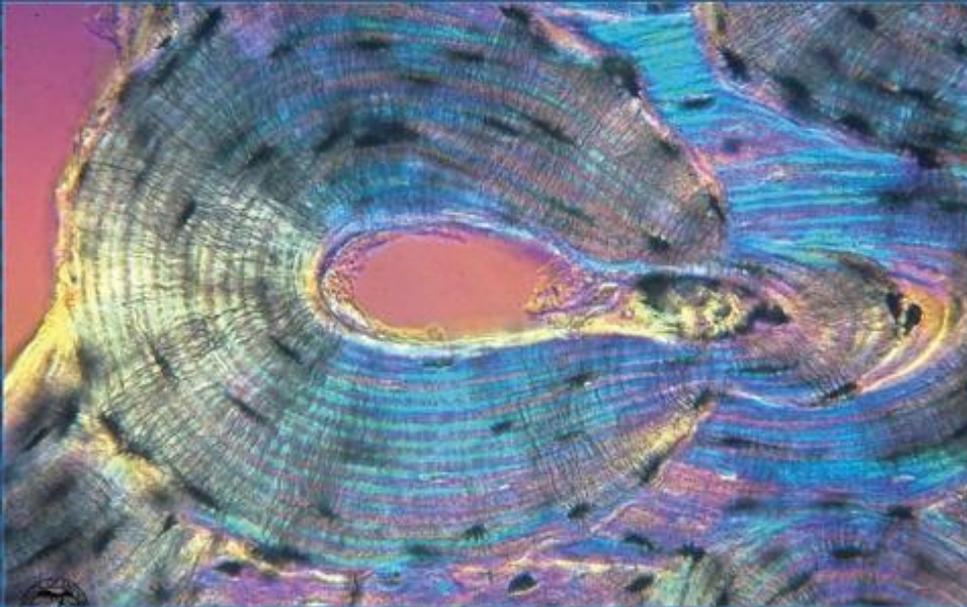




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**Possible Short-Term Biological Effects of Kefir: III: Efficacy of Kefir Beverage on The Cell Biological, Histochemical, Histopathological and Biochemical Changes in Kidney of High-Fat Fed STZ- Induced Diabetic Male Wistar Rat.**

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**ABSTRACT**

This study was designed to investigate the possible protective role of kefir beverage on kidney structure biologically, histochemical and some biochemical parameters such as cholesterol level, MCP-1, and resistin in high-fat diet-fed streptozotocin-diabetic rats. 60 rats were divided into two experiments by six groups: experiment I included 3 non-diabetic ones and experiment II included three STZ-induced diabetes groups. The groups were fed as follows: group 1 received a standard diet and served as control. Group 2 was fed on a standard diet and kefir (0.7 ml/animal/day by gavage). Group 3 received a high-fat diet and kefir (0.7 ml/animal/day by gavage). The diabetic males of groups A, B, and C were fed on the high-fat diet. Group B received besides kefir (0.7 ml/animal/day by gavage), while group C was injected additionally with insulin (0.76 UI/200 mg BW/day). After 5 weeks, animals of all groups were sacrificed. From the cell biological, histochemical, and histopathological conceptions, both kefir and insulin have various effects on cellular activities and different chemical materials contents, such as DNA, RNA, total protein, collagen, polysaccharides, and lipoproteins, in the examined normal and diabetic renal tubule cells of the male rats. Overall, it seems like kefir beverage may, to some extent, completely repair the diabetic pathological side effects such as kidney dysfunction regarding keeping the kidney tissue structurally almost normal with some changes in the histochemical components investigated, also there was an improvement in cholesterol level with a beneficial effect on lowering inflammatory markers. Finally, kefir highly reduces the collagen formation in the renal cells that prevent renal nephron from its function and we propose probably kefir treats kidney failure as a side effect of diabetes, by a mechanism that is opposite to the proposed mechanism, for the formation of this kidney failure induced by diabetes, via us in the present work.

## INTRODUCTION

Diabetes mellitus is important health care for all the aging different populations; approximately one-quarter of people over the age of 65 years have diabetes and one-half of older adults have prediabetes (Boyle *et al.*, 2018).

This percentage is expected to increase speedily in the coming decades. With higher rates of premature death, functional disability, accelerated muscle loss, hypertension, coronary heart disease, and stroke than those without diabetes (Kirkman *et al.*, 2012).

In the meantime, older adults with diabetes are at greater risk than other older adults for several common syndromes such as polypharmacy, cognitive impairment, urinary incontinence, injurious falls, and persistent pain (Institute of Medicine of the National Academies 2018).

Hyperglycemia, increased ketones in the bloodstream, and metabolic acidosis, and it might be caused by several factors, including reduced secretion and action of insulin, and raised levels of anti-insulin hormones (Kitabchi and Nyenwe 2006; Davis and Umpierrez 2007).

The main experimental and clinical studies have focused on the beneficial effects of dairy cultured probiotics (live microorganisms) as

coadjutants in the prevention/treatment of this metabolic disorder (Aune *et al.*, 2013).

## MATERIALS AND METHODS

### 1. 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 6 weeks, weighing about 220-250 g. The animals were obtained from the ENVIGO Company, USA. IACUC Protocol Number (ORA use only): 2017-17.

We placed the adult animals in the Laboratory Animal Research Facility (LARF) building, University of Idaho, USA, under observation for 1 week before experimentation to adapt the animals to the new conditions to live and to exclude any intercurrent infection. During the experimentation, the animals were marked and housed (2-3) rats in each polypropylene cage with bedding from softwood chips, kept at constant environmental conditions at a temperature of  $23 \pm 2^\circ\text{C}$ , light /dark cycle (12 hr), and humidity of  $50 \pm 5\%$  with good ventilation. The animals were fed on a standard rodent pellet diet or high-fat diet (Sririvasan *et al.*, 2004) and allowed to drink ad libitum.

### Composition of HFD:

Ingredients Diet	(g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
dl-Methionine	03
Yeast powder	01
Sodium chloride	01

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

### 2. Induction of Diabetes Mellitus:

Diabetes mellitus was experimentally induced in male animals

by streptozotocin, STZ was dissolved in cold 0.01 M citrate buffer, pH 4.5, and always prepared freshly for immediate use within 5 minutes. Rats were fasted for overnight to induce diabetes by intraperitoneal (IP) injection of streptozotocin (STZ) at the dose of 45

mg/kg body weight (Judiono *et al.*, 2011; Suharyo *et al.*, 2012; Giovana *et al.*, 2014). 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. For glucose assay, blood samples were collected from the tail-tip or tail-vein of the rats and measured using a glucometer.

### 3. Animal Grouping:

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

#### 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).

#### Experiment II:

**Group A-** The 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-** The diabetic group, fed HFD, was subcutaneously injected insulin (0.76 UI/200 mg BW/day).

At the end of the experiment, all rats have fasted 4-6 hours, weighed and collected their blood, were anesthetized, and then sacrificed. The kidney was immediately excised, 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 Hematoxylin and

eosin stain. Tissues were completed preparation 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. The kidney sections were stained according to the different examinations and techniques as follows:

### 4-Experimental studies:

#### 1-Cell Biological Studies:

Karyometric studies were applied to the kidney sections stained with Hematoxylin and eosin stain. 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:

$$V = 4/3\pi r^3.$$

Where: **V= volume of nucleus**, **r = semi diameter** (Lewinski *et al.*, 1984).

