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# Therapeutic and Prophylactic Effect of Zinc and Vitamin D in Alloxan-Induced Diabetic Rats

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**Abstract**

Diabetes mellitus is a serious global public health issue. It is marked by elevated blood glucose levels (hyperglycemia), which trigger inflammations and oxidative damage, factors that contribute significantly to the disease's progress. Zinc is a crucial part of the antioxidant system of body and helps slow down oxidative processes, especially those associated with diabetes mellitus. Vitamin D's involvement in the onset and prevention of diabetes is gaining attention. Our study was designed to assess the effect of zinc and vitamin D on some biochemical parameters and histopathological changes of liver and pancreas in rats with diabetes induced by alloxan. A total of 55 male albino rats weighing 100-150 g were divided into eleven groups, healthy controls rats, diabetic rats, diabetic rats post and pre-treated with zinc, vitamin D and metformin as a reference drug to assess their anti-diabetic, antioxidant and anti-inflammatory activities. Alloxan-induced diabetes not only resulted in notable elevations in serum glucose, liver enzymes and inflammatory markers, but also provoked oxidative stress in pancreatic and hepatic tissue. Significant morphological damage was noted in Langerhans islets and liver of diabetic rats. The administration of zinc and vitamin D ameliorated elevated blood glucose, liver enzyme levels, and other biochemical parameters, with zinc showing the greatest reduction in blood glucose. Histopathological examination of the liver and pancreas also revealed more substantial protection following treatment. In conclusion, the administration of zinc and vitamin D help improve hyperglycemia in alloxan-induced diabetic rats. Therefore, they may be considered as adjunctive therapies alongside other anti-diabetic medications.

**Keywords:** Diabetes mellitus, zinc, vitamin D, IL-6 and TNF- α.

**Full length article***Corresponding Author*, e-mail: *mahmoud.fadl@mu.edu.eg osman.mouftah@mu.edu.eg*, Doi # https://doi.org/10.62877/4-IJCBS-25-27-21-4

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## 1. Introduction

Diabetes mellitus (DM) has become a widespread disease that impacts various organs in the human body. It is a disorder manifested by persistent hyperglycemia, which results from failure in secretion of insulin, action of insulin, or both, leading to disruptions in the metabolism of carbohydrates, fats, and proteins. ß-cells, located in the islets of Langerhans in the pancreas, are responsible for producing the hormone insulin. Damage to these cells can impair insulin secretion, disrupting the cells' ability to utilize glucose from food. Consequently, glucose builds up in the circulation, causing blood sugar levels to rise. A deficiency of insulin can lead to the development of diabetes mellitus [1]. Diabetes is marked by the progressive loss of β cells, and it is widely recognized that reactive oxygen species (ROS) play a role in damaging and impairing pancreatic cells or tissue in both type 1 and type 2 diabetes, although the underlying mechanisms vary [2]. The extent of oxidative stress and the resulting tissue damage may be influenced by imbalance between the overproduction of ROS and the antioxidant defenses within the pancreatic islet [3]. Alloxan functions as a selective inhibitor of glucose-stimulated insulin secretion (by specifically inhibiting glucokinase which plays a key role as a glucose sensor in regulating glucose homeostasis) and the selective necrosis of beta cells due to the formation ROS.

As a result, it is frequently used as an agent to induce diabetes [4]. Alloxan can initiate the formation of ROS through a cyclic reaction with its reduction product, dialuric acid. Additionally, hyperglycemia activates several mechanisms that increase ROS production, including glucose autoxidation, protein glycation, and a reduced antioxidant defense system, which involves enzymes like superoxide dismutase, catalase, and glutathione peroxidase [5]. This results in an increase in free radicals, which is critically important due to their role in pathogenesis and development of complications associated with type 2 diabetes mellitus (T2DM) [6-7]. Chronic inflammation is another crucial factor in progression of T2DM, as inflammation-dependent ROS in tissues like the liver, skeletal muscle, and adipose tissue interact with insulin receptor and its signaling pathways,

leading to an inadequate response to insulin levels. Elevated inflammatory biomarkers are linked to onset and progression of T2DM, as well as its complications, including an increased risk of cardiovascular diseases. [8]. Zinc is an antioxidant and second most prevalent essential metal in human body. It plays a key role in crucial functions like growth, activity of immune system, reproduction, and body's response to oxidative stress, as well as storage of insulin in pancreatic beta cells [9-10].

Zinc is found in all body tissues, with high concentrations in the brain, muscles, bones, kidneys, and liver. It is also present in significant amounts in the prostate and certain parts of the eye. Zinc plays a vital role in immune system function, regulating apoptosis, tissue repair, protein synthesis, and cell division [11-12]. Zinc is involved in the synthesis of vitamin A in the liver, as well as the metabolism of fats, carbohydrates, and proteins in the body. It plays a role in oxidation processes by mediating the functions of superoxide dismutase, aids in the healing of wounds and burns, helps mediate inflammatory reactions, and is essential for the synthesis of deoxyribonucleic acid (DNA) [13]. Vitamin D (VIT D) is a fat-soluble vitamin which is vital for human health. Its receptors are found throughout various tissues, where they exert a wide range of biological effects. Vit D is essential for controlling key genes related to inflammation, oxidative damage, chronic illnesses, and bone metabolism. The main predictor of vitamin D status is the level of circulating 25-hydroxyvitamin D, which has been demonstrated to be inversely correlated with both β-cell dysfunction and insulin resistance (IR) [14-15].

## 2. Material and methods

### *2.1. Animals*

Adult male albino rats, weighing approximately 100 - 150 gm were obtained from the National Research Center, Giza, Egypt and housed in animals house of the Faculty of Pharmacy, Deraya University, Minia, Egypt at constant environmental conditions (humidity 50% ± 10%, temperature 20 – 25 °C) and a 12-hours dark– light cycle, with water and a standard chow diet available at all times during the trial, unless otherwise stated. Prior to the experiment procedures, the animals were kept in separate aerated cages for two weeks to let them to acclimate to the new environment and make sure that they were disease-free. The experiment and rats handling were carried out as stated by the guidelines of the Committee of Research Ethics of Faculty of Pharmacy, Minia University, Egypt (MPEC-230202), and Follow the principles outlined in the Guide for the Care and Use of Lab Animals. Every attempt was made to reduce animal suffering and use as few animals as possible.

### *2.2. