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Original Research Article

Study of the Histological Changes Caused by the Intake of Metformin, Carnitine and Omega-3 in Some Organs of Male Diabetic Rabbits

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ABSTRACT

Diabetes mellitus (DM) refers to a group of diseases that affect how the body uses blood sugar. The condition is often managed with medications. Metformin helps to manage blood sugar levels. Carnitine is an adjuvant treatment for type 2 diabetes, and omega-3 can be taken in conjunction with metformin to reduce diabetic complications. Many of the drugs for managing DM may affect vital organs in the body. Therefore, the present study was conducted to examine the effects of metformin, L-carnitine, and omega-3 fatty acids on the histological changes of the liver and kidney cells in diabetic rabbits. Forty-five (45) male rabbits weighing 1500-1800 g and aged 10-14 months were divided into 5 groups: the control group (1), the alloxan-induced DM group (2), the DM group that received metformin treatment (3), the DM group that received metformin and L-carnitine treatment (4), and the DM group that was administered with metformin and omega-3 (5). The results of the study revealed that several histological changes associated with DM were observed. This was represented by calcification of the bile ducts of the liver, pigmentation of the nuclei, degeneration of some cells, and extensive necrosis. Also, the kidney showed glomerulus defect (necrosis & atrophy), and vacuoles some tubules. The findings of the present study revealed that the histology of the rabbits' livers and kidneys in the treated and untreated groups differed significantly. Therefore, this study recommends caution while using medications to manage diabetes mellitus.

***Keywords*:** Alloxan, Diabetes mellitus, L-carotene, Metformin, Omega-3, T2DM.

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by distinct pathogenic mechanisms that result in hyperglycemia.[1] Type 2 diabetes mellitus (T2DM) is associated with several complications and comorbidities, including diabetic retinopathy, loss of vision, diabetic nephropathy, lower limb amputation, and cardiovascular disease mortality.[2] Type 2 diabetes accounts for nearly 85-95% of total reported cases of diabetes. Obesity and physical inactivity increase the risk of developing T2DM, which is exacerbated by age.[3] Type 2 diabetes mellitus is a complex disease with several pathophysiological abnormalities, including decreased islet function and insulin resistance, resulting in reduced glucose tolerance and abnormally high fasting hepatic glucose production.[4] Metformin is one of the oral hypoglycemic medications, which include biguanides, sulfonylurea, and thiazolidinedione, which are commonly used to treat T2DM. It improves insulin sensitivity and has the unique properties of aiding weight loss and decreasing appetite.[5] L-carnitine is a natural substance that transports long-chain fatty acids from the cytoplasm to the mitochondrial matrix for β-oxidation in diverse tissues.[6]
The liver is one of the primary organs for the production of endogenous carnitine from lysine, methionine, and ascorbate.[7] The presence of adequate carnitine concentrations in the intracellular compartment is essential for normal fatty acid metabolism, which preferentially uses fatty acids for primary energy resources.[8] Free L-carnitine, which is known to control the transfer of acetyl and acyl groups across the mitochondrial inner membrane, can be obtained from food or produced by the liver, kidney, and brain from lysine and methionine.[9-10] Carnitine supplementation has been recently approved by the US Food and Drug Administration (FDA) not only for treatment but also for the prevention of carnitine depletion, making carnitine an essential micronutrient.[11] In studies with healthy people, carnitine infusions improved glucose metabolism evaluated with a hyperinsulinemia euglycemic clamp, mainly by a non-oxidative mechanism that results in the accumulation of glycogen.[12] Omega-3 is a type of polyunsaturated fatty acid in which the first double bond is found between the third and fourth carbon atoms, counting from the methyl end.[13] The long-chain fatty acid has anti-inflammatory properties by reducing the formation of inflammatory prostaglandins and leukotrienes. However, omega-3 fatty acids inhibit adipose fat accumulation by decreasing lipogenic enzymes and boosting β-oxidation.[14] Dietary lipids promote health and play an important function in physiological development. Members of the polyunsaturated fatty acid cluster are injected with therapies since they are thought to be intricate in tissue lipids and are currently gaining importance in biochemistry.[15] Omega-3 fatty acids have been described as inflammation-modulating agents, by stimulating or suppressing the synthesis of pro-and/or anti-inflammatory cell signaling molecules.[16]
Therefore, the present study was aimed at investigating the histological changes associated with metformin, L-carnitine, and omega-3 on kidney and liver cells in diabetic rabbits.

Materials and Methods

Sources of chemicals
Metformin was obtained from SCDM-Samarra, Iraq. L-carnitine 2910 was purchased from Amazing Nutrition, USA. The omega-3 pro and alloxan monohydrate and alloxan monohydrate were bought from Sofia, Bulgaria, EU, and BDH, England), respectively.

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Sources of experimental animals and maintenance
Forty-five (45) male healthy rabbits, 10-14 months old, with a body weight of 1500-1800 g were obtained from Tikritrit University, Tikrit, Iraq. The animals were maintained at the animal house of the Department of Biology, Kirkuk University, Kirkuk, Iraq. They were housed in a well-ventilated hygienic room at 25±1ºC with a 12:12-hour light-dark cycle. The rabbits were given unlimited access to food and water.

Ethical approval
Ethical approval for the study was obtained from the Commission of Ethical Research at Kirkuk University, Kirkuk, Iraq, with certificate number: 236-BS-KU.

Animal groupings and treatment
Rabbits were fasted for 24 hours before receiving a single subcutaneous injection of alloxan (150 mg/kg) to induce diabetes mellitus.[17] The rabbits were given food and glucose solution at a concentration of 10% immediately following the injection to prevent a drop in blood glucose levels and reduce the shock of hypoglycemia as a result of treatment with alloxan.[18] The blood glucose level was monitored every two days for ten days to determine whether diabetes had been induced.[19] The rabbits were divided into five groups of nine animals each, and they received the following treatment for ten days. The first group (control group) consisted of healthy rabbits that received daily doses of distilled water for the duration of the experiment. The second group consisted of diabetic rabbits who received 150 mg/kg of alloxan injections. In the third group, diabetic rabbits were given 21.4 mg/kg of metformin. The fourth group consisted of diabetic rabbits administered metformin and L-carnitine at doses of 21.4 and 96.3 mg/kg, respectively. The fifth group was formed by a diabetic rabbit group that received omega-3 at a dose of 0.33 ml and metformin at a dose of 21.4 mg/kg of body weight. The doses for both omega-3 and metformin were calculated using the following equation:

$$\frac{W1}{V1} \times \frac{W2}{V2}$$

Where w1 is the human weight, v1 is the dose for a human, w2 is the rabbit weight, and v2 is the dose for a rabbit.

Histological analysis
The animals were then anesthetized with chloroform after fasting for 24 hours. The liver and kidneys were removed from the animals after they were sacrificed, fixed in 10% formalin right away, treated with regular alcohol and xylene, embedded in paraffin, and sectioned at a thickness of 5μ. The sections were stained with hematoxylin and eosin stain.[20]

Results and Discussion

Histological alterations in the liver of experimental rabbits
The results of the histological analysis revealed that the parenchymal tissue of the liver of the control group of rabbits had a normal shape with a normal hepatic lobule, a normal arrangement of hepatocytes radially around the vein, and the presence of sinusoids in the cavities located between cells (Figure 1). The group that received the alloxan showed a change in the structure of the liver, as shown in Figure 2. As observed in Figure 3, there was an occurrence of amyloid fluid infiltration around the damaged areas, blood clots, cell degeneration, enlargement of some nuclei, and extensive necrosis with focal necrosis. Figure 4 shows that the diabetic group treated with metformin and L-carnitine had a change in the histological structure of the liver, including an expansion of some sinusoids, pigmentation of the nuclei, and an increase in the number of nuclei with significant focal necrosis. A similar observation was made in the diabetic group administered with metformin and omega-3. The defect in the liver was observed to include significant focal necrosis, some nuclear karyolysis, and a slight pigmentation in the nuclei. In the metabolic process, the liver and kidneys are important organs, particularly to eliminate oxidative damage.[21] The liver is also a crucial organ because it regulates blood sugar levels.[22]

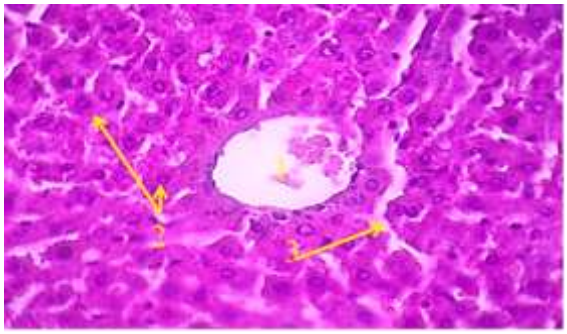

Figure 1: Rabbit's liver in the control group showing: a normal central vein (1), hepatocytes (2), and sinusoids (3). 400X (H&E).

