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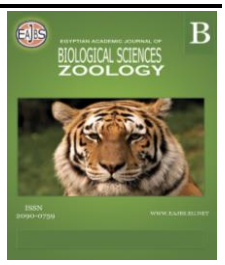

Possible Short-Term Biological Effects of Kefir Beverage: I: Effect of Kefir Beverage on The Cell Biological, Histochemical, Histopathological and Biochemical Changes in Pancreas of High Fat-Fed STZ- Induced Diabetic Male Wistar Rat

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ABSTRACT

The present study was designed to investigate the biological effects of kefir and whether kefir consumption protects and/or repairs the pancreas of high-fat diet-fed streptozotocin-diabetic rats, and how does it do that?.

Our results showed Kefir decreased both the volume of nuclei and RNA content and increased both collagen and lipoproteins material contents in the normal pancreatic cells. Also, kefir increased RNA, protein, and lipoproteins materials content and decreased the volume of nuclei of pancreatic cells in diabetic male rats. Immunohistochemically, kefir showed highly immunoreactive signals of insulin granules inside the Langerhans Islands. Our results showed kefir consumption had a beneficial effect on controlling the glycemic state in diabetic rats by lowering insulin levels and kept the pancreas tissue structurally almost normal.

Kefir probably acts as follows: From the cell biological and histochemical points of view, the results showed an increase in the nuclei volume and DNA, RNA, and total protein contents in the pancreatic cells of diabetic rats treated with kefir. Also, the biochemical and immunohistochemical results showed an increase in insulin hormone (functional protein) of these pancreatic cells, which's probably a result of an increase in the cellular activities or/and the number of beta cells. Finally, therefore we propose that kefir has a stimulatory effect on the cellular activity of beta cells leading to activation of genes responsible for the protein synthesis included insulin hormone, and/or increasing the cell proliferation of beta cells in the pancreas of diabetic rats. From Cell biological, Histochemical, Immunohistochemical, pathological, and

Biochemical points of view, the preclinical treatment with kefir or insulin of normal or diabetic male rats, beneficially highly alternates the cellular activities, histochemical and immunohistochemical materials components, and histological architecture. of the pancreas therefore Kefir and insulin may, to some extent, repair the pathological side effects of type 1 diabetes mellitus.

The positive results of using kefir and insulin in treating the Pathogen effects of type 1 diabetes mellitus on the pancreas of male mice gave us a hope of possibility to obtain positive clinical applications/implications. Therefore, these positive beneficial results make us continue our work and complete the various pre-clinical trial and clinical experiments phases.

INTRODUCTION

Diabetes is an increasing global health challenge. Currently, over 422 million people have diabetes, and this is expected to rise to 700 million by 2045**.** Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2014). In recent years, "Regular consumption of kefir has been associated with improved digestion and tolerance to lactose, antibacterial effect, hypocholesterolaemic effect, control of plasma glucose, anti-hypertensive effect, anti-inflammatory effect, antioxidant activity, anti-carcinogenic activity, anti-allergenic activity, and healing effects" (Rosa *et al.,* 2017). Besides traditional drug treatments for diabetes mellitus (DM), advances have been made in complementary or adjuvant therapy for the treatment of this complex disease (cite) One such example is the use of Kefir as a probiotic (O'Connor *et al.,* 2014), (Reusser & McCarran, 1994; Aihara & Kajimato, 2005).

An increase in kefir consumption has been reported in many countries, due to its unique sensory properties and long history associated with beneficial effects on human health (Farnworth, 2008; Otles & Cagindi, 2003; Wszolek *et al.,* 2006). Kefir benefits include lowering blood glucose as such it is used to control glycemia and reduce or delay the onset of complications associated with diabetes (Michel *et al.*, 2015). The consumption of probiotics may decrease the serum level of glucose and glucose tolerance in diabetes. (Davari *et al.*, 2013; Zhang *et al.,* 2014).

From a biological point of view, the chemistry of cellular structure and function is well established. Therefore, studying the chemical components in their natural locations in the cells and tissues, and tracking the changes that occur to them under abnormal conditions, whether pathological or experimental, is very important, as any change that occurs to these substances is often accompanied by some pathological manifestations" (Aref *et al.,*2021).
While the Kefir mitigates oxidative stress, lowers blood glucose and hyperglycemia, we do not fully understand how it acts. Therefore, we analyzed the pancreas to determine whether it protects pancreatic beta cells and also specify the possible beneficial effects of Kefir on controlling the glycemic state in diabetic rats. As shown in their study, Kefir treatment significantly reduced the progression of oxidative stress and STZ-induced hyperglycemia (type 1 diabetes mellitus) in rats.

MATERIALS AND METHODS

Experimental Animals:

White male albino rats (Wistar rat) (*Rattus norvegicus*) from order Rodentia and family Muridae were used in the present study. Experiments were carried out on 60 albino rats, aged 8 weeks and weighing 220-250 gm. The animals were obtained from the ENVIGO

Company, USA. IACUC Protocol Number (ORA use only): 2017-17.

Rats were kept in the Lab of Animal Research Facility (LARF) building, University of Idaho, USA, for 1 week under observation before experimentation to exclude any intercurrent infection and to acclimatize the animals to the new conditions. The selected animals were housed (3-4) in polycarbonate cages with softwood chips as bedding at a temperature of 23 ± 2°C, relative humidity of 50 ± 5% with good ventilation constant light/dark periods of 12 hours (hr.) each. Rats were either fed on a standard rodent pellet diet (for groups 1,2) or fed with a high-fat diet (Sirrivasan *et al.,* 2004), (groups (3, A, B, C), drinking tap water was provided at libitum for all groups. The composition of HFD is summarized in Table. 1.

Generally, the protocol followed the general guidelines of animal care. All efforts were made to minimize the number used and their suffering.

Induction of Diabetes Mellitus:

Diabetes mellitus was experimentally induced in overnight fasted male animals by an intraperitoneal (ip) injection of streptozotocin (STZ) at the dose of 45 mg/kg (Judiono *et al.,* 2011; Suharyo *et al.,* 2012; Giovana *et al.,* 2014). Streptozotocin was dissolved in cold 0.01 M citrate buffer, pH 4.5, and always prepared freshly for immediate use within 5 minutes. The normal control group was given citrate buffer without STZ. The development of diabetes was confirmed after 48 hours – 7 days of STZ injection. The animals with fasting blood glucose levels of more than 200 mg/dl were considered as diabetic and included in this study.

3. Animal grouping

Male rats were divided into six groups 10 animals each, 3 non-diabetic, and 3 diabetic groups:

A-Experiment I:

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water at a dose of 0.7 ml/animal/day.

Group 2: Animals were fed a standard diet and received oral administration of kefir (0.7 ml/animal/day).

Group 3: Animals received a high-fat diet (HFD) and additionally oral administration of kefir (0.7 ml/animal/day).

B-Experiment II:

Group A: Diabetic group (positive group), was fed HFD and received oral administration of distilled water (0.7 ml/animal/day).

Group B: Diabetic animals received HFD plus oral administration of kefir (0.7 ml/animal/day.

Group C: Diabetic group, fed HFD, was injected insulin (0.76 UI/200 mg BW/day).

By the end of the experimental time of 5 weeks, animals of all groups were fasted 4-6 hours, weighted, anesthetized by isoflurane, and sacrificed. The collected blood was centrifuged, and the serum was stored at -80 ◦C until use. Tissues for the histological investigations were excised immediately, fixed in Formal saline 4% for, and embedded in paraffin. Tissues were prepared for cell biological, histochemical, immunohistochemical, and pathological studies in the Autoradiographic lab. of Cell Biology and Immunology studies, Faculty of Science, South Valley University, Egypt. Under the supervision of Dr. Abdel-baset Aref Mohamed Aref, associated professor of cell biology and histochemistry, the manager of the lab., and the head of cell biology & histochemistry division, zoology depart. Faculty of science, SVU, Egypt, and the vice president of IACUC of SVU in Egypt.

Experimental Studies:

A. Cell biological Studies:

Karyometric studies were applied to the pancreatic cell. The volume of cell nuclei was performed using a camera program (LAS ZA). A total number of (200) nuclei were

measured/animal. The measurements were carried out according to shape nucleus (rounded nuclei) and the following equation was applied: V= 4/3π r3. Where: V= volume of nucleus, r = semi diameter (Lewinski *et al.*, 1984).

