg i.p. of Alloxan to rats resulted in significant oxidative stress, as evidenced by a reduction in antioxidant enzyme activity (CAT, SOD, and GSH) and an elevation in MDA concentration in the pancreas.

Diabetes causes oxidative stress by raising MDA levels and decreasing antioxidants such as GPX, SOD, and CAT [2], which can intensify the harmful effects of free radicals. Glucose auto-oxidation in people with diabetes encourages the production of increased harmful free radicals [38]. In a hyperglycemia condition, glucose is largely utilized in the polyol pathway, which burns NADPH, which is necessary for Glutathione reductase (GSR) regeneration by reduced glutathione enzymes. Ultimately, elevated oxidative stress is brought on (MDA) by GSR depletion, which is brought on by hyperglycemia [39]. The increase in peroxidative damage to lipids from oxidative stress acquired during diabetes may cause the observed rise in malondialdehyde production.

The current study's findings demonstrate that giving diabetic rat niacin supplements dramatically lower the production of lipid peroxides and increases anti-oxidative enzyme activity (CAT, GSH, and SOD). Our findings concur with [19]. They found that giving diabetic rats niacin as a supplement

greatly increased their levels of CAT, GSH, and SOD. It also decreased the production of lipid peroxides and brought protein carbonyl closer to normal. Niacin administration reduces the amount of MDA in the pancreas of diabetic animals by scavenging free radicals and restoring the concentrations of anti-oxidative enzymes [12].

In addition, our research shows that when Alloxan induces diabetes, IL-1β, TNF-α, and IL-6 values markedly rise compared to control. Relative to control normal rats, the control diabetic animals had IL-6 and TNF- α higher concentrations [40]. Following its release by activated CD4+ T cells, this inflammatory process results in localized pancreatic lymphocytic infiltration and necrosis. It eventually ends with the β cell's the apoptosis and activates the pancreas to create TNF-α [3]. Therefore, it facilitates the pathophysiological mechanism of diabetes mellitus, directly aiding the damage done to the pancreatic β cells [41]. Hyperglycemia increases inflammation and causes cytokines to be produced [42]. When blood sugar levels are too high, the build-up of advanced glycation products causes inflammation [43]. Damage caused by hyperglycemia causes pro-inflammatory cytokines like IL-6, IL-1β , TNF-α and to be expressed [36]. Niacin therapy for diabetic rats concurrently lowers cytokine production, which enhances niacin's anti-inflammatory response and may be related to niacin's anti-oxidative qualities, which lessen the severity of inflammation and inflammatory indicators [14].

Our biochemical results are consistent with our pancreatic histological and immunohistochemistry findings. Regarding the results of histopathology and immunohistochemistry, the pancreatic tissue of rats given Alloxan-induced diabetes demonstrated a notable degeneration of the islets of Langerhans β cells and a decrease in their abundance linked to inflammatory lymphocytic infiltration. These deteriorating alterations could be the consequence of Alloxan's ability to kill pancreatic β cells [44] via oxidative stress induction and the generation of ROS, which subsequently causes diabetes [45].

Furthermore, as stated by [46], which could be connected to the enhancement in metabolic stress and endoplasmic reticulum stress, in addition to the induction of inflammatory pathways that cause cell death and apoptosis. Our findings agree with those of [38, 47]. According to [38, 45] the biochemical changes linked to diabetes induced the β cells' apoptosis, the development of insulin resistance, and a drop in its secretion, which reduced insulin immune expression in diabetic rats' pancreas. Conversely, administering niacin to diabetic animals resulted in improved the pancreatic morphology, β cell count, insulin sensitivity, and immune response inside the pancreatic islets [14]. These findings may be linked to niacin's capacity to reduce oxidative damage caused by Alloxan and its role as an antioxidant, which is related to improve antioxidative enzymes activity, as mentioned and identified by [19].

Conclusion

zTo help lessen the harm triggered by free radical accumulation in diabetes mellitus, we have investigated the potential ameliorative remedy of niacin against diabetic symptoms in this study. According to our findings, we advise dietary supplementation of niacin as an essential antioxidant with vitamin B-complex to diabetic subjects. Niacin administration to diabetic rats revealed a reduction in the values of hyperglycemic markers, TNF-α, IL-1β, IL-6, and pancreatic MDA; in addition to an improvement in the levels of plasma insulin, β-cell function indices, pancreatic antioxidant biomarkers, and an enhanced positive insulin immunoreactivity in the pancreatic islets. The anti-diabetic effectiveness of niacin was also confirmed by this investigation, which may be related to its hypoglycemic, anti-inflammatory, and antioxidant qualities.

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Competing interests

The authors declare no competing interests.

Author contributions

All authors contributed to the study concept, design, data acquisition, data analysis, interpretation, manuscript editing, and manuscript review. All authors read and approved of the final version.

Data availability

All data made or analysed in this study are included in this paper

Ethics statement:

The present protocol and guidelines for the care and use of laboratory animals were permitted by the Animal Research and Animal Care Review Committee of the Faculty of Veterinary Medicine at Alexandria University, Alexandria, Egypt (Committee permit number: AU-13-0611-2023-050).

TABLE 1. Effect of Niacin (vit. B3) on hyperglycemic markers and β-cell function indices of diabetic rats

	Groups			
Parameters	**Normal Control**	**Niacin**	**Diabetic control**	**Niacin +Diabetic**
Glucose (mmol/L)	5.03±0.21[c]	4.95±0.18[c]	16.2±1.02[a]	8.43±0.32[b]
Insulin (µU/ml)	6.84±0.26[a]	7.04±0.28[a]	2.87±0.09[c]	4.97±0.18[b]
Glucose/Insulin	0.73±0.03[c]	0.70±0.02[c]	5.64±0.25[a]	1.69±0.02[b]
Insulin^{-1}	0.14±0.001[c]	0.14±0.001[c]	0.34±0.015[a]	0.20±0.002[b]
HOMA β	23.69±1.41[a]	24.94±1.26[a]	0.04±0.002[c]	8.29±0.42[b]
HbA1c%	5.37±0.14[c]	5.17±0.11[c]	12.3±0.89[a]	7.21±0.33[b]

Values are means ± standard error. Means in the same row without a common superscript letter differ significantly ($P \leq 0.05$).

TABLE 2. Effect of Niacin (vit. B3) on plasma pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) of diabetic rats

	Groups			
Parameters	**Normal Control**	**Niacin**	**Diabetic control**	**Niacin +Diabetic**
TNF-α (pg/ml)	184.2 ± 10.78[c]	179.2 ± 11.21[c]	282.6 ± 19.13[a]	207.6 ± 13.60[b]
IL-1β (pg/ml)	112.8 ± 7.67[c]	107.9 ± 6.34[c]	174.5 ± 9.23[a]	124.4 ± 8.55[b]
IL-6 (pg/ml)	18.2 ± 1.07[c]	16.9 ± 1.12[c]	38.2 ± 2.39[a]	24.9 ± 2.08[b]

Values are means ± standard error. Means in the same row without a common superscript letter differ significantly ($P \leq 0.05$).

TNF- α = tumour necrosis factor alpha; IL-1β = interleukin 1β; IL-6 = interleukin 6.

TABLE 3. Effect of Niacin (vit. B3) on pancreatic oxidative/antioxidant parameters of diabetic rats

Parameters	**Groups**			
	Normal Control	**Niacin**	**Diabetic control**	**Niacin +Diabetic**
MDA (nmol/mg protein)	3.05 ± 0.14[c]	2.97 ± 0.09[c]	10.6 ± 0.87[a]	5.31 ± 0.26[b]
GSH (mmol/mg protein)	11.68 ± 0.96[a]	12.2 ± 0.84[a]	4.15 ± 0.12[c]	8.72 ± 0.52[b]
SOD (U/mg protein)	4.38 ± 0.18[a]	4.64 ± 0.19[a]	1.28 ± 0.08[c]	3.17 ± 0.07[b]
Catalase (U/mg protein)	6.72 ± 0.47[a]	7.04 ± 0.58[a]	2.13 ± 0.05[c]	4.89 ± 0.12[b]

Values are means ± standard error. Means in the same row without a common superscript letter differ significantly ($P \leq 0.05$).

MDA= malondialdehyde; GSH = reduced glutathione; SOD= superoxide dismutase.

TABLE 4 Effect of Niacin (vit. B3) on the percent of insulin-positive area

	Groups			
	Normal Control	**Niacin**	**Diabetic control**	**Niacin +Diabetic**
% of insulin-positive area	70.05 ± 4.26[a]	73.97 ± 3.89[a]	20.43 ± 3.16[c]	58.34 ± 4.86[b]

Values are means ± standard deviations. Means in the same row without a common superscript letter differ significantly ($P \leq 0.05$).

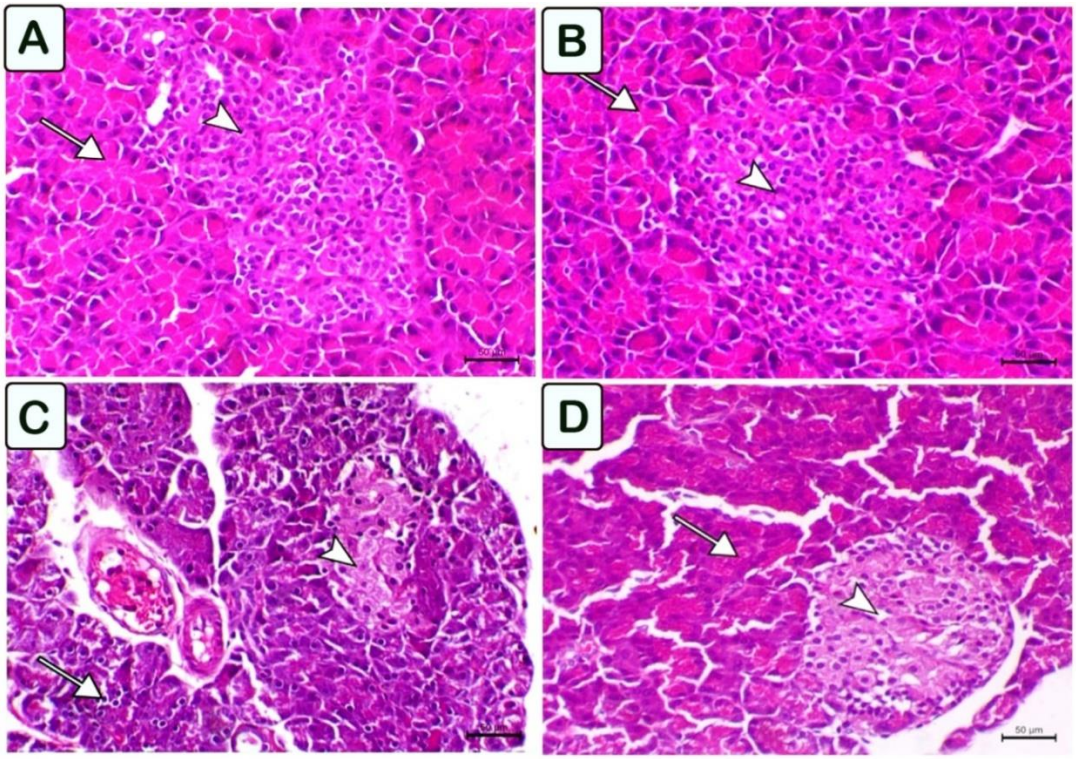

Fig.1. Representative photomicrograph for pancreatic tissues (H&E stain, X200, scale bar =50 µm) of (a) control, (b) Niacin- treated rats showing normal pancreatic glands (arrow) and β cells in islets of Langerhans's (arrowhead), (c) diabetic rats showing pancreatitis associated with lymphocytic infiltration between the pancreatic glands (arrow) and marked degeneration of β cells in islets of Langerhans's (arrowhead) and (d) Niacin- treated diabetic rats showing marked decrease degenerative changes within the β islets of Langerhans's (arrowhead) and glands (arrow).

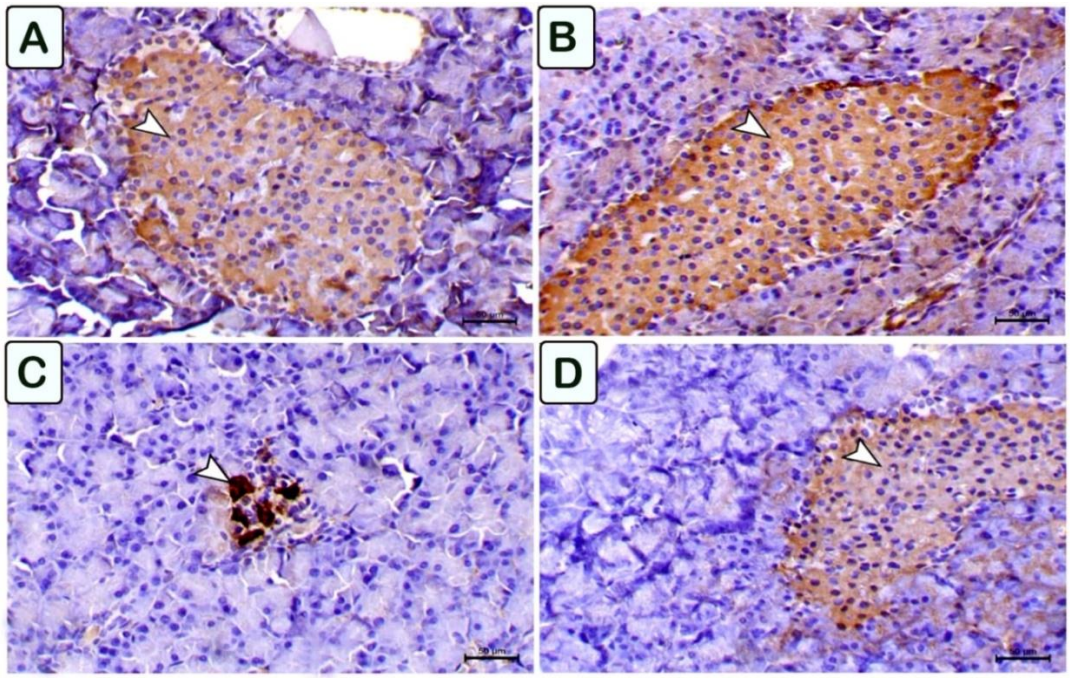

Fig.2. Representative photomicrograph for pancreatic tissues (Anti-insulin antibody IHC stained, X200, scale bar =50 µm) from (a) control, (b) Niacin- treated rats showing marked cytoplasmic insulin antibody expression within the β cells in islets of Langerhans (arrowhead), (c) diabetic rats showing marked decrease insulin antibody expression within the β islets of Langerhans (arrowhead) and (d) Niacin- treated diabetic rats showing an increase of insulin antibody immunostaining within the β islets of Langerhans (arrowhead).

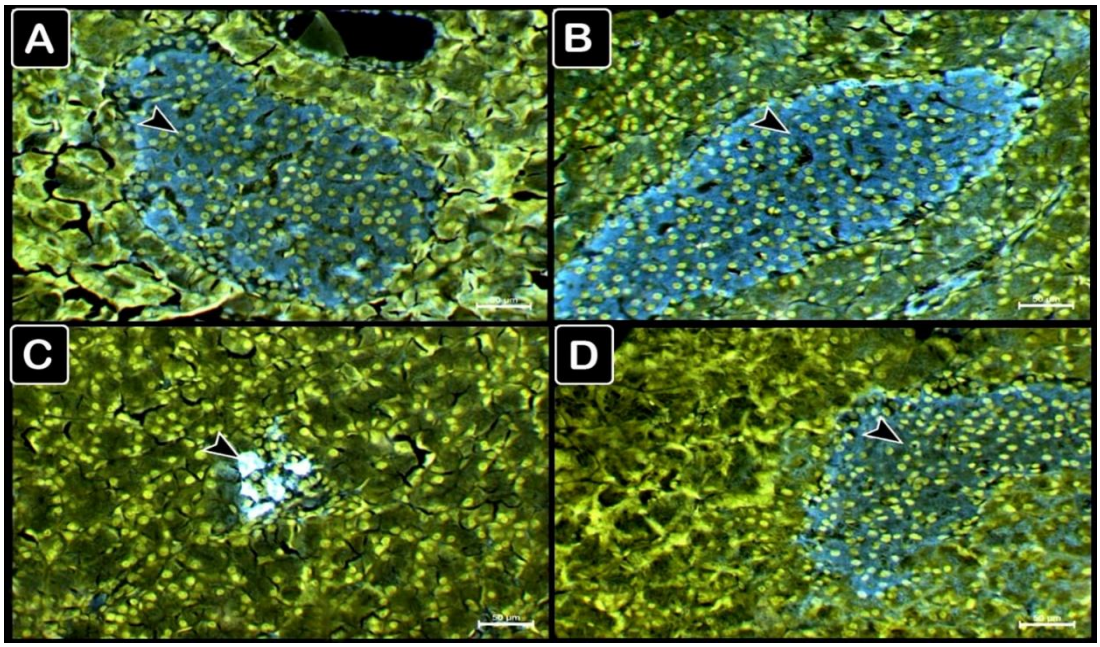

Fig.3. Negative image of Representative photomicrograph for pancreatic tissues (Anti-insulin antibody IHC stained, X200, scale bar =50 µm) from (a) control, (b) Niacin- treated rats showing marked cytoplasmic insulin antibody expression within the β cells in islets of Langerhans (arrowhead), (c) diabetic rats showing marked decrease insulin antibody expression within the β islets of Langerhans (arrowhead) and (d) Niacin- treated diabetic rats showing an increase of insulin antibody immunostaining within the β islets of Langerhans (arrowhead).