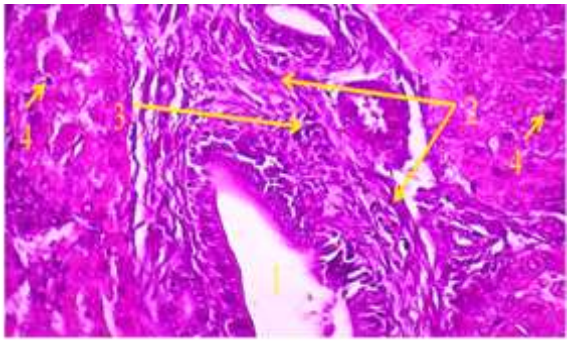

Figure 2: Rabbit's liver in the alloxan-treatment group showing: sclerosis bile duct (1), fibrocytes (2), inflammatory infiltration (3), and pyknotic (4). 400X (H&E).

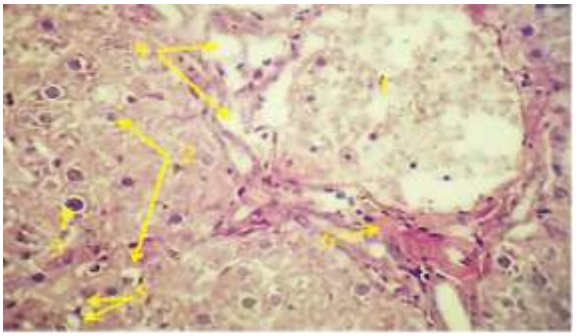

Figure 3: Rabbit's liver in the diabetic group that received metformin treatment, showing: congestion (1), degeneration of cells (2), enlargement of some nuclei (3), necrosis (4), necrosis (5) amyloidosis (6). 400X (E&H).

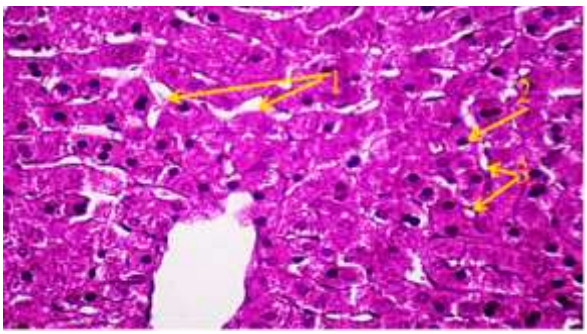

Figure 4: Rabbit's liver in the diabetic group that received metformin and creatinine treatment, showing: the expansion of sinusoids (1), picnotic of many nuclei (2), and several necroses (3). 400X (E&H).

The results of this study were consistent with those of earlier studies,[23] including a histological analysis of a group of metformin-treated local diabetic rabbits that showed the presence of calcification in the bile ducts, pigmentation of the nuclei, inflammatory cells infiltrating the hepatic thallus, and fibroblasts in comparison to the normal shape of hepatocytes. By observing several bi-nucleated parenchymal cells, the current study's results demonstrated the division of parenchymal cells in this group. This division in hepatocytes can be attributed to an important adaptive response when the liver is infected with acute or chronic diseases.[24] The occurrence of an expansion in a few sinuses corroborated the findings of the researcher,[25] who discovered a minor expansion in the sinusoids of the liver of diabetic rabbits that had been given an alcoholic extract. Blood flow through the hepatocytes was stopped or disrupted in some areas due to poor blood drainage brought on by hepatic vein obstruction, which was consistent with the findings of the study conducted by Mir et al.,[26] who observed the presence of blood congestion in diabetes. When rabbits were given metformin treatment, fat droplets were discovered inside the central vein, indicating that fatty degeneration had taken place within the blood vessels. One of the causes of chronic hepatitis is changes in fat. The results of the present study were not consistent with the findings of Lin et al.,[27] who found that the use of metformin improves lipids. Hepatitis, as well as the occurrence of degenerative changes, results in an inflammatory reaction. This is due to the fact that degenerative cells' byproducts are chemotactic factors that draw inflammatory cells to the degenerative area to defend the tissue by consuming the degenerative substances and removing the damage-causing factor.[28] Additionally, amyloid protein (one of the body's proteins that is deposited in the walls of damaged blood vessels and around the cells) causes amyloidosis around the areas that have been damaged. This condition also occurs in chronic hepatitis.[29] These results disagreed with those of the findings by Gomaa and Abd-EL-Aziz,[30] which found that omega-3 supplementation alone did not affect the histological structure of the liver in its normal state, and with those of the study by EL-Moghazy et al.,[31] which discovered that omega-3 supplementation in rabbits for six weeks did not affect the kidney's histological structure. The kidneys of the control group were depicted in Figure 6 as having normal-shaped glomeruli and tubules, whereas Figure7 the alloxan-treatment group rabbits' kidneys showed damage to the glomerulus (necrosis and atrophy of the glomerulus), vacuoles in some tubules, congestion of blood vessels, thickening of the blood envelope, and the presence of fatty degeneration within the vessels. The histological structure of the kidney changed in the group of rabbits that received both metformin and alloxan treatments, as displayed in Figure 8, which was represented by the occurrence of glomerular hypertrophy, large vacuoles in the tubules, and some degenerating tubules. When metformin and L-carnitine were administered to the diabetic rabbit group, as observed in Figure 9, some had closed lumen, swollen tubule cells, initiation, and shedding of some epithelial cells of the tubules, and the presence of acidifying substances. As observed in the diabetic group of rabbits treated with metformin and omega-3, the kidneys exhibited tubule lumen narrowing, epithelial cell loss, tubule lumen closure, and glomerular hypertrophy (Figure 10). The results of this study are in agreement with those of the previous studies.[32.33] The kidney inflammation that led to the observation of a histological structural change in the alloxan-treated kidneys. Roy et al.[34], observed a change in the histological structure of the kidney treated with alloxan, which resulted from kidney inflammation. Damage in the glomerulus includes atrophy and necrosis, and this consequently affects the glomerular filtration, as the glomeruli have a network of capillaries that filter blood to the urinary tubules.[35] Atrophy and necrosis have been observed in the glomerulus, which results from the effect of free radicals, which disrupt metabolic processes and cause cells to swell and necrosis in the glomeruli.[36] The results of this study differ from those of Zhang *et al.*,[37] who found that metformin protected the histological structure of the kidneys of mice treated with streptozotocin. The researchers opined that the narrowing of the hollow and the absence of the hollow in some cases are caused by the swelling of the epithelial cells in the tubules, bringing them close together and causing the narrowing of the hollow.[38] Tubule necrosis is caused by a state of poisoning, which results in the death of the cell as a result of not receiving enough oxygen, and the necrosis of the tubules indicates renal failure.[39] Additionally, the presence of acidic substances in the injured urinary tubules suggests that acute inflammation occurred as a result of the inabilitys to control the influencing factor.[39]

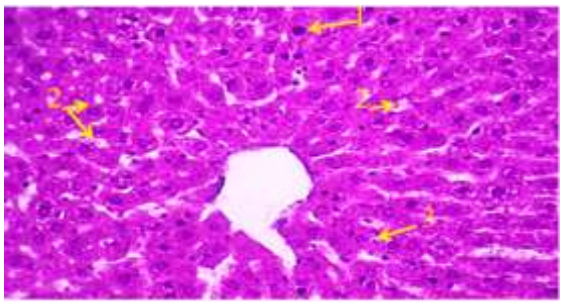

Figure 5: Rabbit's liver in the diabetic group that received omega-3 and metformin treatment, showing: picnotic of small cells (1), several necrosis (2), karyolysis of some nuclei (3). (400X E&H).