B. Histochemical Examinations Include:

I. DNA content changes (Feulgen reaction).
II. RNA materials content changes (toluidine blue technique).
III. Protein contents changes (bromophenol blue technique).
IV. Collagen contents changes (Masson's trichrome method).
V. Phospholipids materials content changes (Sudan Black B technique).

C. Histopathological Examination of Pancreatic Tissue:

The pancreas was immediately excised. Small tissue blocks were prepared and fixed in 4% neutral buffered formalin, then transferred to Washington State University, Veterinary School, Pathological lab, Pullman, WA, USA, for complete tissue process, 5 µm sections were stained in specific dyes such as Haematoxylin and eosin stain.

D. Immunohistochemical Examination: (Detection of insulin granules in pancreatic tissue)

Insulin granules were immunoassayed by rabbit insulin polyclonal antibody, labeled streptavidin biotin-complex (LSABC) technique, Harris hematoxylin counterstain.

E. Gross Morphology of Pancreatic Tissue:

The pancreas was dissected out and dried on filter paper. The absolute weight of the organ was determined, and its relative weight was calculated.

F. Biochemical Examination: Oral glucose tolerance test (OGTT) and insulin level.

This test was performed on normal and diabetic rats biweekly starting from the first day of the experiment. Overnight fasted (10-12 hours) animals were given 3 g/kg b.wt. glucose by gastric intubation. After oral administration, blood samples were taken at zero-time, 30 min., 60 min, and 90 min. from the caudal vein by using lancets and glucose measured immediately using AlphaTRAK 2 Blood Glucose Monitoring Kit obtained from ADW Diabetes company, USA. The test was done at the beginning of the experiment (Baseline), after 2 and 4 weeks.

Insulin measured in duplicate using a Millipore Rat Insulin (Billerica, MA, USA) RIA kit on 3/8-3/9/18 (Jia *et al.*, 2013).

Statistical Analysis:

Variables with a normal distribution were expressed as mean ± standard deviation. Variables with no normal distribution were expressed as median (25th -75th percentile). One-Way ANOVA test was used for comparing between groups mean of normally distributed variables. For multiple comparisons between different groups were done using the Post Hoc Tukey test. For not normally distributed variables, Kruskal- Wallis 1-way ANOVA test was used. Data were analyzed by using SPSS (Statistical Package for Social Science) version 24 software. P value < 0.05 was considered significant.

RESULTS

Cell biological Studies (Karyometric studies):

In experiment I:

In the pancreas of male rats of groups 1, group 2, and group 3, the values of mean volume nuclei of pancreatic cells were 141.9 ± 7µm, 139.4 ± 8 µm, and 76.3 ± 4 µm respectively (Fig. 1).

From the quantitative point of view, the daily receiving of kefir and standard food for 35 days decreased 1.8% the value of mean volume nuclei of pancreatic cells in the pancreas of male rats (group 2) versus those of control animals (group 1). While the daily receiving of kefir and high diet food for 35 days decreased 45.3% the value of mean volume nuclei of

pancreatic cells in the pancreas of male rats (group 3) versus those of rats which daily received kefir and standard diet food for 35 days (group 2) (Fig. 1).

In experiment II:

The pancreas of diabetic male rats (group A) showed value 31.6 ± 3 µm of the mean volume nuclei of pancreatic cells, while these values, of pancreatic cells of diabetic male rats which daily treated with kefir and insulin separately for 35 days (group B and group C) ware 25.2 ± 1 µm and 85.6 ± 9 µm respectively (Fig. 1).

From the quantitative point of view, the daily treatment with kefir and insulin separately for 35 days decreased 20.2% and increased 170.8% respectively, the values of mean volume nuclei of pancreatic cells of pancreas of diabetic male rats of group B and group C versus those of diabetic rats (group A).

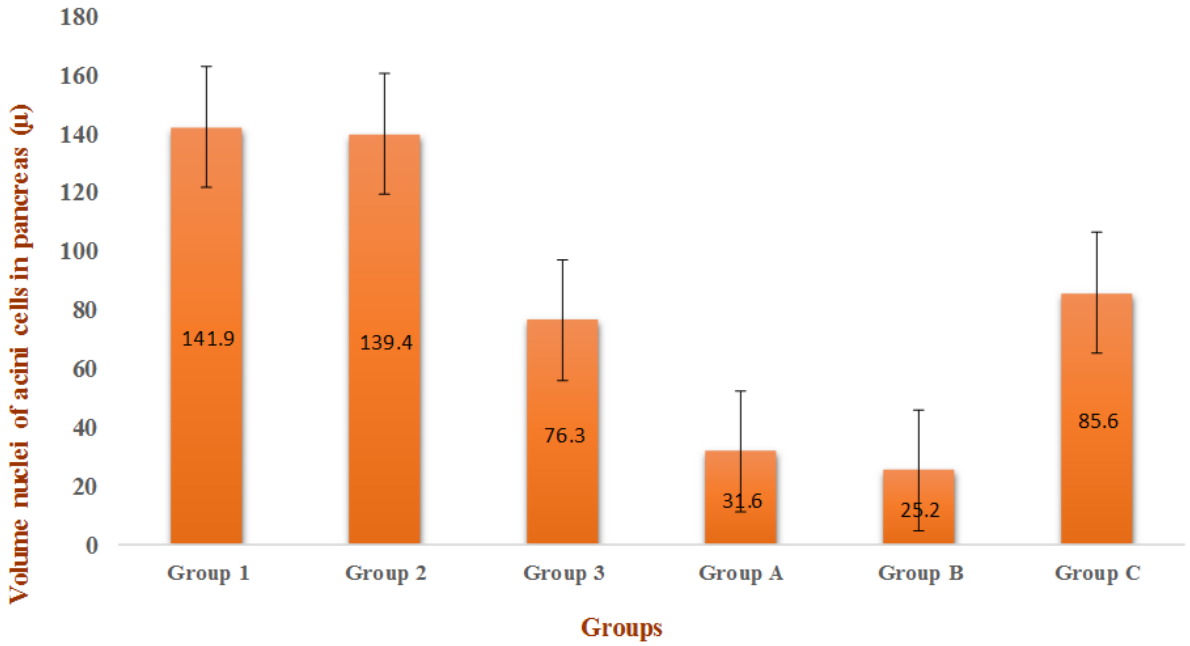

Fig. 1. The volume of nuclei in the pancreatic acinar cells of male rats in experiments I and II. The Mean volume of nuclei in the pancreatic acinar cells in the pancreas of male rats of groups 1, 2, and 3 of experiment I and groups A, B, and C of experiment II was calculated. The percentage of mean volume stimulation (S %) or inhibition (I %) was given.

Histochemical Examinations:

I. DNA Content Changes (Feulgen reaction):

Experiment I:

The pancreas of rat in C-N (group 1), C-N + Kefir (group 2), and CHFD+ Kefir (group 3) showed deeply stained coloration with high DNA content in cytoplasm and nuclei, according to that the DNA content showed no changes in comparison with the three groups (Fig.2).

Experiment II:

The pancreas of rats in Diab-HFD (group A), Diab-HFD+Kefir (group B), and Diab-HFD+ Insulin (group C) showed deeply stained coloration with high DNA content. Therefore, DNA content appeared no changes between the compared groups (Fig.2).