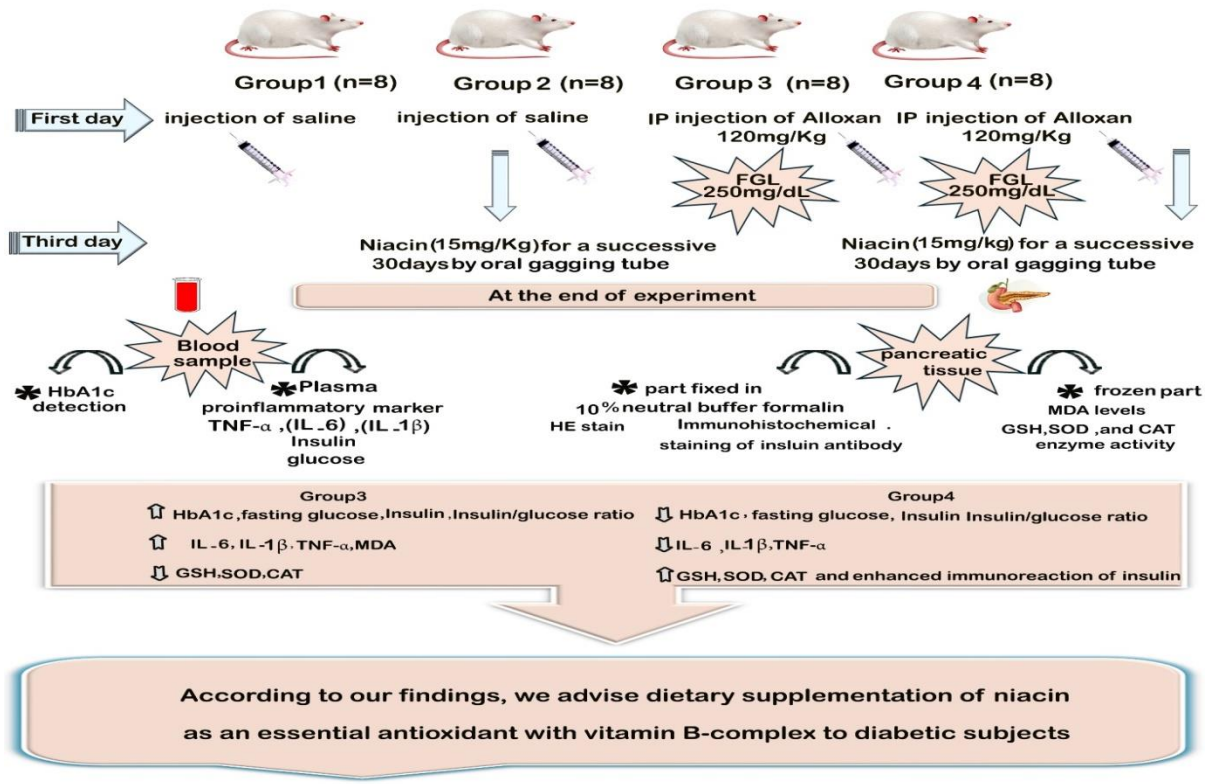

Fig.4. shows the experimental design and conclusion.

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التأثيرات الوقائية للنياسين (فيتامين ب3) على وظائف البنكرياس، وارتفاع سكر الدم، والعلامات الالتهابية، والإجهاد التأكسدي في الفئران المصابة بمرض السكري الناتج عن الألوكسان.

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الملخص

كشفت العديد من الدراسات والتجارب أن بدء وتطور داء السكري من النوع الثاني يُعزى بشكل رئيسي إلى الإجهاد التأكسدي. يُعد النياسين، المعروف بفيتامين ب3، فيتامينًا أساسيًا يُعتبر مُضافًا غذائيًا مهمًا، إذ يُزيل أضرار الجذور الحرة. هدفت هذه الدراسة إلى اختبار مدى تأثر البيئة المصابة بمرض السكري بمضادات الأكسدة الغذائية. أُنشئت أربع مجموعات علاجية تجريبية لـ 32 فأرًا بعد حقن الألوكسان (120 ملغ/كغ من وزن الجسم) داخل الغشاء البريتونى لتحفيز الإصابة بمرض السكري. تشمل هذه المجموعات المجموعة الضابطة القياسية، ومجموعة النياسين (15 ملغ/كغ من وزن الجسم)، ومجموعة التحكم بمرض السكري، ومجموعة السكري المعالجة بالنياسين. جُمعت عينات دم وأنسجة بنكرياسية من حيوانات أُعطيت بعد أربعة أسابيع لتقييم مختلف التغيرات البيوكيميائية والنسيجية. كما قُيّمت مستويات الأنسولين في جزر بيتا باستخدام تحليل الكيمياء المناعية. في الفئران المصابة بداء السكري، لوحظت عدة تغيرات فسيولوجية، منها ارتفاع مستويات سكر الدم، وعامل نخر الورم ألفا، ومالونديالدهيد البنكرياسي (مؤشر حيوي لأكسدة الدهون)، والإنترلوكين (IL-1β و IL-6) بالإضافة إلى ذلك، انخفضت مستويات الأنسولين في البلازما ومضادات الأكسدة البنكرياسية، وتحديدًا انخفاض تركيزات إنزيمي سوبر أكسيد ديسميوتاز ، والغلوتاثيون المختزل، والكاتالاز. علاوة على ذلك، ارتبطت استجابة مناعية إيجابية متواضعة للأنسولين بتغيرات نسيجية في جزر البنكرياس. استعادت خلايا بيتا نشاطها وبنيتها بشكل ملحوظ بعد العلاج بالنياسين. وكان هناك انخفاض ملحوظ في مؤشرات الإجهاد التأكسدي مع انخفاض مستويات الجلوكوز في الدم أثناء الصيام. يمكن أن تُخفف مكملات النياسين الغذائية أعراض داء السكري بفضل خصائصها الخافضة لسكر الدم، ومضادات الأكسدة، والمضادة للالتهابات.

الكلمات الدالة: وظائف خلايا بيتا، داء السكري، الالتهاب، النياسين، الإجهاد التأكسدي، البنكرياس.