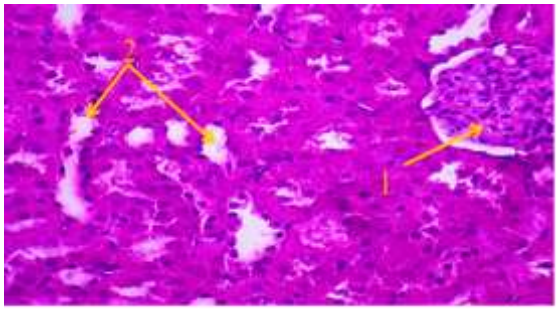

Figure 6: Rabbit's kidney in the control group showing: normal glomerulus (1), and tubules (2). 400X (E&H).

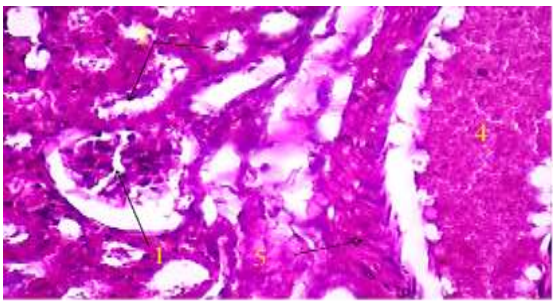

Figure 7: Rabbit's kidney in the alloxan-treatment group, showing: necrosis in the glomerulus (1), vacuoles in some tubules (2), fatty degeneration (3), and thickening in the wall of blood vessels (4). 400X (E&H).

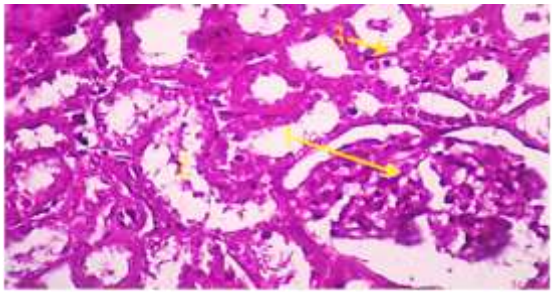

Figure 8: Rabbit's kidney in the diabetic group that received metformin treatment, showing: hypertrophy of the glomerulus (1), vacuole (2), and degeneration of some tubules (3). 400X (E&H).

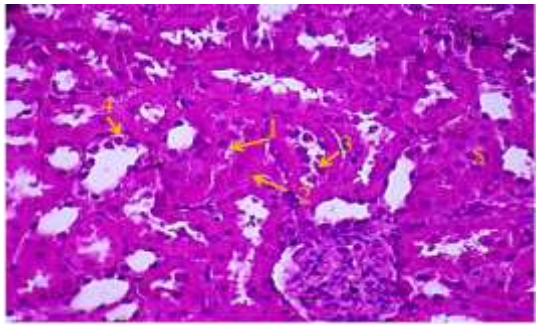

Figure 9: Rabbit's kidney in the diabetic group that received metformin and creatinine treatment, showing: narrowing of the tubule's lumens (1), closure of some tubule's lumens (2), pinging of sloughed cells (3), degeneration (4), and swelling of cells (5). 400X (E&H).

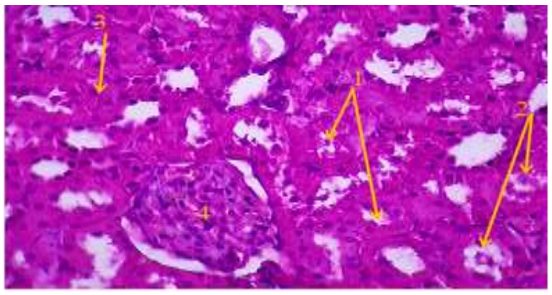

Figure 10: Rabbit's kidney in the diabetic group that received creatinine and omega-3 treatment, showing: narrowing of the tubule's lumen (1), sloughed cells (2), closure of some lumens (3), and hypertrophy of glomerulus (4). 400X (E&H).

Conclusion

The findings of this study revealed a significant difference between treated and untreated animals in terms of the nuclei's size, lysis, and presence of focal necrosis in the liver of rabbits. Additionally, the kidneys of the treated animals displayed necrosis, atrophy of the glomerulus, dorsal cell sloughing and collapsing in the urinary tubules, and congestion of blood vessels. Therefore, it is strongly recommended to use extreme caution when using drugs to treat diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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