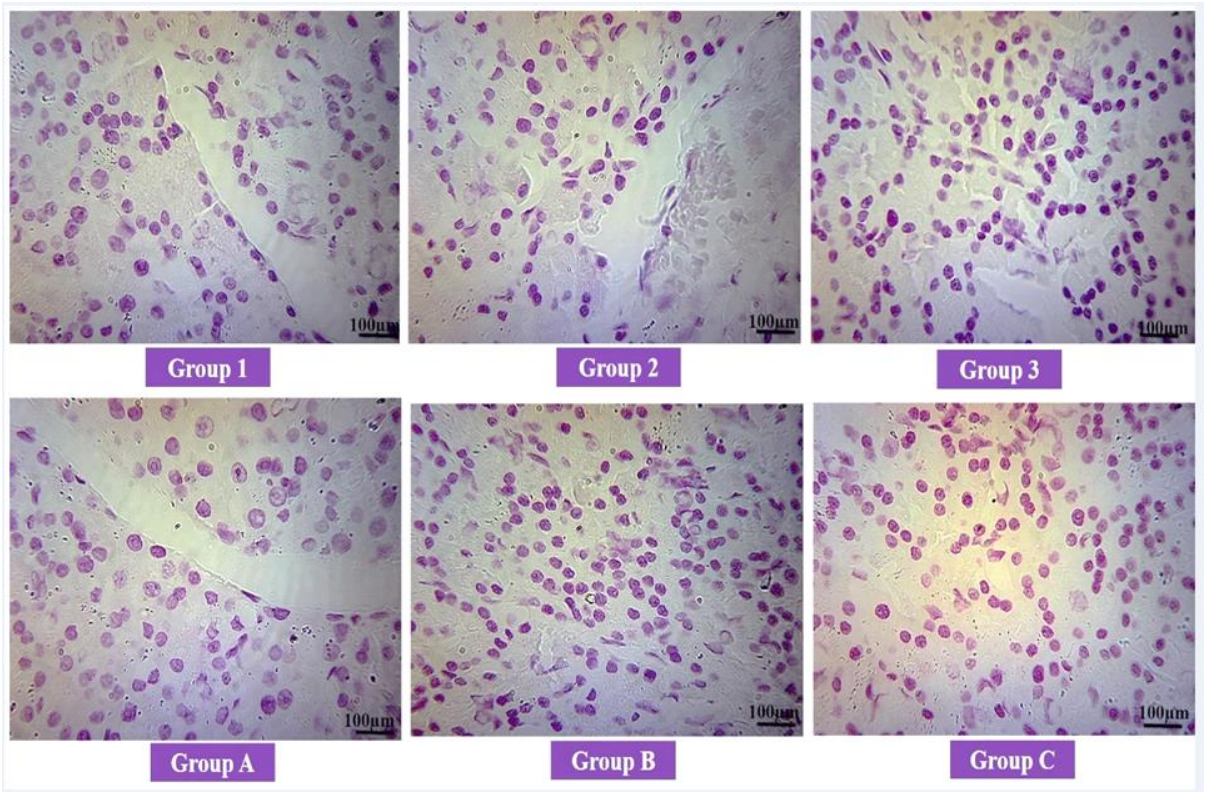

Fig. 2. Photomicrographs of the pancreatic tissue in the six rat groups in experiment I (groups 1, 2, and 3) and experiment II (groups A, B, and C) stained with the Feulgen method showing the DNA content in the pancreatic acinar cells.

II. RNA Materials Content Changes (toluidine blue):

Experiment I:

The pancreas of rats in group 1 and group 2 showed deeply stained coloration with high RNA materials content in cytoplasm and nucleolus of pancreatic tissue, but the pancreas of rats in group 3 showed moderately stained coloration with moderate RNA materials content. (Fig.3). RNA materials content revealed no changes in comparison between group 2 and group 1, while it was in group 3 slightly decreased than those in group 2.

Experiment II:

The pancreas of rats in group A showed moderately stained coloration with moderate RNA materials content, while the pancreas of rats in group B and group C showed deeply stained coloration with highly RNA materials content. RNA materials content in both group B and C slightly increased than those in group A (Fig. 3).

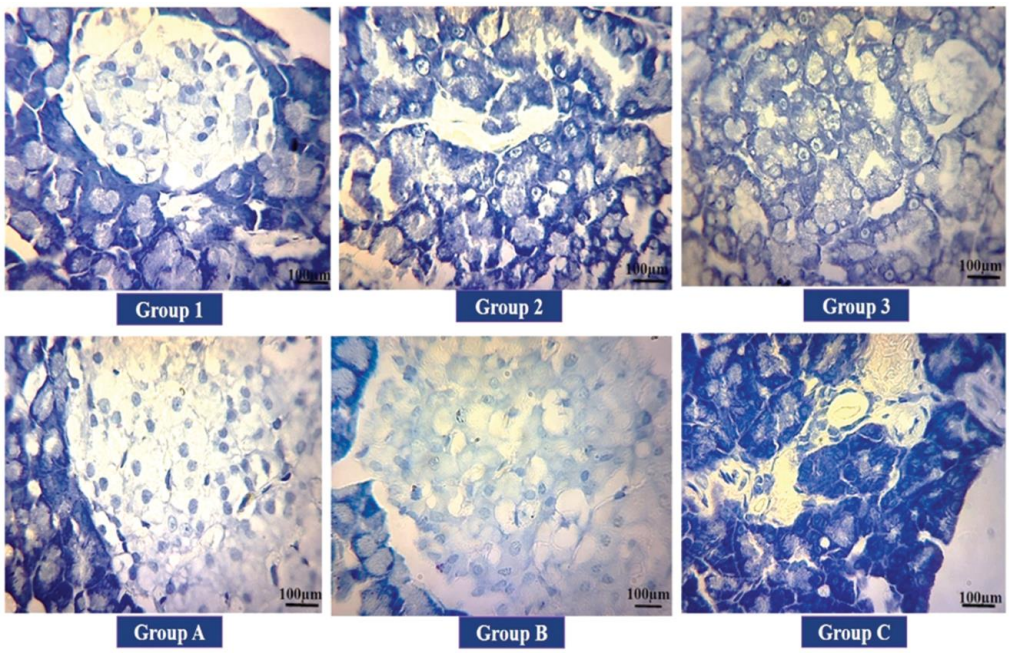

Fig. 3. Photomicrographs of the pancreatic tissue in the six rat groups in experiment I (group 1, 2, and 3) and experiment II (group A, B, and C) stained with Toluidine Blue method showing the RNA material content of the pancreatic acinar cells.

III. Protein Contents Changes (bromophenol blue technique):

Experiment I:

The pancreas of rats in group 1, group 2, and Group 3 revealed a deeply stained blue color with high protein contents in the islets of Langerhans's and exocrine pancreatic acini. The total protein contents appeared no changes in comparison between the three groups (Fig. 4).

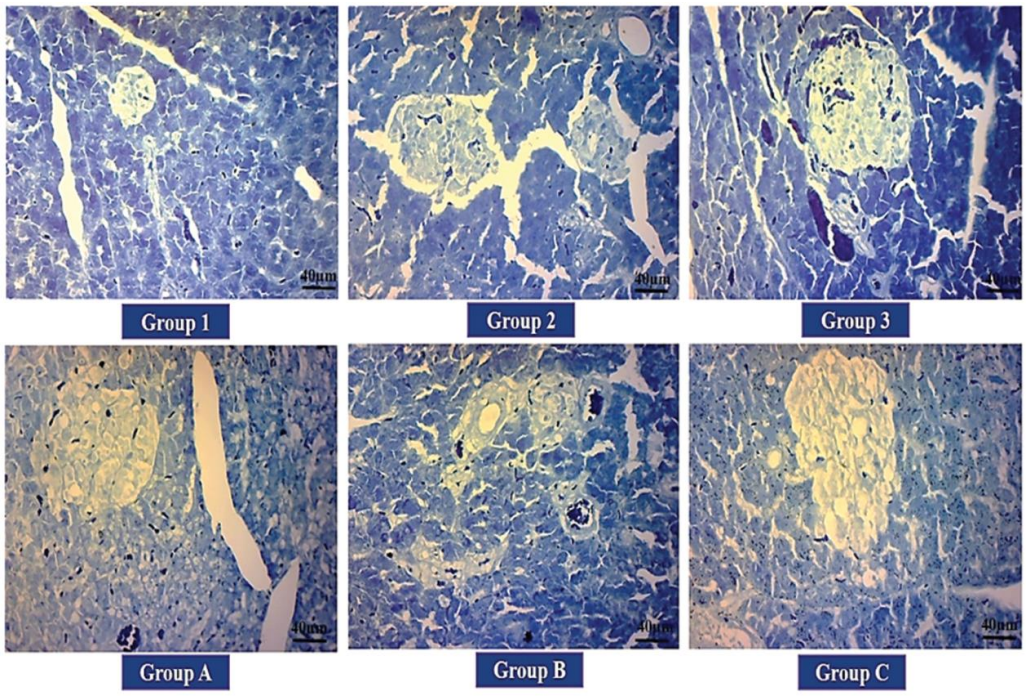

Fig. 4. Photomicrographs of the pancreatic tissue in the six rat groups in experiment I (group 1, 2, and 3) and experiment II (group A, B, and C) stained with Mercuric Bromophenol Blue method showing the total protein content of the pancreatic acinar cells.

Experiment II:

The pancreas of rats in group A and group C revealed moderate blue color with medium protein contents, while the pancreas of rats in group B revealed a deeply blue color with high protein contents. The total protein contents in group B slightly increased than those in group A and group C, while it in group C showed no changes versus group A (Fig. 4).

IV. Collagen Contents Changes (Masson's trichrome):

Experiment I:

The pancreas of rat in group 1 and group 2 showed moderate blue color with moderate collagen fibers; perivascular and interstitial fibrosis, while the pancreas of rat in group 3 showed deeply blue color with highly collagen fibers; perivascular and interstitial fibrosis. Collagen fibrosis content revealed no changes in comparison between group 2 and group 1, while it was in group 3 slightly increased than those in group 2 (Fig. 5).

Experiment II:

The pancreas of rats in group A, group B, and Group C showed a deeply blue color with high collagen fibers content, perivascular and interstitial fibrosis. Collagen fibrosis content appeared no changes in comparison between the three groups (Fig. 5).

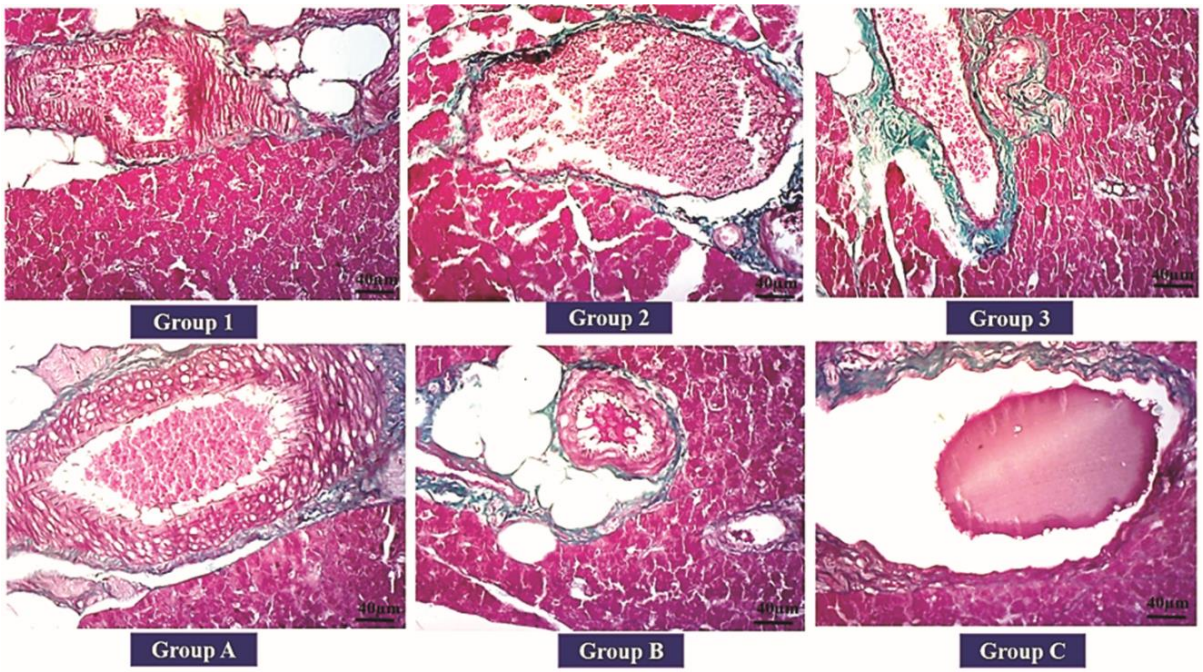

Fig. 5. Photomicrographs of the pancreatic tissue in the six rat groups in experiment I (group 1, 2, and 3) and experiment II (group A, B, and C) stained with Masson Trichrome method showing the collagen content of the pancreatic acinar cells.

V. Phospholipids Materials Content Changes (Sudan Black B technique):

Experiment I:

The pancreas of rats in group 1 exhibited faint black-blue coloration with few phospholipids materials content, while the pancreas of rats in group 2 and group 3 revealed moderate black-blue coloration with moderate phospholipids materials content. The Phospholipids materials content in group 2 slightly increased than those in group1, meanwhile, it was no changes between group 3 and group 2 (Fig. 6).

Experiment II:

A moderately black-blue coloration with moderate phospholipids materials content showed in the pancreas of rat in group A and group C, while a very deeply blue-black color with very highly phospholipids materials content appeared in the pancreas of rat in group B.

The Phospholipids materials content in group B highly increased than those in group A and group C, while it in group C showed no changes versus group A (Fig. 6). All data concerning DNA, RNA, total protein, collagen, polysaccharides, and lipoproteins content in the pancreatic acinar cells of experiments I and II were summarized in table 1.

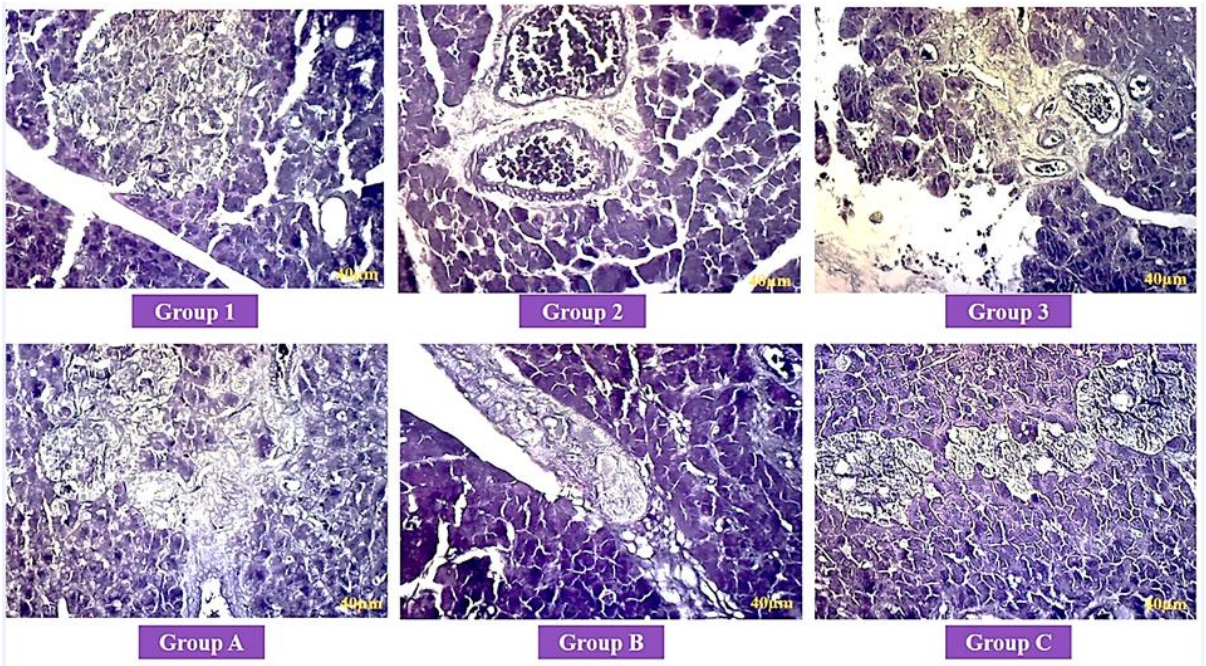

Fig. 6. Photomicrographs of the pancreatic tissue in the six rat groups in experiment I (group 1, 2, and 3) and experiment II (group A, B, and C) stained with Sudan Black B method showing the lipoprotein content of the pancreatic acinar cells.

Table 1: The histochemical score of the rat pancreas

Experiments	**Experiment I**			**Experiment II**		
Groups	**Group 1**	**Group 2**	**Group 3**	**Group A**	**Group B**	**Group C**
Feulgen reaction for DNA content in pancreas						
Red stained coloration	+++	++++	+++	+++	+++	+++
DNA contents	+++	++++	+++	+++	+++	+++
Toluidine blue for RNA content in pancreas						
Blue stained coloration	+++	+++	++	++	+++	+++
RNA contents	+++	+++	++	++	+++	+++
Bromophenol technique for protein contents in pancreas						
Blue stained coloration of protein content	+++	+++	+++	++	+++	++
Protein distribution inside cells	+++	+++	+++	++	+++	++
Masson's trichrome technique for collagen contents in pancreas						
Blue stained coloration of dense collagen fibers	++	++	+++	+++	+++	+++
Interstitial fibrosis	++	++	+++	+++	+++	+++
Perivascular fibrosis	++	++	+++	+++	+++	+++
Sudan Black B for phospholipids in pancreas						
Black stained coloration	+	++	++	++	++++	++
Lipoproteins contents	+	++	++	++	++++	++

The histochemical score of the rat pancreas of groups (1, 2 and 3) in experiment I and groups (A, B and C) in experiment II stained with Feulgen reaction for DNA, toluidine blue for RNA, bromophenol blue for total protein, Mallory trichrome technique for collagen, PAS for polysaccharides and Sudan black B for lipoprotein. Results severity were classified according to number of (+).

Histopathological Examination of Pancreatic Tissue.

Experiment I:

The pancreas of rat in C-N (group 1) revealed islets of Langerhans's and exocrine pancreatic acini lined with cuboidal epithelium, the pancreas of rat in C-N + Kefir (group 2) revealed well-developed cells of Beta islets of Langerhans and exocrine pancreatic acini, also, the pancreas of rat CHFD+ Kefir (group 3) revealed the normal histological structure of exocrine and endocrine parts (Fig. 7).

Experiment II:

The pancreas of rats in Diab-HFD (group A) revealed degeneration of beta cells and shrinkage and atrophy of acini also necrosis of beta cells. The pancreas of rats in Diab-HFD+Kefir (group B) revealed an almost similar histological picture to the control group; the same description at the higher magnification, it almost similar histological picture to the control group. The pancreas of rats in Diab-HFD+ Insulin (group C) revealed an almost similar histological picture to the control group (Fig. 7).

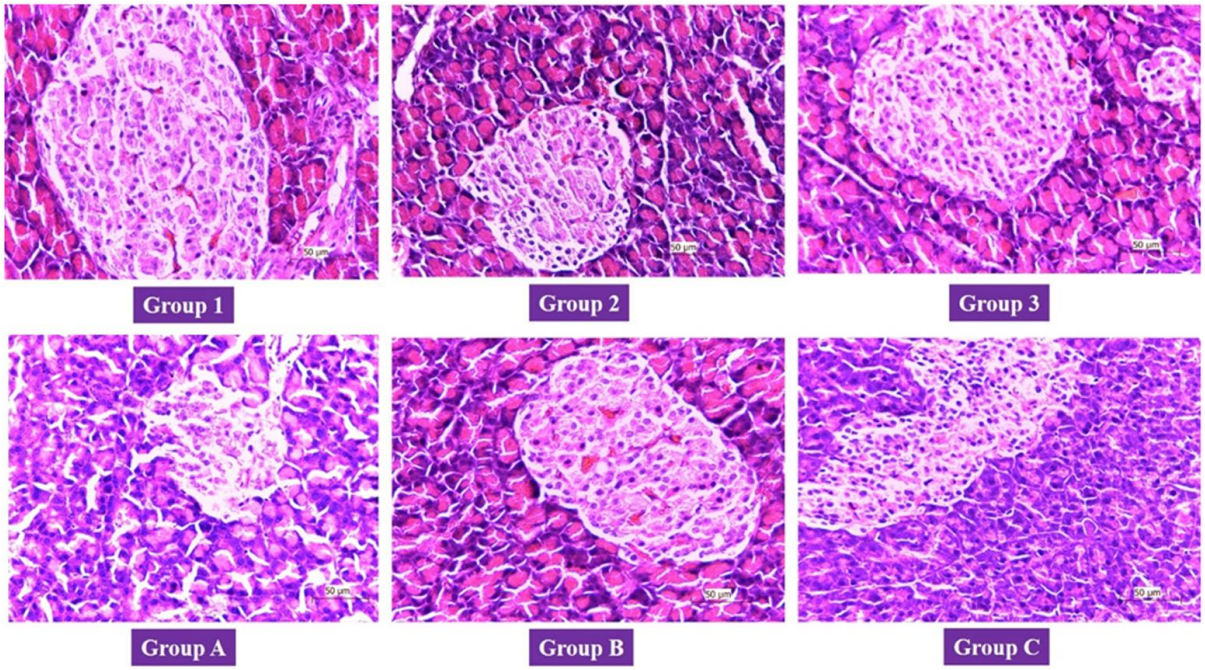

Fig. 7. Photomicrographs of the pancreatic tissue in the six rat groups in experiment I (group 1, 2, and 3) and experiment II (group A, B, and C) stained with H&E showing the pathological changes in pancreatic acinar cells.

Immunohistochemical Examination (Detection of insulin granules in pancreatic tissue):

Experiment I:

The pancreas of animal rats in group 1 and group 2 revealed beta-cells contained brown insulin granules immunostained by rabbit insulin polyclonal antibody, labeled streptavidin biotin-complex (LSABC) technique, Harris hematoxylin counterstain. While pancreas of rats in group 3 revealed beta-cells contained brown insulin granules with degenerated periphery zone of cells immunostained by the same method (Fig. 8).

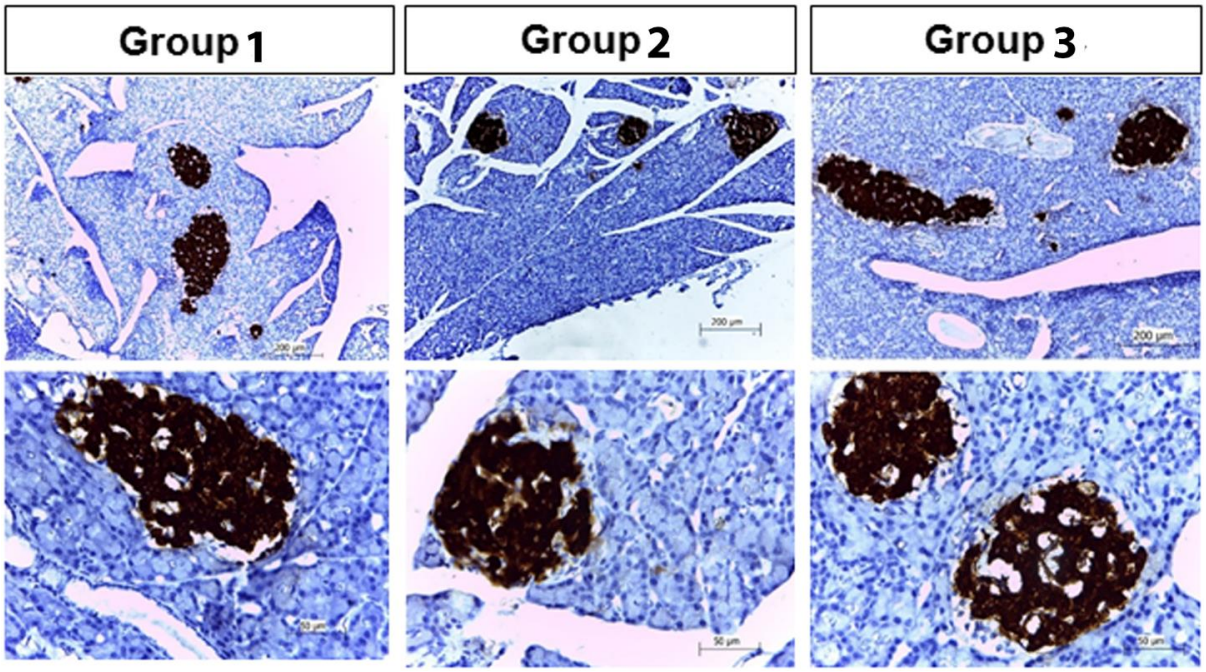

Fig. 8. Photomicrographs of the rat pancreatic tissue in the three groups of experiment I (1, 2, and 3) showing the immunoreactive signals of insulin granules inside the Langerhans Islands.

Experiment II:

The pancreas of rats in group A revealed beta-cells contained brown insulin granules with degenerated periphery zone of cells in Langerhans islets immunostained by rabbit insulin polyclonal antibody, labeled streptavidin biotin-complex (LSABC) technique, Harris hematoxylin counterstain (Fig. 9).

However, in group B and group C staining revealed well beta-cells contained brown insulin granules with few islands that were degenerated and atrophied, cells immunostained by the method before.

