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Honey versus Metformin: Effects on Pancreatic Beta- Cells in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Morphological and morphometric alterations to pancreatic beta- cells were evaluated in streptozotocin (50 mg/kg; i.p) induced diabetic rats treated with honey, metformin or their combination. To clarify Biochemical, histological and morphometric changes that occur in the pancreatic islets of induced diabetic rats treated by honey. Forty rats were randomized into five groups of eight rats. Diabetic rats received distilled water (0.5ml), honey (4.8 gm/kg) , metformin (100 mg/kg), metformin (100 mg/kg) and honey (4.8 gm/kg). Treatments were taken once daily orally for 30 days. Serum blood glucose was measured. Antioxidant activities of beta- cells were evaluated by measuring tissue catalase, malondialdehyde, paraoxonase and fraction of DNA strands. Sections of animal pancreases were fixed into paraffin and stained with hematoxylin & eosin and Van Gieson. Morphologic and morphometric analysis of pancreatic sections including area, perimeter, and percentage of fibrotic area were determined. Combination of honey and metformin reduced the elevated blood glucose level, malondialdehyde , DNA damage% and increased catalase, paraoxonase activities almost to normal. Treatment with either metformin, honey or their combination ameliorated the degenerative effects of streptozotocin on pancreatic beta- cells. The protecting effect of honey on shrinkage of cells and fibrosis % was more than that of metformin alone. Honey could protect against the biochemical and pathological alterations of pancreatic beta-cells induced by diabetes. Clover honey can be considered as a potential adjuvant to metformin in reducing oxidative stress and beta –cell damage.

Keywords: diabetes ; honey ; metformin; biochemical; histological and morphometric analysis; rats.

INTRODUCTION

The composition of honey which is a natural product of bees is dependent on plant nectar type. Plant nectar is influenced by the botanical sources, geographical and climate states [1]. Among the major components of honey are the flavonoids, phenolic compounds, enzymes, nitric oxide metabolites, organic acids, vitamins, amino acids, Maillard reaction products, monosaccharides and oligosaccharides [2]

Although the interest in investigating the health benefits of honey is increasing nowadays [3], honey was one of the folk remedies dating back to ancient Egyptian medicine [4]. Several medicinal values of honey have been recognized from evidence based experimental studies and clinical trials as being hypoglycemic and antioxidant [5,6]. Some investigated the role of honey as adjunct to commonly used diabetic drugs and proved its potential role in achieving better glycemic control, reducing oxidative stress and reversing metabolic dysfunction [7].

In experimental animal studies to induce diabetes with pancreatic beta cells destruction , streptozotocin (STZ) was suggested to be given in a dose of 50 mg/kg . The aetiology of beta cell destruction in this case could be attributed to

the oxidative stress with the release of cytokines and reactive radicals leading to inability to produce insulin and finally to beta-cell death [8].

The accumulated free radicals attack macromolecules in a trial to stabilize themselves such as DNA, proteins, and lipids. DNA damage will lead to disturbance in cell function. In addition to cell mutation and lipid peroxidation which may lead to membrane malfunction by altering fluidity of cell membrane and activity of membrane-bound receptors and enzymes [9]. Oxidative damage and stress has been indicated by measuring lipid peroxidation of unsaturated fatty acids [10].

Metformin, the common antidiabetic drug has several mechanisms of action as the support of peripheral glucose uptake, inhibition of glucose intestinal absorption and glucose hepatic production. Metformin enhances insulin post receptor transport. However, the role of metformin regarding pancreatic beta-cell function is not clear. In vitro studies indicated that metformin's action on muscle and hepatic cells is mediated through activation of adenosine monophosphate-activated protein kinase [11].

Studies on morphometric analysis of islets of Langerhans demonstrated that beta- cell changes were relevant to discriminate glucose intolerance [12]. However, studies testing natural substances versus known anti-diabetic drugs were few.

The present study aimed to compare the effect of clover honey and metformin ingestion on the biochemical, histological and morphometric alterations to beta cells of pancreatic islets following the experimental induction of diabetes using streptozotocin (STZ).

MATERIALS AND METHODS

Ethical approval:

The study protocol was approved by the Animal Ethics Committee of National Research Center, Egypt. All animal procedures were performed in strict compliance with the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes and in accordance with the Recommendations from Helsinki Declaration.

Animals:

Forty healthy female albino rats weighing 110-130 g were enrolled in this study. Rats were obtained from the Experimental Animal House of National Research Centre, Cairo Egypt. All animals were maintained under standard laboratory conditions of temperature (22±2 °C), humidity (45±5%) and 12 h day: 12 h night cycle and were allowed free access to food (standard pellet diet) and water ad libitum. The animals were acclimatized to the animal room condition for at least one week prior to the experiment.

Preparation of honey and metformin:

Natural honey (clover) was purchased from the Ministry of Agriculture Market , Dokki, Egypt. This honey has the following composition: pH (2.7), ash (0.37%) , moisture (16.3%),total protein (0.96), total lipids (0.72), total sugars (82.1%) and total reducing sugar (72.2%) [fructose (38.2 %), glucose (29.2 %), sucrose (4.8 %)] . Honey was freshly diluted with distilled water just before each administration. Metformin (100 mg/kg body weight) was dissolved in distilled water before administration.

Induction of diabetes:

Diabetes was induced in 40 fasted rats by intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1M cold citrate buffer (pH = 4.5) [13]. Diabetes was stabilized in these STZ treated rats over a period of 3 days. After that time the blood was collected by sinocular puncture and the plasma glucose level of each rat was determined. Rats with a fasting plasma glucose range of ≥ 250 mg/dl were considered diabetic and included in the study [14].

Treatment:

Rats were randomly divided into five groups, eight rats in each. Distilled water, clover honey, metformin or their combinations were administered once daily by oral gavage for 30 days as follows:

Group 1: Non-diabetic + Distilled water (0.5ml) (Non-diabetic control)

Group 2: Diabetic + Distilled water (0.5 ml) (Diabetic control)

Group 3: Diabetic + honey (4.8 mg/kg)

Group 4: Diabetic + Metformin (100 mg/kg)

Group 5: Diabetic + Metformin (100 mg/Kg) + honey (4.8 mg/kg)

Tissue preparation:

At the end of the experiment (30 days), rats were fasted overnight and blood samples were withdrawn through the retro-orbital plexus under light ether anaesthesia using a heparinized glass capillary and collected in tubes. Blood was allowed to clot and serum separated by centrifugation at 4000 rpm for 10 min to measure serum glucose. Pancreases were removed into two parts, first part washed with ice-cold saline solution (0.9 % NaCl), weighed and stored at -80 °C for the biochemical analyses. The tissues were homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 20 % w/v for the biochemical assays. Second part fixed in formalin saline mixture for pathological study.

Determination of glucose:

Serum glucose was determined by the glucose oxidase method [15].

Determination of antioxidant activities:***Catalase (CAT) Assay:***

CAT activity was measured according to the method of Gott [16]. Briefly, this assay involves the incubation of 0.5ml of hydrogen peroxide and 0.1 mL of tissue homogenate in a sample test tube. After incubation at 37 °C for 60 sec, the enzymatic reaction was stopped by addition of 0.5 mL of ammonium molybdate solution. The yellow complex of ammonium molybdate and hydrogen peroxide was then measured spectrophotometrically at 405 nm. One unit of CAT was defined as the amount of enzyme that catalyzes the decomposition of 1 μ mol of hydrogen peroxide per minute.

Paraoxonase (PON) Assay:

Arylesterase activity of paraoxonase was measured spectrophotometrically in supernatants using phenylacetate as a substrate [17]. In this assay, arylesterase/ paraoxonase catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol is measured by monitoring the increase in absorbance at 270 nm at 25 °C. The working reagent consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM CaCl₂ and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer are added and the change in absorbance is recorded following a 20 s lag time. Absorbance at 270 nm was taken every 15 s for 120 s. One unit of arylesterase activity is equal to 1 mM of phenol formed per minute. The activity is expressed in kU/L, based on the extinction coefficient of phenol of 1310 m/cm at 270 nm, pH 8.0 and 25 °C. Blank samples containing water are used to correct for the spontaneous hydrolysis of phenylacetate.

Lipid Peroxidation Assay:

The extent of lipid peroxidation was determined as the concentration of malondialdehyde (MDA) according to the method of Ohkawa *et al.* [18]. Briefly, 100 μ L of tissue homogenates or MDA standards were pipetted into test tubes containing 1.5 mL of 20% (w/v) glacial acetic acid (pH 3.5), 200 μ L of 8.1% (w/v) sodium dodecyl sulphate (SDS), 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) and 700 μ L of distilled water. The test tubes were incubated at 95 °C for 60 min with a marble on top of each test tube. After incubation, the test tubes were cooled and then centrifuged at 3000 \times g for 10 min. The amount of malondialdehyde (MDA) formed was measured spectrophotometrically at 532 nm. 1,1,3,3-Tetraethoxypropane (TEP), a form of MDA, was used as standard in this assay. TBARS concentration was expressed as nmol of malondialdehyde (MDA) per mg protein.

Protein Assay

Protein concentration was estimated using a Bio-Rad protein assay kit based on the method of Bradford [19]. The assay is a dye-binding assay in which a differential color change of a dye, with maximum absorbance at 595 nm, occurs in response to various concentrations of protein.