#### 2-Histochemical Examinations Include:

The kidney sections were stained according to the different histochemical examinations by histochemical techniques as follows

- 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. Polysaccharides content changes (Periodic acid-Schiff reaction).
- VI. Phospholipid's materials content changes (Sudan Black B technique).

#### 3-Histopathological Examination of Kidney Tissue:

For pathological studies, the kidney sections were stained in specific dyes such as Hematoxylin and eosin stain. All histochemical and

pathological methods were applied according to Carleton *et al.* (1980).

#### **4-Gross Morphology of Kidney Tissue:**

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

**5-Biochemical examination:**  
Determination of total cholesterol, MCP-1, and resistin:

Collected blood sera from each group were frozen after the sacrificed process in  $-80^{\circ}$  C, then send to Metabolism Core Laboratory/ Human Physiology Core Laboratory, University of Alabama (USA).

**-Total Cholesterol** was measured using a Sirus Stanbio Analyzer (Boerne, TX) using a colorimetric reagent (Root *et al.*, 2013).

**-MCP-1** was measured in duplicate with two MesoScale Discovery (Rockville, Maryland 20850-3173, USA) Rat MCP-1 kits using chemiluminescence. assays on 2/20/18 & 2/21/19 (Lee *et al.*, 2017).

**-Resistin** run in two Mesoscale Discovery (Rockville, Maryland 20850-3173, USA) Mouse/Rat Resistin kits using chemiluminescence on 2/21/18 & 2/22/18 (Cludts *et al.*, 2017).

#### **5-Statistical analysis:**

Variables with a normal distribution were expressed as mean  $\pm$  standard deviation. Variables with no normal distribution were expressed as median (25th -75th percentile). One-Way ANOVA test was used for comparing the mean of variables that were normally distributed between groups. Multiple comparisons between different groups were done using the Post hoc Tukey test. For variables that were not normally distributed, 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**

### **1-Cell Biological Studies:**

#### **Cell Biological Changes in Renal Tubules Cells in The Cortex (Karyometric studies):**

##### **In Experiment I:**

In the kidney of male rats of groups 1, group 2, and group 3, the values of mean volume nuclei of renal tubules cells were  $61.5 \pm 16 \mu\text{m}$ ,  $100.8 \pm 23 \mu\text{m}$ , and  $74.3 \pm 11 \mu\text{m}$  respectively (Fig. 1).

From the quantitative point of view, the daily receiving of kefir and standard food for 35 days increased 63.9% the value of mean volume nuclei of renal tubules cells in kidney of male rat (group 2) versus those of control rat (group 1). While the daily receiving of kefir and high diet food for 35 days decreased 26.3 % the value of mean volume nuclei of renal tubules cells in kidney of male rat (group 3) versus those of rats which daily received kefir and standard diet food for 35 days (group 2).

From a Cell biological point of view, kefir has a stimulatory effect on the cellular activity of the normal renal tubule cells.

##### **In Experiment II:**

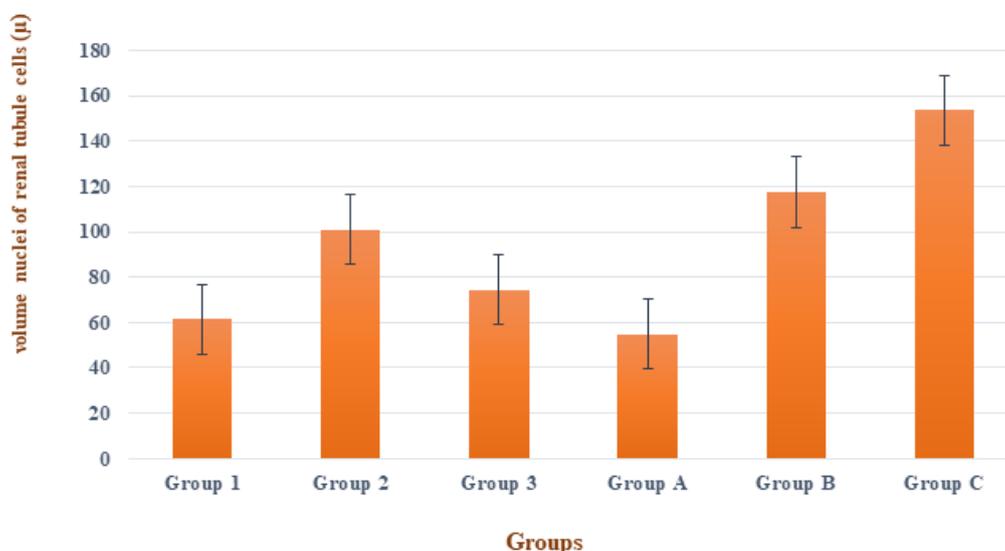
The kidney of diabetic male rats (group A) showed value  $54.7 \pm 12 \mu\text{m}$  of the mean volume nuclei of renal tubules cells, while these values, of renal cells of diabetic rats which daily treated with kefir and insulin separately for 35 days (group B and group C) were  $117.4 \pm 38 \mu\text{m}$  and  $153.7 \pm 36 \mu\text{m}$  respectively (Fig. 1).

From the quantitative point of view, the daily treatment with kefir and insulin separately for 35 days increased 114.6% and 180.9%, respectively, the value of mean volume nuclei of renal tubules cells of group B and group C versus those of (group A). The kidney of male rats which were treated with insulin (group C) showed that the value of mean volume nuclei in renal cells

was higher 30.9% than those of rats treated with kefir (group B).

From a Cell biological point of view, both kefir and insulin have a

stimulatory effect on the cellular activity of both the examined normal and diabetic renal tubule cells.



**Fig.1:** Mean Nuclear Volume in the Renal Tubular Cells of the Control and Treated Mice in Experiments I (Groups 1, 2, and 3) and II (Groups A, B, and C).

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.

**2-Histochemical Examinations Include:**

**1-DNA Content Changes (Feulgen reaction):**

**Experiment I:** The kidney of rat in C-N (group 1) showed deeply stained coloration with high DNA content in nucleus and nucleolus of renal cells; while kidney of rat in C-N + Kefir (group 2) and C-HFD+ Kefir (group 3) revealed very deeply stained coloration with very high DNA content (Table 1& Fig. 2). DNA content in group 2 slightly increased than those in group 1, while

there were no changes in comparison between groups 3 and 2.

**Experiment II:** The kidney of rats in Diab-HFD (group A), Diab-HFD+Kefir (group B), and Diab-HFD+ Insulin (group C) showed very deeply stained coloration with very high DNA content. DNA content revealed no changes in comparison between the three groups (Table 1& Fig. 2).

From the histochemical concept, kefir does not affect the DNA content of both the normal and diabetic renal tubule cells.

**Table 1:** The histochemical score of kidneys of rat 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 (+).

	Experiment I			Experiment II		
	Group 1	Group 2	Group 3	Group A	Group B	Group C
<b>Feulgen reaction for DNA content in kidney</b>						
Red stained coloration	+++	++++	++++	++++	+++++	+++++
DNA contents	+++	++++	++++	++++	+++++	+++++
<b>Toluidine blue for RNA content in kidney</b>						
Blue stained coloration	+++	+++	++	++	++	++
RNA contents	+++	+++	++	++	++	++
<b>Bromophenol technique for protein contents in kidney</b>						
Blue stained coloration of protein content	+++	+++	++	++	+++	++
Protein distribution inside cells	+++	+++	++	++	+++	++
<b>Masson's trichrome technique for collagen contents in kidney</b>						
Blue stained coloration of dense collagen fibers	+	+	+	++	+	+
Interstitial fibrosis	+	+	+	++	+	+
Perivascular fibrosis	+	+	+	++	+	+
<b>PAS for polysaccharides in kidney</b>						
Red stained coloration of glycogen content	++	++	++	++	++	++
Polysaccharides precipitation inside cells	++	++	++	++	++	++
<b>Sudan Black for phospholipids in kidney</b>						
Black stained coloration	+++	++	+++	++	++++	++
Lipoproteins contents	+++	++	+++	++	++++	++

**Group 1<sub>N</sub>:** Control animals, (negative group) were fed standard diet plus oral administration of distilled water

**Group 2:** Animals were fed standard diet and received oral administration of kefir

**Group 3:** Animals received high fat diet (HFD) and additionally oral administration of kefir

**Group A:** Diabetic group (positive group), was fed HFD and received oral administration of distilled water

**Group B:** Diabetic animals received HFD plus oral administration of kefir

**Group C:** Diabetic group, fed HFD, was injected insulin

## 2-RNA Materials Content Changes (toluidine blue):

**Experiment I:** The kidney of rats in group 1 and group 2 showed deeply stained coloration with high RNA materials content in cytoplasm and nuclei of renal cells, but the kidneys of rats in group 3 showed moderately stained coloration with moderate RNA materials content (Table 1 & Fig. 3). RNA materials content showed no changes in comparison between group 2 and group 1, while RNA in group 3 slightly decreased than those in group 2. **Experiment II:** The kidney of rats in groups A, B, and C showed moderately stained coloration with moderate RNA materials content. There were no changes in RNA materials content in comparison between the three groups (Table 1 & Fig. 3). From the

histochemical concept, kefir does not affect the RNA content of both the normal and diabetic renal tubule cells.

## 3-Protein Contents Changes (bromophenol blue technique).

**Experiment I:** The kidney of rats in both group 1 and group 2 revealed a deeply stained blue color with high protein contents inside glomeruli and renal tubule cells. While kidney of rat group 3 revealed moderate blue color with moderate protein contents inside glomeruli and renal tubule cells (Table 1 & Figure 4). There were no changes in protein contents between group 2 and group 1, while the protein contents in group 3 slightly decreased than those in group 2.

**Experiment II:** The kidney of rats in group A and group C revealed moderate blue color with moderate protein

contents inside glomeruli and renal tubule cells. Moreover, while kidney of the rat in group B revealed a deeply blue color with high protein contents. The protein contents in group B were slightly raised than those in groups A and C (Table 1 & Fig. 4).

Histochemically, Kefir has a slight stimulatory effect on protein synthesis in diabetic renal cells.

#### **4-Collagen contents changes (Masson's trichrome).**

**Experiment I:** The kidney of rats in groups 1, 2, and 3 showed faint blue color with few distributions of collagen material contents; perivascular and interstitial fibrosis (Table 1 & Fig. 5). There were no changes in comparison between the three groups.

**Experiment II:** The kidney of rats in group A showed moderate blue color with moderate periglomerular, perivascular and interstitial fibrosis, while the Kidney of the rat in both group B and group C showed faint blue color with few distributions of collagen fibers; perivascular and interstitial fibrosis. The collagen fibrosis contents in both group B and group C were moderately decreased than those in group A (Table 1 & Fig. 5).

Histochemically, both kefir and insulin have a slight inhibitory effect on collagen synthesis in diabetic renal cells.

#### **5-Polysaccharides Content Changes (Periodic acid-Schiff reagent) (PAS):**

**Experiment I:** The kidney of rats in groups 1, 2, and 3 revealed the same moderate red coloration with moderate polysaccharides materials content inside glomeruli and renal tubules cells (Table 1 & Fig. 6). In comparison between the three groups, the amount of polysaccharides content showed no changes.

**Experiment II:** A moderately stained red coloration with moderate polysaccharides content was detected inside glomeruli and renal tubule cells in the kidney of rats in groups A, B, and C. Polysaccharides content showed no

changes between the compared groups (Table 1 & Fig. 6).

From the concept of histochemistry, both kefir and insulin do not affect polysaccharides synthesis in both normal and diabetic renal tubule cells.

#### **6-Phospholipids Materials Content Changes (Sudan Black B technique):**

**Experiment I:** The kidney of rats in group 1 and group 3 showed deeply black blue coloration with highly lipoproteins materials contained within the cells of the renal tubules, while the kidney of rats in group 2 revealed moderate black blue coloration with moderate lipoproteins materials content (Table 1 & Figure 7). The Phospholipids materials content in group 2 was slightly lowering than those in group 1, while phospholipids materials content in group 3 slightly increased compared with group 2.

**Experiment II:** A moderately black blue coloration with moderate lipoproteins materials content inside glomeruli and renal tubules cells in the kidney of rat in group A and group C, while a very deeply blue-black color with a very highly lipoproteins materials contents observed in the kidney of rat in group B. The Phospholipids materials content in group B highly increased than those in group A and group C (Table 1 & Fig. 7).

From the Histochemical point of view, kefir has a highly stimulatory effect on lipoproteins materials in the diabetic renal cells, in contrast, it has an inhibitory effect in the normal renal cells.

#### **3-Histopathological Examination of Kidney Tissue:**

**Experiment I:** Figures (8 & 9) showed the pathological changes that occurred in the kidney in the six rat groups using different magnifications. The kidney of rats in C-N (group 1) and C-N + Kefir (group 2) revealed normal renal corpuscles contained tufts of capillaries and convoluted tubules lined with

cuboidal epithelium, also kidney of rat CHFD+ Kefir (group 3) revealed no microscopic alteration in renal tissue. Histopathologically, kefir does not affect the normal pathological features

**Experiment II:** The kidney of rat in Diab-HFD (group A) revealed vacuolization of renal tubules especially distal convoluted tubules and focal fatty changes in interstitial renal tissue; higher magnification revealed vacuolization of distal convoluted tubules; hyaline casts of renal tubules especially distal convoluted tubules; focal fatty changes in interstitial renal tissue and renal necrotic changes with minimal calcium precipitation. The kidney of rat in Diab-HFD+Kefir (group B) revealed almost normal renal tissue except for few vacuolated renal tubules; also, under the higher magnification revealed almost normal renal tissue except for few vacuolated renal tubules, while kidney of rat in Diab-HFD+ Insulin (group C) revealed mild focal vacuolization of renal tubules; Higher magnification revealed mild vacuolization of the apical portion of lining epithelium of few renal tubules (Figs. 8 & 9).

From the Histopathological point of view, kefir has beneficial efficacy and it is possible to repair the diabetic pathological changes in the renal tubule cells in the male rat.

#### **4-Gross Morphology of Kidney Tissue:**

The relative weight of kidneys was explained in figure 10 there was non-significance between the normal groups in experiment I according to the P value; while the relative kidney weight in the diabetic groups was in experiment II significantly increased between group C and B but there was non-significance between group B and A.

#### **5-Biochemical Examination: Determination of Total Cholesterol, MCP-1, And Resistin:**

**-Total Cholesterol (mg/dl):** Results obtained from measurement of the total cholesterol level in the blood serum for the six groups in both experiments at the end of the experiment represented in figure 11 there was non-significance in experiment I according to the P value; however, the results showed a highly significant decrease in diabetic ones which treated with kefir beverage and insulin.

Biochemically, both kefir and insulin have a high inhibitory efficacy in the total cholesterol level in the blood serum of diabetic male rats.

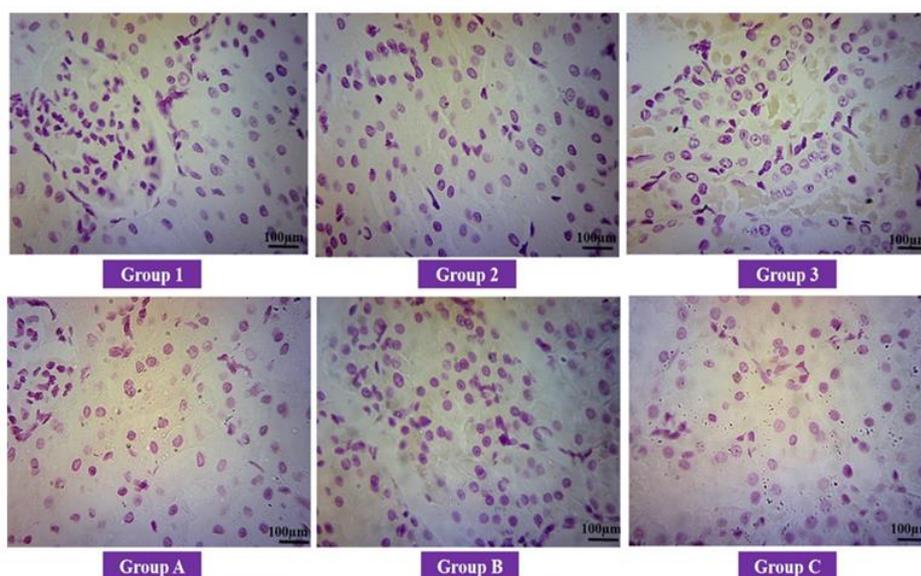
**-Serum MCP-1 (pg/ml):** Figure 12 showed the mean serum MCP-1 level in the six rat groups; there was non-significance in experiment I according to the P value; however, the results showed a highly significant decrease in diabetic ones treated with kefir beverage and insulin compared with the non-treated diabetic one.

Biochemically, both kefir and insulin have a high inhibitory efficacy of the serum MCP-1 of diabetic male rats.

**- Serum Resistin (pg/ml);** The mean serum resistin was non-significant between normal animal groups with less value in experiment I, the data represented in figure 13; while the diabetic ones in the experiment II had high significance changes between the groups.

Biochemically, kefir has high significantly inhibitory efficacy serum resistin of diabetic male rats.

From the biochemical point of view, both kefir and insulin have high significantly inhibitory efficacy in the total cholesterol level, MCP-1, and resistin in the blood serum of diabetic male rats, exceptionally insulin does not affect the serum resistin.



**Fig.2:** Photomicrograph of the kidney (cortex) in the six rat groups in experiment I (groups 1, 2, and 3) and experiment II (groups A, B, and C) stained with Feulgen method to the identification of DNA, bar = 100x.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

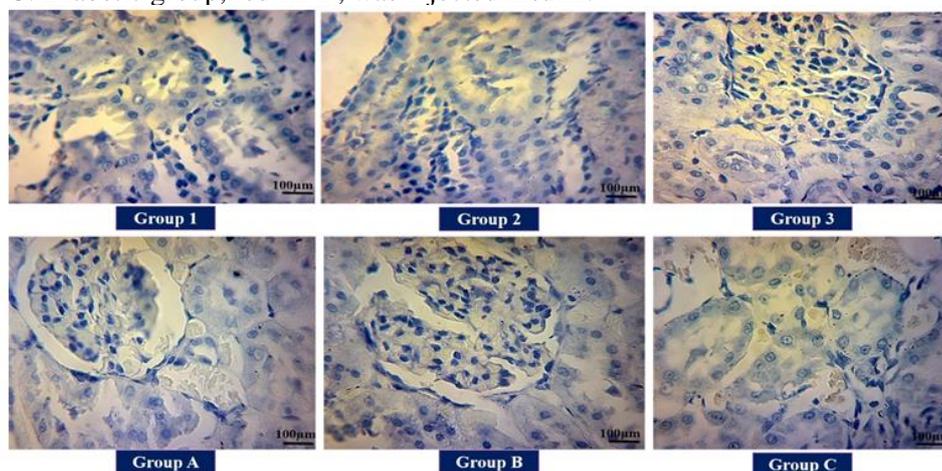
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.3:** Photomicrograph of the kidney (cortex) in the six rat groups in experiment I (groups 1, 2, and 3) and experiment II (groups A, B, and C) stained with Toluidine blue method to the identification of RNA, bar = 100x.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

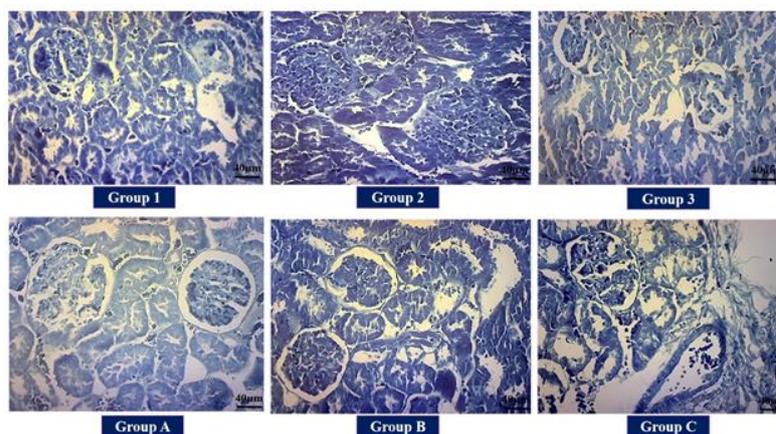
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.4:** Photomicrograph of the kidney (cortex) 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 to the identification of the total protein, bar = 40x.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

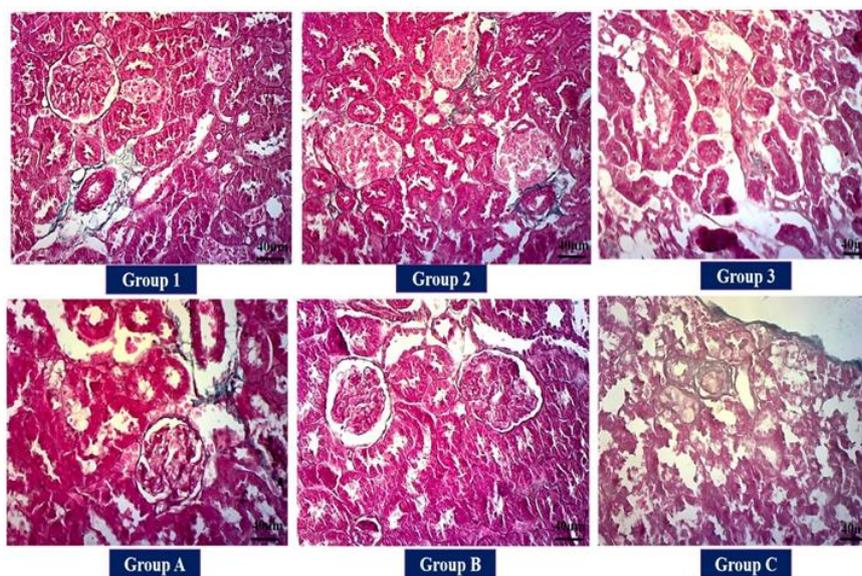
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.5:** Photomicrograph of the kidney (cortex) in the six rat groups in experiment I (groups 1, 2, and 3) and experiment II (groups A, B, and C) stained with Masson trichrome method to the identification of collagen, bar = 40x.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