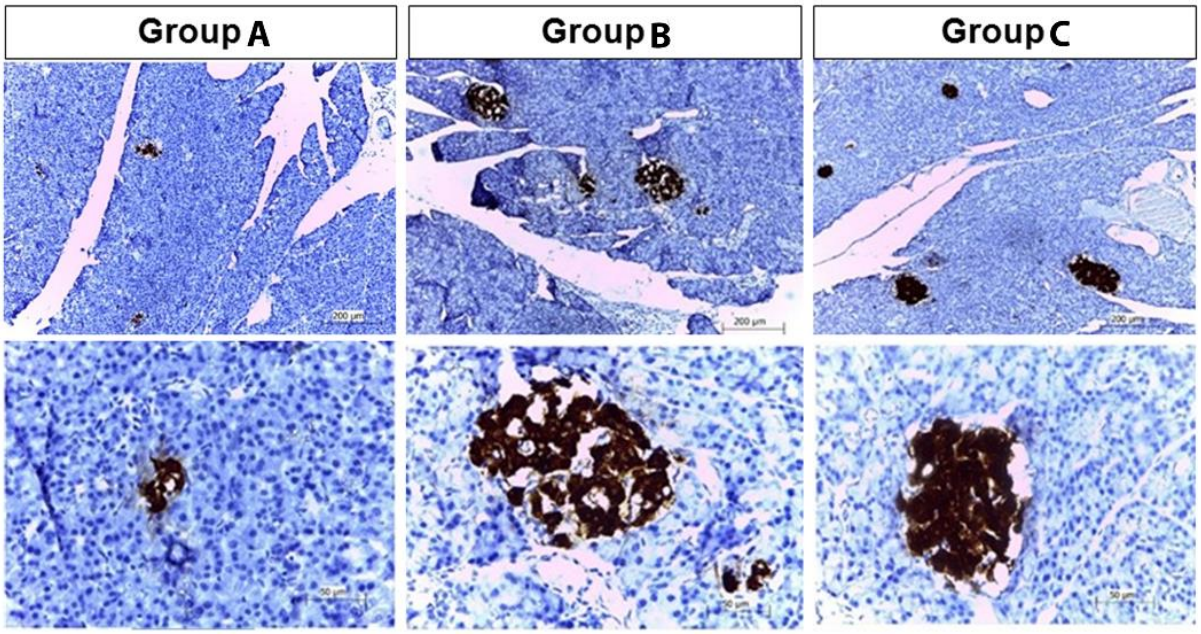

Fig. 9. Photomicrographs of the rat pancreatic tissue in the three groups of experiment II (A, B, and C) showing the immunoreactive signals of insulin granules inside the Langerhans Islands.

Gross Morphology of Pancreatic Tissue:

The mean relative weight of pancreas in both experiments I and II at the end of the experiment were calculated, P value showed non-significance between group 2 and group 1 or group 3 and group 2 in experiment I; similarity in experiment II, there was neither significance in comparison between group B and A nor group C and B (Fig. 10).

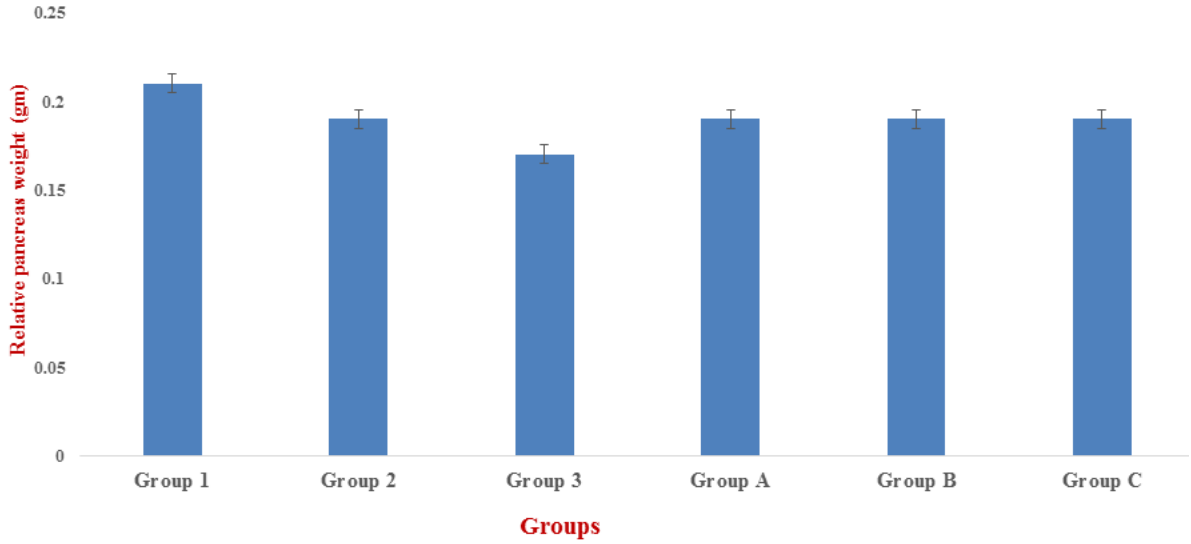

Fig. 10. The relative weight of the pancreas of both experiments at the end of the experiment.

Biochemical Examination:

1-Oral glucose tolerance test (OGTT):

A- Oral Glucose Tolerance Test (Week 0) Baseline:

The results of OGTT at week 0 is shown in figure 11 in experiment I there was non-significance in the glucose level between the groups at 0 times of the experiment while in experiment II it became significant through the interval times at 30 min., 60 min, and 90 min. after glucose administration between group B and A and began with non-significance between group C and B at 0 times then became significance ended with non-significance.

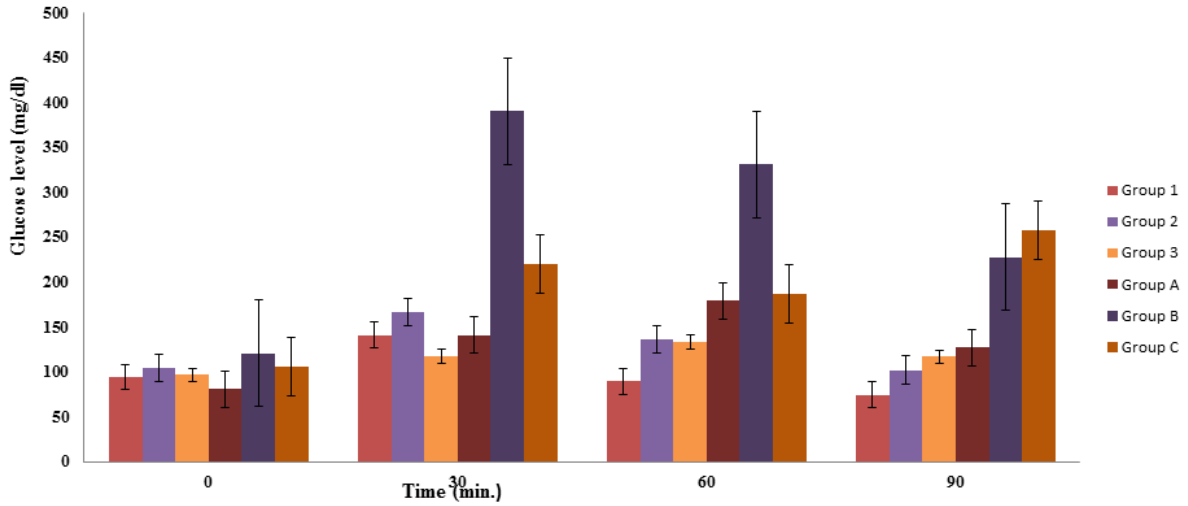

Fig. 11. Blood Glucose level curve at week 0 after oral glucose administration of two experiments (I and II) groups at different experimental periods.

B- Oral Glucose Tolerance Test (2 Weeks);

The results of OGTT data after 2 weeks are shown in figure 12; Summarizing the OGTT after two weeks of treatment all the groups in the experiment I showed normal glucose level after 90 min (group one, two, and three) ranging from 83 to 107 mg\dl, while in experiment II it significantly between group B and A but it showed non-significance between group C which treated kefir and group B which had insulin injected meanwhile showed an improvement in the glycemic state.

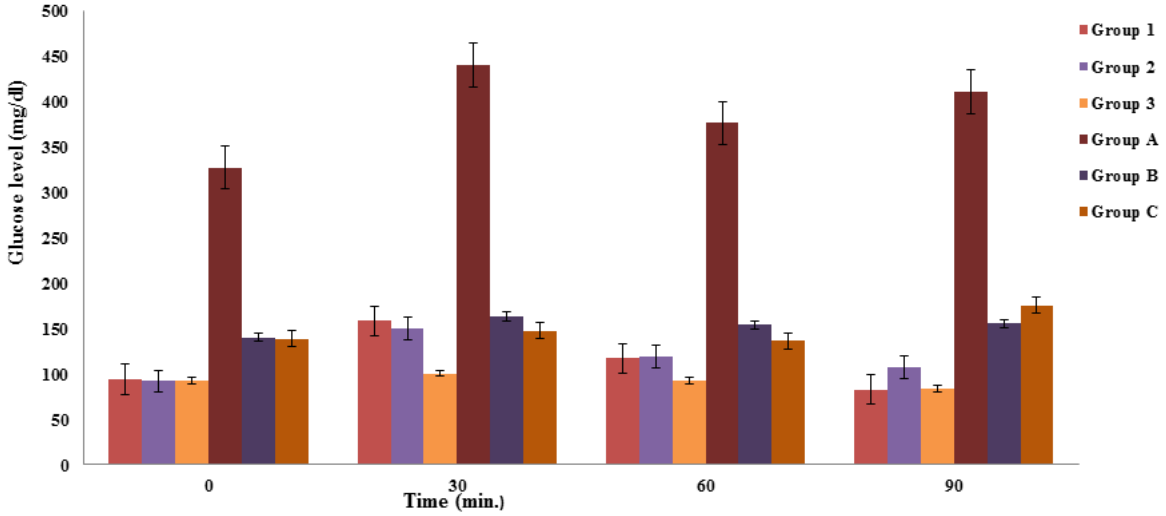

Fig. 12. Blood Glucose level curve at 2 weeks after oral glucose administration of two experiments (I and II) groups at different experimental periods.

C - Oral Glucose Tolerance Test (4 Weeks):

Figure 13 summarizes the data observed after 4 weeks of treatment, after 4 weeks in experiment I the glycemic state of non-diabetic rats after 90 min was still within the normal range although the group feed on a high-fat diet and treated with kefir showed the lowest value.

While in experiment II, the diabetic group's values are still higher blood glucose levels, but groups treated with kefir beverage and/or insulin dose showed improvement comparing with 2 weeks, it reached 156; 185 mg\dl respectively.

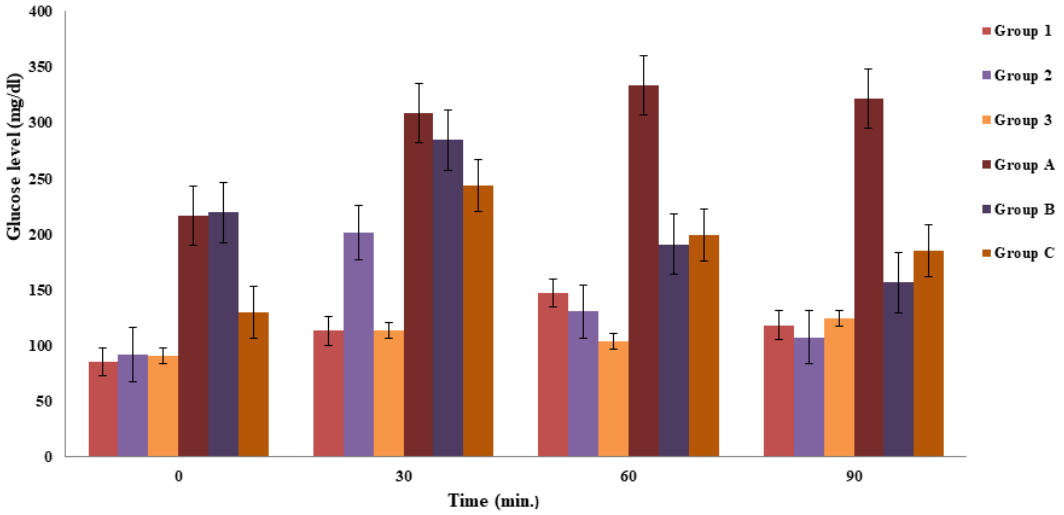

Fig. 13. Blood Glucose level curve at 4 weeks after oral glucose administration of two experiments (I and II) groups at different experimental periods.

2-Serum insulin (ng/ml):

Figure 14 explained the results of measuring the serum insulin level for all the six groups in both experiments, it was non-significant between the normal groups in experiment I, while in experiment II, there was non-significance between group B and A than significance between group C and B.

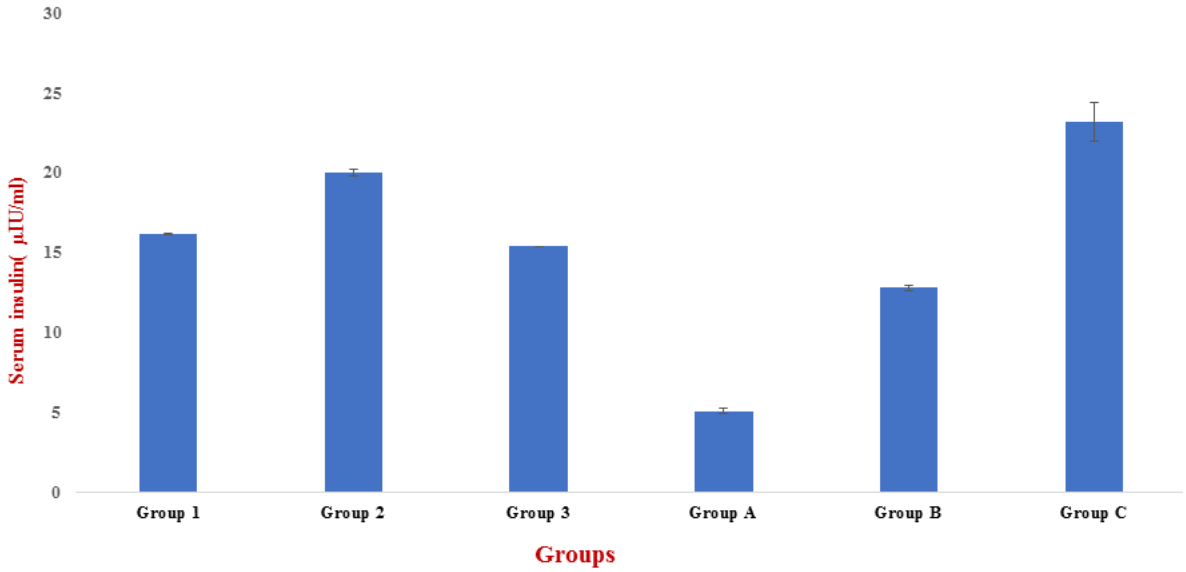

Fig. 14. Serum insulin (µIU/ML) of two experiments (I and I) groups at the end of the experiment.

DISCUSSION

In experiment I: the cell biological and histochemical results showed Kefir decreased both the volume of nuclei and RNA content, and increased both collagen and lipoproteins materials content in the normal pancreatic cells.

In experiment II: Our results showed kefir increased RNA, protein, and lipoproteins materials content and decreased the volume of nuclei of pancreatic cells in diabetic male rats. Immunohistochemically, kefir showed highly immunoreactive signals of insulin granules inside the Langerhans Islands.

Diabetes mellitus (DM) is a major public health problem all over the world and diseases rapidly. It is estimated that the number of adults with DM will be increased by 69% in developing countries and 20% in developed countries during the period between 2010 and 2030 (Shaw *et al.*, 2010).

The death rate in DM adults is 2-4 times higher than for non-DM adults, with cardiovascular diseases (CVD) being the most common cause of death (Roger *et al.*, 2012). The consumption of probiotics may decrease the serum level of glucose and glucose tolerance in diabetes (Davari *et al.*, 2013; Zhang *et al.*, 2014).

Kefir is one of the fermented milk derived from various species of lactobacilli. It is known that kefir has a lot of biological activities including antitumor, immunostimulating effect, an antioxidant action by reducing the lipid peroxidation, an antidiabetic effect, antibacterial and antifungal properties (Quiro's *et al.*, 2005). Therefore, the present study tends to investigate the effect of kefir consumption on and diabetic rats fed HFD. Diabetes was experimentally induced with streptozotocin. The effect of streptozotocin on the glycemic state of different animal species has been published in several reports (Gunnarson, *et al.*, 1974; Annamala & Augusti, 1980; Yamamoto *et al.*, 1981; Uchigata *et al.*, 1983; Okamoto,

1984; Al-Awadi *et al.,* 1985; Noreen *et al.,* 1988, 1992; El-Seifi *et al.,* 1993 a, b; Rawi, 1995; Rawi *et al.,* 1996; Abdel-Moneim *et al.,* 1997, 1999; Judiono *et al.,* 2011; Suharyo *et al.,* 2012; Giovana *et al.,* 2014).