Measuring the fraction of DNA strands:

This is measured by Comet assay based on Singh *et al.* [20] with slight modification. Fresh blood about 5 μ L was mixed with 0.6% low melting point agarose (LMA) (Sigma-Aldrich, USA) and rapidly pipetted onto 0.6% normal melting point agarose (NMA)(ICN Biomedicals, USA) layer and covered with a coverslip. The mix was solidified for 15min. Then, the coverslip was removed and the slides were immersed in cold lysing solution for 1 hour in 4°C. After 1 hour, the slides were removed from lysing solution and were placed in a horizontal gel electrophoresis platform in the freshly prepared and cooled (1–10°C) electrophoresis buffer to the depth of approximately 0.25 cm. The slides were kept in the solution for 20 minutes. The electrophoresis was conducted at 1–10°C for 20 minutes using 25V with the current being adjusted to 300mA by a change of the buffer volume.

After electrophoresis, the slides were placed horizontally and neutralization buffer was dropped. The slides were allowed to stand for 5 min. This was done for 3 times. The slides were drained and 30 μ L EtBr was added to each slide. All the slides were placed in a humidified air-tight container in a refrigerator to prevent drying of the gel.

Following that, slides were analyzed as soon as possible under 200x magnification using a fluorescence microscope (AxioCam MRC, Carl Zeiss, Germany). Scores assigned on an arbitrary scale of 0–4 were based on perceived comet tail length migration and relative proportion of DNA in the comet tail. Five hundred nonoverlapping cells were randomly selected on each slide by categorizing cells as undamaged cells without tail (type 0), cells with tiny cell (type 1), cell with a dim tail (type 2), cells with a clear type (type 3), and only tail (type 4). Total damage score for each slide can be calculated by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades:

$$\text{Arbitrary unit} = \text{score } 0 \times (N) + \text{score } 1 (N) + \text{score } 2 (N) + \text{score } 3 (N) + \text{score } 4 (N)$$

N =the number of cells assigned to each grade of damage.

Histopathological study:

The removed pancreases were fixed in 10% neutral buffered formalin (pH 7.4) for 24 h; the organs were routinely processed using paraffin blocks and sectioned at 4–5 m thickness. Sections of pancreas were stained with hematoxylin and eosin (H&E) and with Van Gieson stain [21]. Stained sections were qualitatively (morphology) and quantitatively (morphometry) evaluated.

Morphometric measurements:

Morphometry means measurement of form. The increasing interest for application of morphometry in diagnostic pathology is due to its objectivity, reproducibility possibility of detecting minor differences or variations in a specimen that would otherwise escape subjective evaluation.

In this study morphometric analysis was performed using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England,) that consists of Leica DM-LB microscope with JVC color video camera attached to a computer system Leica Q 500IW.

Morphometric analysis of pancreatic islets was done to estimate:

1- Pancreatic islets parameters:

Images of pancreatic sections stained by H&E were captured by the colour video camera attached to the light microscope. Boundaries of the pancreatic islets were measured on real time image using interactive software with magnification of 50X. The maximum distance (perimeter) in μm of the surface pancreatic islets area, X coord and Y coord (the long and short axis of the islets) were measured using magnification of 400X. The average area of the islets was determined by measuring the area of 4 islets in each section of one rat [22].

2- Roundness of the pancreatic islets by the equation:

Roundness = (perimeter)² / (4 × π × area) Fig. 1A,B. Screen photo of the morphometry program in image analysis system showing how to detect the parameters of the islets (green coloured area).

3-Fibrotic area %:

Van Gieson stained slides was used, which stain the tissue yellow and the fibrotic area red colour Fig. 2 A,B. The average area of the fibrosis was determined by measuring the area of fibrosis in 4 islets in each section of one rat [22].

Statistical analysis: Statistical analysis was performed using SPSS statistical software, version 15.0 (SPSS Inc., Chicago, IL, USA) for windows. Data were analysed and presented as means \pm SEM or \pm SD. Differences between continuous data were analysed using one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of honey and metformin on blood glucose:

At the start of this study, blood glucose levels in all groups were similar to control non-diabetic rats. However, 3 days after injection with STZ, blood glucose levels increased and remained significantly elevated in the non-treated rats (Table 1). Treatment with honey or metformin significantly lowered the increased blood glucose levels in diabetic compared to control non-diabetic rats (Table 1). Combination of honey with metformin reduced the elevated blood glucose levels to normal levels of control non-diabetic rats (Table 1).

Effect of honey and metformin on lipid peroxidation, paraoxonase and catalase:

MDA as a measure of lipid peroxidation was elevated in diabetic compared to non-diabetic pancreas (Table 1). Treatment with honey, metformin or their combination led to decrease in MDA concentrations compared to diabetic control rats.

Catalase (CAT) activity was reduced in pancreas during diabetes compared to that of the non-diabetic control group. Metformin had no effect on CAT activity after induction of diabetes. On the other hand, treatment with honey in diabetic group showed significantly increased CAT activity compared to diabetic control rats. While combination of metformin with honey increased CAT activity back to normal (Table 1). The paraoxonase (PON) activity level was decreased in diabetic rats compared to non-diabetic rats. Administration of metformin or honey alone significantly ($p < 0.05$) increased the decreased PON activity. While, treatment of diabetic rats with combination of honey and metformin increased PON activity up to normal levels (Table 1)

Table 1. Honey, metformin, and their combinations on blood glucose and oxidative stress markers in the pancreatic tissue of control and diabetic rats

Group	Glucose	MDA	PON	CAT
Non-Diabetic control	79.83± 3.75	15.16± 0.41	11.6± 0.77	22.3± 1.60
Diabetic control	249.3± 17.69 ^a	22.97± 0.99 ^a	4.90± 0.75 ^a	12.6± 0.9 ^a
Diabetic+ Honey	153.3± 13.42 ^{ab}	16.85± 0.75 ^b	8.1± 1.4 ^{ab}	17.8± 0.6 ^{ab}
Diabetic+ Metformin	158.1± 15.62 ^{ab}	16.11± 0.95 ^b	7.80± 0.5 ^{ab}	14.8± 0.8 ^a
Diabetic+ Metformin + Honey	89.38± 3.52 ^b	14.23± 0.52 ^b	11.1± 0.7 ^b	22.6± 1.2 ^b

Values are represented as mean ± SEM. 8 animals in each group. Significant values at $p < 0.05$, ^{a, b} compared with non-diabetic and diabetic control rats respectively.

Effect of honey and metformin on DNA damage % in pancreatic tissue:

Induction of diabetes increased the DNA damage %. Treatment with metformin did not lower DNA damage%. While treatment of honey alone or in combination with metformin reduced the DNA damage % (Table 2).

Table 2. Effects of honey and metformin on DNA damage % in pancreatic tissue

	Non-diabetic control	Diabetic control	Diabetic + honey	Diabetic + metformin	Diabetic + metformin+ honey
DNA damage%	11.0± 1.06	22.16± 1.7 ^a	11.83± 1.7 ^b	20.5± 0.84 ^a	11.50± 1.17 ^b

Values are represented as mean ± SEM. 8 animals in each group. Significant values at $p < 0.05$, ^{a, b} compared with non-diabetic and diabetic control rats respectively.

Pancreatic islets morphology:

Normal pancreatic sections of non-diabetic rats stained with H&E showed the normal appearance of islets of Langerhans as non-capsulated pale stained oval or rounded areas (Fig. 3A).

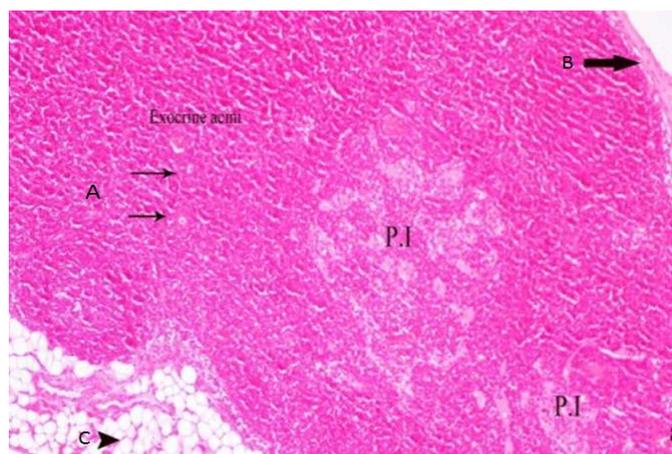


Fig 3a. Photomicrographs of a pancreatic section from the control non-diabetic rats showing normal pancreatic tissue where pancreatic islets of Langerhans (P.I) are surrounded by the exocrine acini, exocrine ducts (thin arrows) "A", all the tissues are covered by the pancreatic capsule (thick arrow) "B" and surrounded by the pancreatic fat (arrow head) "C". H&E staining, 100X

They were formed of irregular, anastomosing and, branching cell cords separated by blood capillaries (Fig. 3B).

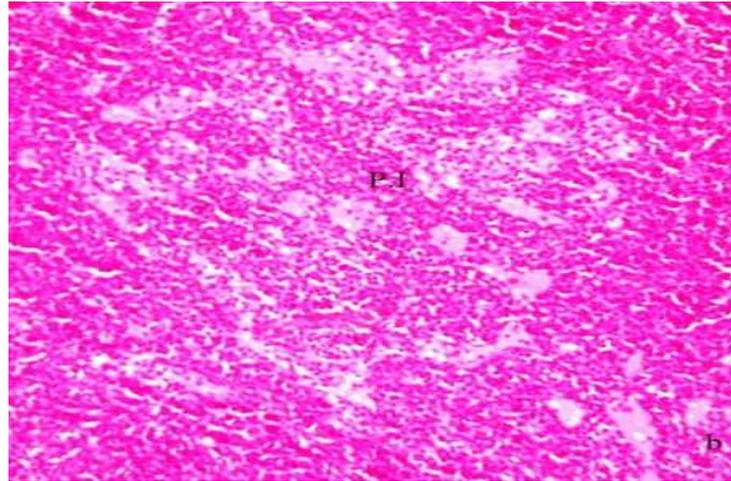


Fig 3b Photomicrographs of a pancreatic section from the control non-diabetic rats showing granulated cytoplasm of islet cells with small, dark nuclei on the periphery (alpha-cells), or with light and large nuclei (beta- cells) in the centre. H&E staining, 200X

STZ caused marked degenerative changes of pancreatic islets in diabetic rats with reduction in size and number, mainly at the centre of the islets (Fig. 3C).

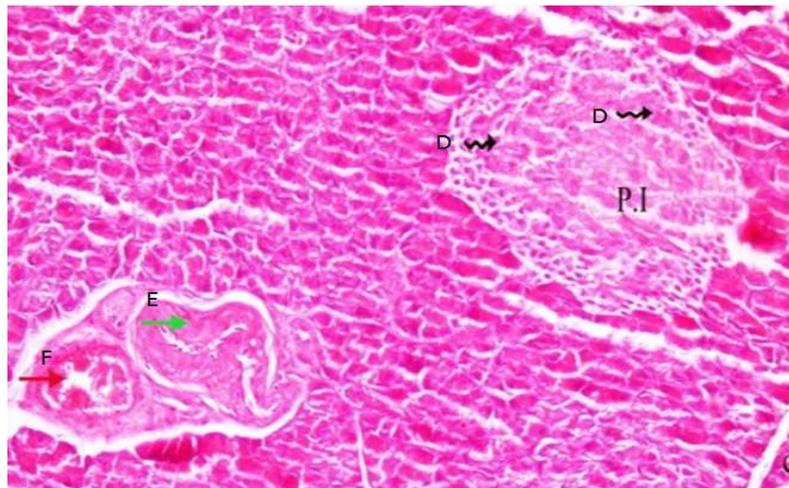


Fig 3c. Photomicrograph of STZ-induced diabetic pancreatic section showing cytoplasmic degenerative changes in most P.I especially in the centre (beta-cells) which are replaced by fibrous tissue (curved arrow) "D" while the surrounded exocrine tissue, pancreatic duct (green arrow) "E" and the blood vessels (red arrow) "F" are looking normal. H&E staining, 200X

Sections from the pancreas of diabetic rats treated with metformin (Fig. 3D),

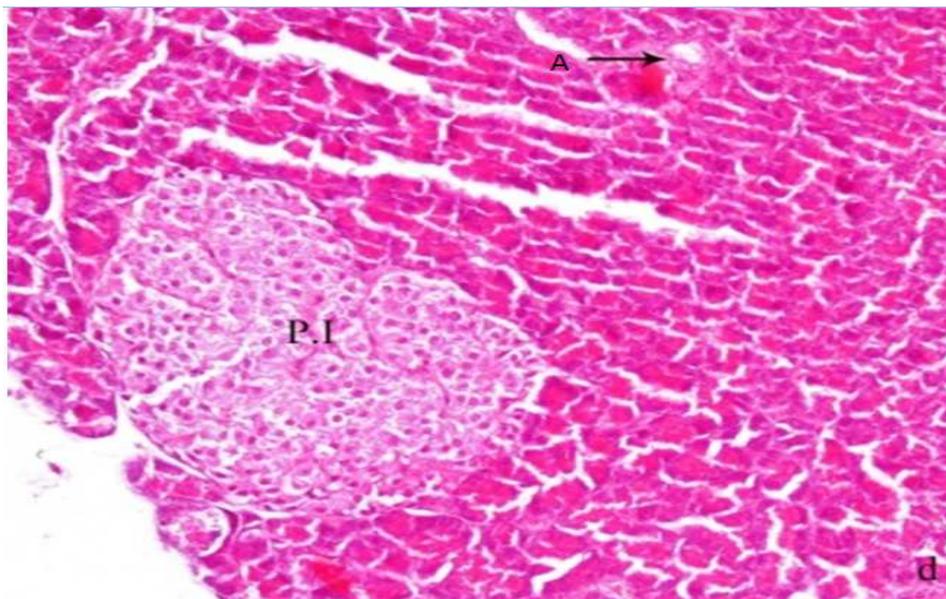


Fig 3d. Photomicrograph of diabetic islet treated with metformin showing regeneration of P.I cells with the exocrine duct (thin arrow) "A" looking normal with nearly regular outline of an islet with apparently normal appearance of most cells. H&E staining,200X. honey (Fig. 3E) or combination of honey and metformin (Fig. 3F) appeared similar to those of the normal islets regarding outlines , cell morphology and count but with little fibrosis

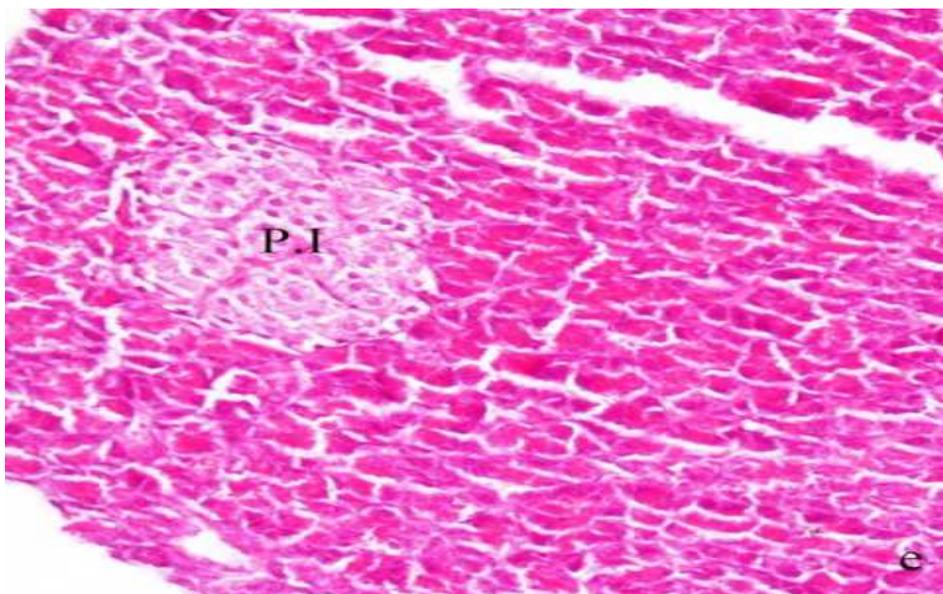


Fig 3e. Photomicrograph of diabetic islet treated with honey showing regeneration of P.I more or less similar to normal but still less cellular. H&E staining,200X

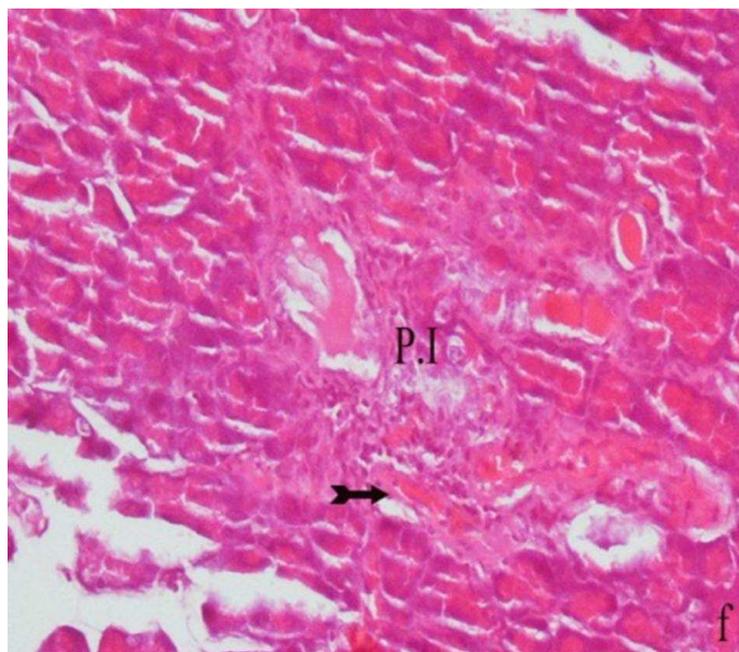


Fig 3f. Photomicrograph of diabetic islet treated with honey and metformin showing regeneration of P.I similar to normal. H&E staining,200X

Morphometric analysis:

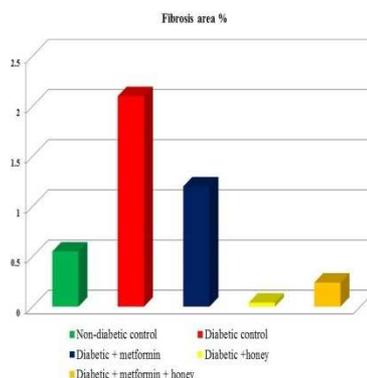
All diabetic groups showed significant decrease in area and perimeter of pancreatic islets compared to non-diabetic control rats (table 3).

Table 3: Morphometric parameters of islets of Langerhans in all studied groups

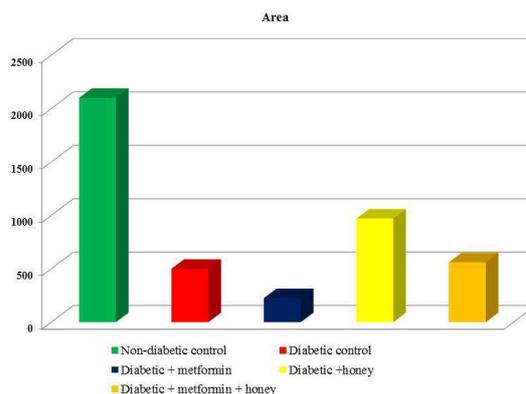
	Area	Long axis	Short axis	Perimeter	Round-ness	Fibrosis area%
Non-diabetic control	2108.97± 903	281.6± 85.95	150.6± 59.59	178.58± 42.92	1.27± 0.16	0.55± 0.18
Diabetic control	507.59± 139.8 ^a	258± 67.89	207.8± 64.36	90.42± 12.88 ^a	1.27± 0.128	2.171± 0.385 ^a
Diabetic+ metformin	925.1± 133.82 ^{ab}	175.6± 60.19	209± 80.62	102.13± 24.69 ^a	1.39± 0.324	1.29± 0.225 ^a
Diabetic+ honey	1973.5± 396.6 ^b	206± 85.42	226.4± 51 ^{ab}	162.52± 23.29	1.27± 0.09	0.61± 0.04 ^b
Diabetic+ metformin+ honey	1765.18± 433.4 ^b	170.8± 57.85 ^a	229.3± 49 ^{ab}	166.4± 45.1	1.43± 0.376	0.59± 0.07 ^b

Values are represented as mean± SD; Significant values at $p < 0.05$, ^{a, b} compared with non-diabetic and diabetic control rats respectively.

The protecting effect of honey alone or combined with metformin on shrinkage of cells was more than that of metformin alone (Fig.4A). On the other hand the roundness of the pancreatic islets didn't show any significant differences between the studied groups.



The diabetic groups showed significantly increased fibrotic area % in the pancreatic islets when compared to non-diabetic control group (table 3). The protecting effect of honey alone or combined with metformin on fibrosis % was more than that of metformin alone (Fig. 4B).



In this study we compared the effect of clover honey and metformin ingestion on the biochemical, histological, morphometric alterations to pancreatic beta- cells after streptozotocin (STZ) induction of diabetes.

STZ has a selective cytotoxic action on the beta cells and therefore is used to induce diabetes mellitus in experimental animals [23]. STZ is a N-methyl nitrosocarbamil-glucosamine-structured substance synthesised by *Streptomyces achromogenes*. Its cytotoxic action on pancreatic beta cell is through destruction of cell DNA induced by increasing polyadenine diphosphate ribose synthetase activity or by decreasing NAD levels thus blocking pro-insulin synthesis and leading to insulin insufficiency [24].

In this study, in agreement with others [23,25] honey supplementation significantly reduced blood glucose concentrations in STZ-induced diabetic rats. Moreover, we found that the combination of honey with metformin reduced the elevated blood glucose levels nearly as normal levels.

Honey is a supersaturated sugar solution of glucose and fructose. Besides carbohydrates, it contains a wide range of substances such as vitamins, minerals, enzymes, organic acids, proteins, and antioxidants [23]. The hypoglycemic effect of honey can be exerted by its major component fructose, which does not increase plasma glucose and its metabolism does not require insulin [26]. The ability of honey to prevent oxidative damage in diabetic rats might be due to its phenolic antioxidants content or through the action of CAT activity found in honey in reducing hydrogen peroxide. [27]. Some attributed the hypoglycemic action of honey to its anti-inflammatory property that suppresses beta cell inflammation or its direct stimulating action to insulin secretion [28].

The main process leading to hyperglycaemia in diabetes mellitus includes the excess production of glucose and the decreased tissue utilization of glucose [29]. In this study, metformin similar to honey had antidiabetic potentials as has been established by several studies [30]. This is quite obvious when starting drug administration, there is rapid fall of blood sugar levels [29].