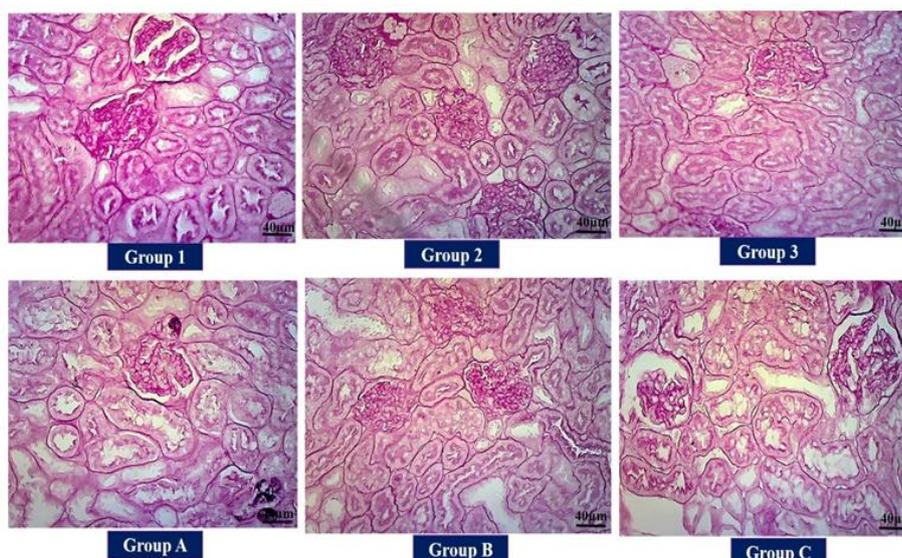
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.6:** The carbohydrates distribution and condenses in the kidney tissue in the different rat groups; PAS, bar= 50 µm.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

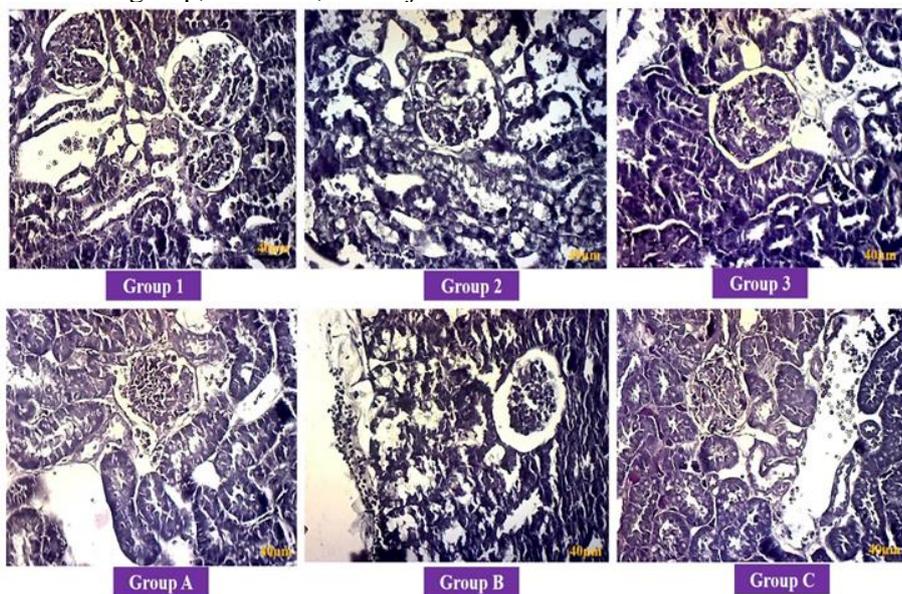
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.7:** Photomicrograph of the kidney (cortex) in the six rat groups stained with Sudan black B method to the identification of lipoproteins, bar = 40x.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

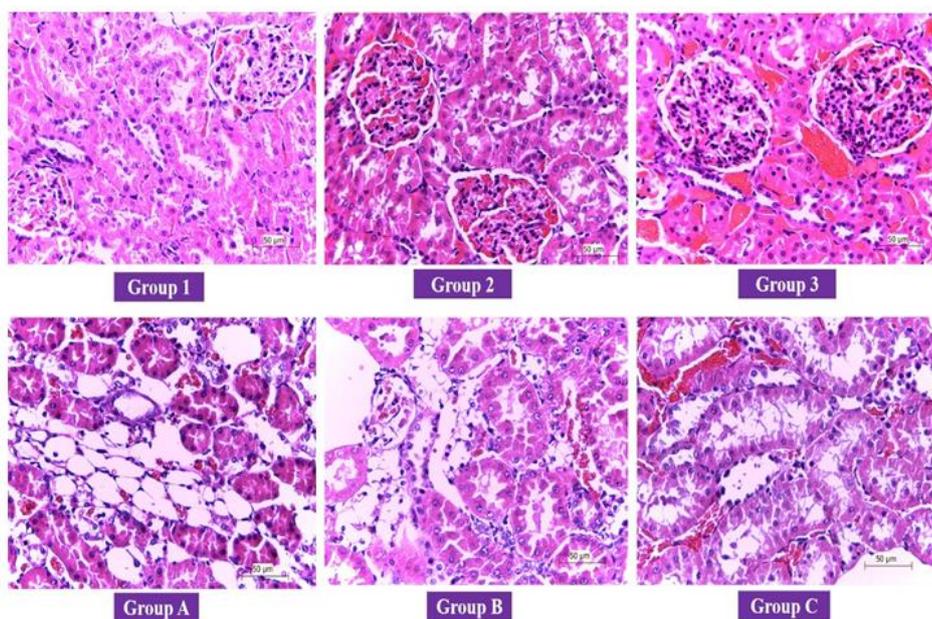
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

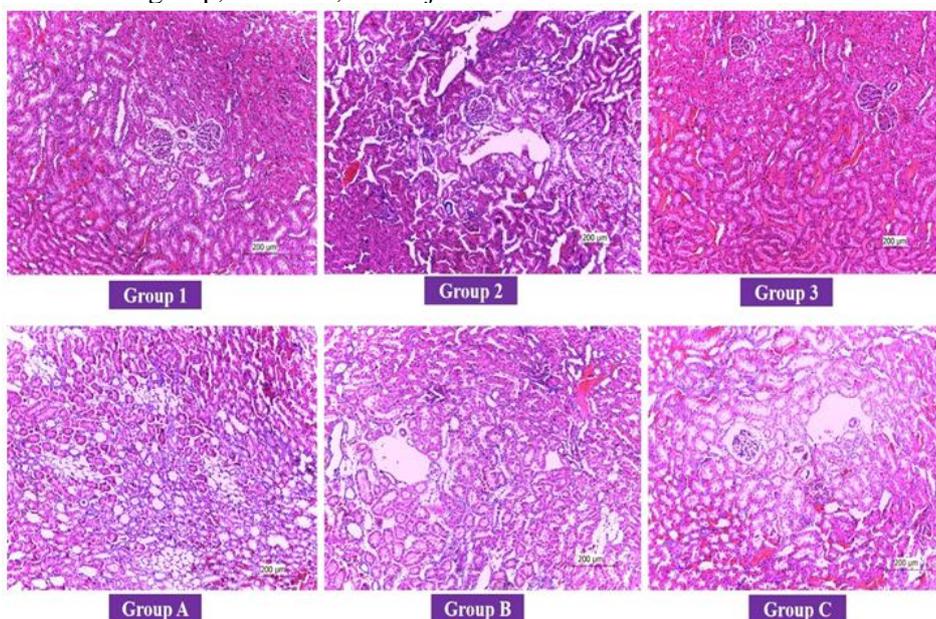
Group A: Diabetic group (positive group), was fed HFD and received oral administration of distilled water.

Group B: Diabetic animals received HFD plus oral administration of kefir.

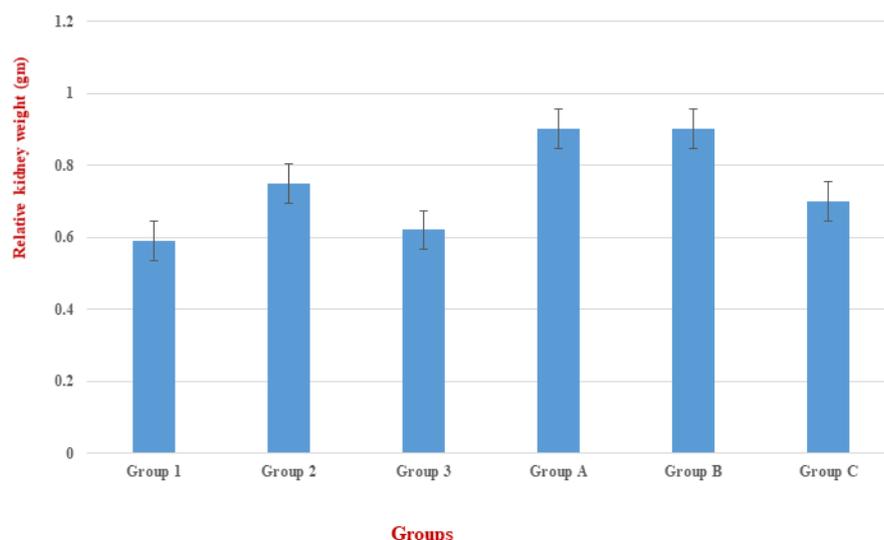
Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.8:** Pathological changes in the kidney in the six rat groups, H&E, bar = 50µm.  
 Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.  
 Group 2: Animals were fed a standard diet and received oral administration of kefir.  
 Group 3: Animals received a high-fat diet (HFD) and additionally oral administration of kefir.  
 Group A: Diabetic group (positive group), was fed HFD and received oral administration of distilled water.  
 Group B: Diabetic animals received HFD plus oral administration of kefir.  
 Group C: Diabetic group, fed HFD, was injected insulin



**Fig.9:** Pathological changes in the kidney in the six rat groups, H&E, bar = 200µm.  
 Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.  
 Group 2: Animals were fed a standard diet and received oral administration of kefir.  
 Group 3: Animals received a high-fat diet (HFD) and additionally oral administration of kefir.  
 Group A: Diabetic group (positive group), was fed HFD and received oral administration of distilled water.  
 Group B: Diabetic animals received HFD plus oral administration of kefir.  
 Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.10:** The relative weight of the kidney of the two experiments (I and II) groups at the end of the experiment.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

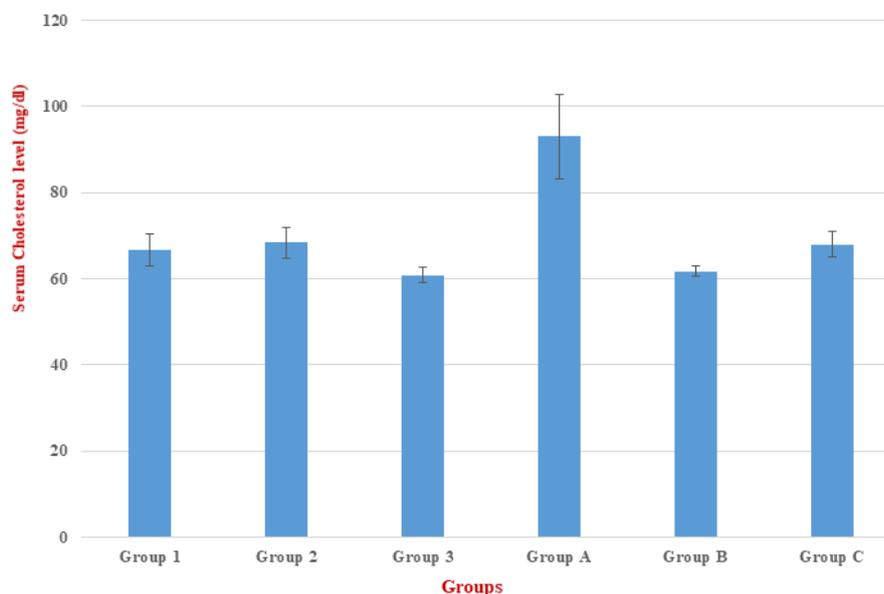
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.11:** The serum cholesterol (mg/dl) of the two experiments (I and II) groups at the end of the experiment.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

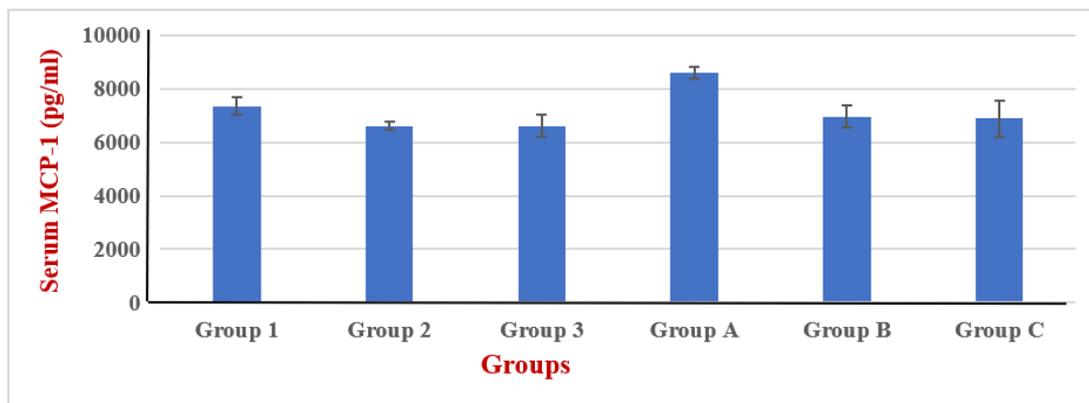
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig.12:** Serum MCP-1 (pg/ml) of two experiments (I and II) groups at the end of the experiment.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

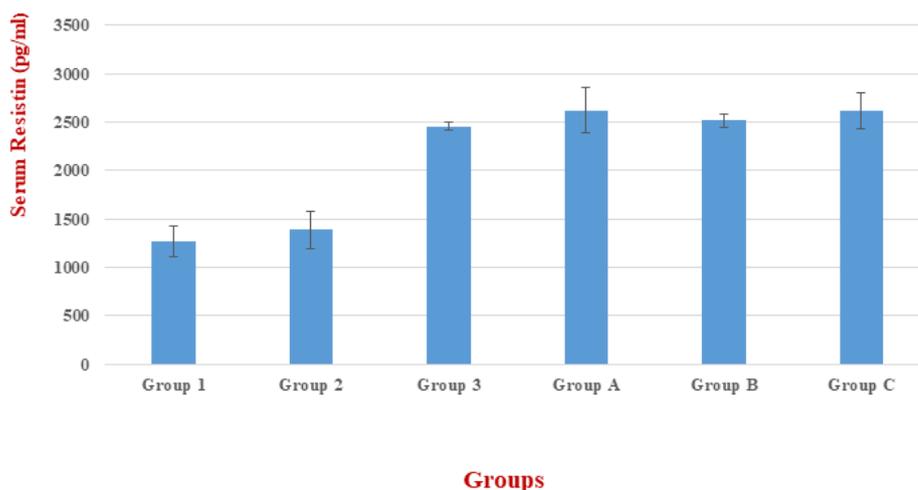
Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.



**Fig. 13:** Serum Resistin (pg/ml) of the two experiments (I and II) groups at the end of the experiment.

Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water.

Group 2: Animals were fed a standard diet and received oral administration of kefir.

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

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

Group B: Diabetic animals received HFD plus oral administration of kefir.

Group C: Diabetic group, fed HFD, was injected insulin.

## DISCUSSION

The present cell biological data showed both kefir and insulin highly increased the cellular activities of both the examined normal and diabetic renal tubule cells of male rats.

the present histochemical results showed, both kefir and insulin showed slightly histochemical changes in DNA, RNA, and polysaccharides contents, except remarkably changes in both protein and collagen contents in both the examined normal and diabetic renal tubule cells of male rats. Also, kefir highly increased lipoproteins material contents in the diabetic renal cells, in contrast, kefir decreased these substant in the normal renal cells.

Histopathologically, kefir repaired the diabetic pathological changes in the renal tubule cells in the male rats.

From the present biochemical results, both kefir and insulin highly significantly decreased the total cholesterol level, MCP-1, and resistin in the blood serum of diabetic male rats, exceptionally insulin did not change the serum resistin.

Animals with chemically induced diabetes have been used to study either insulin-dependent diabetes mellitus (IDDM) (Wilson *et al.*, 1986; O'Brien *et al.*, 1993; Mathe 1995; Ulicna *et al.*, 1996; Rawi *et al.*, 1996; 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). Kefir has the efficacy to prevent the loss of body weight of diabetic male rats (Aref *et al.*, 2020).

"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).

In reviewing the previously published literature, it is obvious that there is little or no amount of research work that concerning the effect of Kefir or HFD, or insulin on the volume of nuclei and histochemical contents such as nucleic acids, proteins, and polysaccharides components in the kidney of both control and HFD-diabetic male rats.

According to these data and reviewing the deficiencies relative to the effect of Kefir and/or insulin on the volume of nuclei and histochemical contents such as nucleic acids, proteins, and polysaccharides components in the kidney of both normal and HFD-diabetic male rats, so in this discussion, we described the results of every study of every investigated organ without comparing it to publications of others authors dealing with the same study /organ.

### Cell Biological Changes in Renal Tubules Cells of Kidney (Karyometric studies):

**In experiment I:** From the cytological point of view, the present results showed the kefir has a stimulatory effect on the volume nuclei of renal tubule cells in the kidney of control male rats. In contrast, the high diet food has an inhibitory effect on the cellular activities of renal tubules cells of kefir-received male rats.

**In experiment II:** From the cytological point of view, our present results showed that although both kefir and insulin have a stimulatory effect on the cellular activities of renal tubules cells in the kidney of diabetic male rats the stimulatory effect of insulin is higher than that of kefir.

From a Cell biological point of view, both kefir and insulin have a stimulatory effect on the cellular

activity of both the examined normal and diabetic renal tubule cells.

#### **The Histochemical Examination:**

The histochemical examination methods used for the determination of DNA content, RNA materials content, total proteins content, collagen fibers, polysaccharides and phospholipids content in the kidney revealed variable differences between the treated groups. In the kidney tissue, in both experiments (I, II) there were variable results between the different histochemical methods.

Our results revealed no changes in DNA contents between the compared groups in both experiments I or II. From the histochemical concept, kefir does not affect the DNA content of both the normal and diabetic renal tubule cells.

There were no changes in RNA materials content between the compared groups in both experiments I or II. From the histochemical concept, kefir does not affect the RNA content of both the normal and diabetic renal tubule cells.

In experiment I, the total protein content in the animal group which fed on HFD+kefir slightly decreased than the others, while the diabetic animal group treated with kefir showed a slight increase in the total protein contents than the other groups. Histochemically, Kefir has a slight stimulatory effect on protein synthesis in diabetic renal cells.

Related to the collagen contents in experiment I, there were no changes or effects by the treatment or type of the diet, while in experiment II, the untreated diabetic animal group showed a slight increase in collagen fibrosis than the treated ones. Histochemically, both kefir and insulin have a slight inhibitory effect on collagen synthesis in diabetic renal cells.

Regarding the polysaccharides content, in both experiments I and II, there were no differences by feeding different diets or variable treatments. From the concept of histochemistry, both kefir and insulin do not affect polysaccharides synthesis in both

examined normal and diabetic renal tubule cells.

In the experiment, I, the animal group fed on SD+kefir had fewer phospholipid materials content than the other groups, so maybe due to the kefir beverage; vice versa in the diabetic treated animals with kefir, the phospholipid materials content were higher than the others, so maybe due to the HFD+kefir effect. These results agree with Kefir has efficacy to prevent the loss in body weight of diabetic male rats (Aref *et al.*, 2020). From the Histochemical point of view, kefir has a stimulatory effect on lipoproteins materials in the diabetic renal cells, in contrast, it has an inhibitory effect in the normal renal cells.

Pathologically, kefir repaired the diabetic pathological changes in the renal tubule cells in the male rats.

From the Histopathological point of view, kefir has beneficial efficacy and it is possible to repair the diabetic pathological changes in the renal tubule cells in the male rat.

Histopathological findings in some studies supported that the kidney of the kefir-added group, close to the normal histological structure was observed; it concluded that consumption of kefir beverage would be beneficial against T2DM which causes serious damage to different body organs especially the liver and kidney (Bülent *et al.*, 2017).

It was clear that the mean relative weight of kidneys in the normal rat male groups was significantly decreased than the diabetic rat male groups; we expected the increased weight of kidneys in the treated and untreated diabetic rat males.

However, the studies by Agerholm-Larsen *et al.* (2000) and Lee *et al.* (2014) were the only studies reporting either increased amounts of fat mass or lower reduction in fat mass in the intervention group compared with the control group,

Urdaneta *et al.* (2007); Sahin

and Yardimci (2009) showed that using a kefir supplemented diet had no significant differences in the weight of the body organs examined.

For the biochemical markers that analyzed in the blood serum, it was included the total cholesterol, MCP-1 and resistin, the results showed significance between all the group trials, it found a gradual decrease in groups five and sixth which treated with kefir beverage and insulin dose. From the biochemical point of view, both kefir and insulin highly significantly decreased the total cholesterol level, MCP-1, and resistin in the blood serum of diabetic male rats, exceptionally insulin did not change the serum resistin.

Most of the previous studies go with the noticeable effect of kefir dose for decreasing the cholesterol level and the lipid profile (Mann and Sperry 1974; Anderson and Gilliland 1999; St-Onge *et al.*, 2000; Xiao *et al.*, 2003; Nakajima *et al.*, 1992; De Roos *et al.*, 1998; St-Onge *et al.*, 2000; Xiao *et al.*, 2003).

Also, isolated yeast from kefir, when applied to dairy products, was reported to provide a hypocholesterolemic effect in the product (Tamai *et al.*, 1996; Noh *et al.*, 1997).

Wojtowski *et al.* (2003) was observed that sheep milk has more beneficial effects to produce kefir about hypocholesterolemia activity as compared to cow and goat milk.

Two substances, i.e., orotic acids and/or hydroxymethyl glutaric are thought to restrict the rate-limiting enzyme that is important in the synthesis of cholesterol (Shahani and Chandan 1979). Another researcher explained a new concept for the reduction of cholesterol in humans that is based on the loss of orotic acids during kefir fermentation (Ozer and Ozer 1999).

Several scientists reported the role of exopolysaccharides in the

reduction of serum cholesterol levels in rats when they consumed excessive dietary cholesterol (Maeda *et al.*, 2004).

Oliveira Leite 2013, indicated that the reduced cholesterol levels in the serum may be ascribed to the suppression of 3HMG-Co A, an intermediary compound of mevalonate, during the production of cholesterol from acetyl-Co A in fermented milk products. The researchers also reported cholesterol assimilation properties of *L. Plantarum* and *L. paracasei* and certain Bifidobacterium species in Kefir (Yoon 1999).

Vujicic *et al.* (1992) showed that a wide range of kefir grains exhibited a high cholesterol assimilation capacity and decreased the cholesterol by 62% in milk under incubation at 20 ° C for 24 hours.

A research analysis conducted by Kalavathy (2009) the depletion of cholesterol by Lactobacillus strains may be dependent on the type of strain.

Additionally, researchers have also observed a reduction in the serum cholesterol concentrations in kefir-supplemented chickens in a dose-dependent fashion (Cenesiz 2008).

The cholesterol-lowering effect was also found in two different rat models fed with kefir and supplemented with cholesterol and orotic acid. Kefiran enhanced the excretion of elimination of sterol and prevented liver injury damage (Maeda 2005).

Administration of LAB in rats showed a significant decrease in the concentrations of total cholesterol, triglyceride, and lipoprotein cholesterol in serum as well as liver compared to rats fed with a high cholesterol diet in absence of LAB supplementation (Huang 2013).

Zheng *et al.* have confirmed the probiotic characteristics of three Lactobacillus species namely, *Lactobacillus acidophilus* LA15, and *Lactobacillus kefir* D17, *Lactobacillus Plantarum* B 23, which demonstrated

resistance to acid and bile salts and facilitated in-vitro attachment to Caco-2 cells (Zheng 2013).

The researchers reported a significant reduction ( $P < 0.05$ ) in total cholesterol, triglyceride, and low-density lipoprotein fractions in the rats treated with LAB, relative to the rats fed with high cholesterol diet with no LAB administration (Zheng 2013).

Güven (2003) has shown a sizeable decline in the malondialdehyde concentrations in plasma, suggesting an antagonistic action of fermented products on the peroxidation of lipids.

Wang *et al.* showed a sizeable reduction in the serum levels of total cholesterol, triglycerides, and low-density lipoproteins, whereas the high-density lipoproteins remain unaffected in mice administered with a cholesterol-rich diet augmented with *Lactobacillus Plantarum* MA2 (Wang 2009).

The latter effect could be a consequence of a kefir-induced reduction in the inflammatory response (Firouzi *et al.*, 2016).

In agreement with these results, it has been shown that kefir reduced pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), in DM (Hadisaputro *et al.*, 2012; Tonucci *et al.*, 2015).

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

Moreover, kefir treatment of T1DM rats led to a decrease in the pro-inflammatory cytokines IL-1 and IL-6 as well as an increase of anti-inflammatory IL-10 compared to control groups (Aune *et al.*, 2013).

Consumption of kefir has been associated with several health-promoting properties, such as antimicrobial (Rodrigues *et al.*, 2005), anti-inflammatory (Lee *et al.*, 2007).

There is evidence that kefir and its polysaccharide extract possess anti-inflammatory activity (Rodrigues *et al.*, 2005).

Hyperglycemia, increased ketones in the bloodstream, and metabolic acidosis, and it might be caused by several factors, including reduced secretion and action of insulin, and raised levels of anti-insulin hormones (Kitabchi and Nyenwe 2006; Davis and Umpierrez 2007).

Aref *et al.* (2021) showed that from the cell biological, histochemical, and histopathological point of view; we can say that logically and biologically, any biological stress factor affects the size of cells nuclei that change the cellular activities such as metabolism and cellular membranes permeability and then lead to quantitatively and qualitatively changes in the chemical components in cells and tissues such as nucleic acids, protein, carbohydrates, and lipids, then these changes are reflected in a form of pathological manifestations and then finally dysfunction of the organ occurs in the organism.

Our results showed diabetes, like stress, highly stimulate collagenous fibers in renal tubule cells which may finally cause kidney dysfunction, by a mechanism like a mechanism of forming tramadol-induced kidney dysfunction in Aref *et al.* (2021), where we propose may Diabetes intensely provided collagen fibers in form intense (1) glomerular fibrosis (in the basement membrane of endothelium and white and reticular fibers around the blood capillaries), (2) periglomerular and perivascular fibrosis, and (3) interstitial renal tubular and basement membrane of epithelial cells in renal tubules fibrosis: these collagen fibers may obstruct or block the passage of various substances through the basement membranes (via the selective permeability process) of epithelial cells in the walls of both blood vessels in the glomeruli and renal tubules that forming nephrons. Consequently, the toxic substances, water, and various components of urine are not filtered from the glomeruli to the renal tubules, and on contrary, also water and

beneficial materials are not restored from renal tubules to beside blood vessels, ultimately the kidneys lose their blood purification function and maybe kidney failure occurs. In contrast, our results showed that kefir highly reduces the collagen formation in the renal cells that prevent renal nephron from its function. Probably kefir treats kidney failure as a side effect of diabetes, by a mechanism that is opposite to the proposed mechanism for the formation of this kidney failure induced by diabetes.

### Conclusion

From the cell biological, histochemical, and histopathological conceptions, both kefir and insulin have various effects on cellular activities and different chemical materials contents, such as DNA, RNA, total protein, collagen, polysaccharides, and lipoproteins, in the examined normal and diabetic renal tubule cells of the male rats.

Biochemically, both kefir and insulin have a highly significant inhibitory effect on the total cholesterol level, MCP-1, and resistin in the blood serum of the diabetic male rats, exceptionally insulin does not affect the serum resistin. Therefore, kefir beverages may, to some extent, repair the diabetic pathological side effects such as kidney dysfunction.

The positive impact of using kefir beverage in treating the pathogenic effects of streptozotocin, STZ on the kidneys of the rats yielded beneficial clinical applications/implications. Therefore, these findings encourage us to continue working and completing various preclinical and clinical trial phases long-term.

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