Animals with chemically induced diabetes have been used to study either insulin-dependent diabetes mellitus (IDDM) (Wilson *et al.,* 1986; Mathe, 1995; O' Brien *et al.,* 1993; Ulicna *et al.,* 1996; Rawi *et al.,* 1996, 1998; Ohno *et al.,* 1998; Abdel-Moneim *et al.,* 1999) or non-insulin-dependent diabetes mellitus (NIDDM) (Ostenson *et al.,* 1989; Ali *et al.,* 1993; Masiello *et al.,* 1998).

In reviewing the previously published literature, it is obvious that a little or no amount of research work concerning the effect of Kefir or HFD or insulin on the volume of nuclei and histochemical contents; nucleic acids, proteins, and polysaccharides components, in the pancreas cells of control or HFD-diabetic-induced male rats has been performed.

According to these data and in reviewing the deficiencies relative to the effect of Kefir or insulin on the volume of nuclei and histochemical contents; nucleic acids, proteins, and polysaccharides components in the pancreas cells of normal or HFD-diabetic-induced male rats, therefore in this discussion, we described the present results of every single parameter of organ investigated without comparing it to publications of other authors dealing with the same parameter/organ.

Cell Biological Changes in The Pancreas (Karyometric studies): -

In experiment I: -

From the cytological point of view, although the kefir has a slightly inhibitory effect on the volume nuclei of pancreatic cells in the pancreas of normal male rats. Also, the high diet food has an inhibitory effect on the cellular activities of pancreatic cells in the pancreas of kefir-received male rats.

In experiment II: -

From the cytological point of view, kefir has an inhibitory effect while insulin has a high stimulatory effect on the cellular activities of pancreatic cells in the pancreas of diabetic male rats. The histochemical examination methods used for the determination of DNA content, RNA materials content, total proteins content, collagen fibers, and phospholipids content in the pancreas showed variable differences between the treated groups.

DNA content was nearly the same amount in both experiments I and II, so there were no effects by type of feeding diets or treatment doses.

In experiment I, the RNA material content was slightly decreased in animals fed HFD+kefir, so maybe it is the effect of HFD, while in experiment II, the RNA material content was slightly increased in the diabetic animals treated with kefir or insulin.

In experiment I, there was no effect by feeding different diets or kefir on the total proteins content, while in experiment II feeding diabetic animals with kefir increased the total proteins content than the other one's cause of the beneficial effects of kefir.

Regarding the collagen content in experiment I, there was a slight increase in the animal group fed on HFD+kefir and maybe it’s the diet effect. While the collagen contents were the same in the animal groups of experiment II.

The phospholipid materials content in experiment I was slightly decreased in the animal's group fed on SD only than the others, so perhaps feeding on kefir or HFD+kefir caused this increase. While in experiment II the diabetic animals treated with kefir had highly increase in their phospholipid material contents more than the other groups, so maybe the kefir caused this increase.

The present results show that the total protein and phospholipids materials contents in pancreatic cells of high fat-fed-streptozotocin-induced diabetic male rats which daily treated with kefir (group B) slightly and highly increased respectively than those in group A and group C, this is agreed with that kefir affects keep the bodyweight balanced on high fat-fed-

streptozotocin-induced diabetic male rats which fed with a daily kefir gavage dose (Aref *et al.*, 2020).

The histopathological images of the pancreas of rat in experiment I (normal animals) revealed islets of Langerhans's and exocrine pancreatic acini lined with cuboidal epithelium, that's mean there were no differences in the histological structures of pancreas between the three groups; while pancreas of rat in diabetic animals (experiment II) revealed degeneration of beta cells and shrinkage and atrophy of acini also necrosis of beta cells but treated diabetic animals with kefir and insulin revealed almost similar histological picture to the normal ones.

Immunohistochemically for pancreas tissue revealed beta-cells contained brown insulin granules in normal animals while the pancreas in diabetic animals revealed beta-cells contained brown insulin granules with degenerated periphery zone of cells but diabetic animals after treatment with kefir and insulin showed much improvement in their beta-cells.

The mean relative weight of the pancreas was calculated in each group at the end of the experiment, p-value showed non-significance between the six groups; the values were nearly the same in the untreated and treated rat males.

Urdaneta *et al*. (2007) and Sahin & Yardimci (2009) showed that using a kefir supplemented diet had no significant differences in the weight of the body organs examined.

Also, in the present study, it appeared the glycemic state was in the normal range in the normal males' rat even in group three which is treated with kefir beverage and feed on a high-fat diet. while the diabetic groups suffered from high blood glucose level, but groups treated with kefir beverage and insulin showed gradual improvement comparing within 2 weeks, it reached 178; 163 mg\dl respectively; Considering increasing daily kefir dose and the time of the treatment, perhaps the improvement of the glycemic state might be even better as it reported by several studies. In STZ-induced DM, it has been shown that daily administration of kefir caused an improvement in the increased levels of glycemia and glucose tolerance compared to conventional fermented milk (Yadav *et al.,* 2007; Hadisaputro *et al.*, 2012; Punaro *et al.*, 2014) Interestingly, kefiran, which is an exopolysaccharide isolated from kefir grains, has been shown to decrease blood pressure and blood glucose in animal models of hypertension (Maeda et al., 2004a) and an animal model of intolerance to glucose overload (Maeda *et al.*, 2004b).

The beneficial effects of cultured probiotics have also been demonstrated in experimental type 2 DM. Administration of a strain of the probiotic microorganism Lactococcus lactis in rats with type 2 DM induced by a high-fructose diet resulted in significantly lower fasting blood glucose, HbA1c, insulin, free fatty acids, and triglyceride levels than untreated DM rats (Yadav *et al.,* 2007).

The kefir effects observed on primary outcomes included decreased fasting blood glucose and HbA1c levels as well as improved insulin resistance (Ejtahed *et al.,* 2011; Ostadrahimi *et al.,* 2015; Hulston *et al.,* 2015; Firouzi *et al.,* 2016).

The consumption of probiotics may help decrease the serum level of glucose and glucose tolerance in diabetes (Davari *et al.,* 2013; Zhang *et al.,* 2014).

Prebiotics and probiotics have a great effect on insulin sensitivity, inflammatory markers, and glucose tolerance (Musso *et al.,* 2010).

Giovana *et al.,* (2014) showed in their study that Kefir treatment significantly reduced the progression of STZ-induced hyperglycemia and oxidative stress in rats.

Besides drug treatment for diabetes; in recent years, many efforts have been made on traditional medicines as complementary therapy in the treatment of diabetes. In this regard, probiotics have been considered in diabetic patients. (Guarner *et al.,* 2005).

Studies in vitro support that kefir is lowering blood glucose (Hadisaputro, 2011).

The underlying mechanism is probably via its bioactive components such as exopolysaccharide, peptide, antioxidants (Brown & Valiere, 2004; Virtanen, 2004). Maeda.

et al., (2004a) in vitro studies supported that kefir lowers blood glucose. Kefir can potentially be a useful choice for patients with diabetes who are required to control their blood glucose levels (Kong, 2009). It was proven with another study that kefir has hypoglycemic activities (Honda *et al.*, 2011; Wu *et al.*, 2011).

Results of measuring the serum insulin level for all the six groups in both experiments, it was non-significance between the normal groups in experiment I, so diets and treatments did not affect; while in experiment II, there was nonsignificant between untreated diabetic rats and the one treated with kefir, other than there was significance between the group treated with insulin and the one treated with kefir.

Hulston *et al.* (2015) reported that probiotic consumption has a positive effect on blood glucose concentrations and insulin sensitivity.

Probiotics may enhancement insulin resistance by reducing the inflammatory response in diabetes (Lye *et al.*, 2009).

The kefir effects observed on primary outcomes included decreased fasting blood glucose and HbA1c levels as well as improved insulin resistance (Ostadrahimi *et al.*, 2015; Hulston *et al.*, 2015; Ejtahed *et al.*, 2016; Firouzi *et al.*, 2016).

Kefir probably acts as follows: from the cell biological and histochemical points of view, the results showed an increase in the nuclei volume and DNA, RNA, and total protein contents in the pancreatic cells of diabetic rats treated with kefir. Also, the biochemical and immunohistochemical results showed an increase in insulin hormone (functional protein) of these pancreatic cells, which's probably a result of an increase in the cellular activities or/and the number of beta cells. Finally, we propose that kefir has a stimulatory effect on the cellular activity of beta cells leading to activation of genes responsible for the protein synthesis included insulin hormone, and/or increasing the cell proliferation of beta cells in the pancreas of diabetic rats.

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