Metformin main action is through increasing insulin sensitivity through unknown mechanisms; however it could be attributed to the reduction in hepatic glucose production, the increase of glucose utilization by muscles or the decrease of intestinal glucose absorption [31]. In addition, metformin increases low and high affinity receptors of insulin, and reduces insulin resistance [29].

Oxidative stress represents an essential factor in the pathogenesis and complications of diabetes mellitus [28]. Untreated hyperglycaemia can lead to high production of ROS which may produce cellular oxidative damage to DNA, lipids, and proteins [32]. Malondialdehyde (MDA) – a measure of lipid peroxidation- can reflect the degree of oxidative stress in cells and tissues. Insulin deficiency stimulates the activity of fatty acyl Coenzyme A oxidase in diabetics, which leads to fatty acids oxidation and increased formation of hydrogen peroxide. Hydrogen peroxide and other peroxides induce harmful effects on cell membrane's lipids, proteins and polyunsaturated fatty acids (PUFAs). These harmful effects can occur directly by the formation of reactive hydroxyl radical or indirectly through toxic aldehydes resulting from reaction with transition ions such as iron or copper. Lipid peroxidation products are also liable to be radicals, thus leading to more oxidative damage [33].

In the present study, MDA level in pancreatic tissues of diabetic rats were significantly elevated. This is similar to other studies [33,34]. The increased MDA levels indicated the existence of lipid oxidative damage which is responsible for the development of pancreatic beta cell damage [31]. The antioxidant defence system enzymes like

CAT and PON are decreased in diabetes because of excessive levels of glucose in mitochondria resulting in overdrive of electron transport chain producing excess free radicals [28].

It was reported that honey after four weeks of treatment exerts a hypoglycemic effect and ameliorates the oxidative damage produced in kidneys of diabetic rats [35]. In this study honey after similar treatment duration had tissue antioxidant effect where it decreased the elevated tissue MDA and increased the reduced tissue CAT and PON activities. Metformin alone had no effect on the elevated CAT activity.

When combining metformin with honey, marked tissue antioxidant activities were noticed. The potential antioxidant properties of honey could be due its high content of phenolic compounds and CAT that reduced hydrogen peroxide [25].

The produced oxidative stress in diabetic rats led to damage of DNA fragments which can be produced by different internal or external factors including oxidative and metabolic stress [32]. In our study we found that diabetic rats had elevated DNA damage % than normal control rats. Administration of metformin had no effect on DNA damage%. While administration of honey alone or in combination with metformin reduced the DNA damage %.

Marked damage of DNA in diabetic humans and rodents due to oxidative stress was found to be associated with an increase of reactive oxygen species. Increased level of biomarkers for oxidative DNA damage has been found in diabetics as 8-hydroxy 2'-deoxy-guanosine [36] or P53 activation that ends in cell-cycle arrest and apoptosis. The DNA protecting effects of honey could be referred to its contents of flavonoids and phenolic compounds with strong free radical scavenging activities [37].

The pancreatic beta-cell dysfunction can be attributed to hyperglycemia, inflammatory cytokines, hyperlipidemia or oxidative stress. The low levels of antioxidant enzyme expressions in the beta cells make them liable to oxidative stress by reactive oxygen and nitrogen species [38].

In diabetic rats of this study, STZ caused severe degenerative changes of pancreatic islets with reduction in size and number, mainly at the centre of the islets (beta cells). Morphometric analysis of pancreatic islets revealed decrease in their total area and perimeter with increased fibrotic area %. Treatment with metformin, honey or their combination led to regeneration of pancreatic beta – cells with normal morphology , outline and count, although the protecting effect of honey alone or combined with metformin on shrinkage of cells was more than that of metformin alone. On the other hand the roundness of the pancreatic islets didn't show any significant differences between the studied groups.

In addition, the protecting effect of honey alone or combined with metformin on reducing fibrosis % was more than that of metformin alone.

The histochemical findings and the morphometric measurements presented in our study as a result of treatment with honey in diabetic rats confirmed the results reported by Erejuwa et al who reported in addition, disturbed beta cells ultrastructure and reduced insulin immunoreactive cells [39].

Honey could improve the function of beta cells partly due to its anti-inflammatory action and partly to its high content of the powerful antioxidants; flavonoids. A study has proved that injection of flavonoid (quercetin) had a protective effect against beta cell damage in STZ-induced diabetic rats [40].

CONCLUSION

Honey could protect against the biochemical and pathological alterations of pancreatic beta-cells induced by diabetes. Clover honey can be considered as a potential adjuvant to metformin in reducing oxidative stress and beta –cell damage.

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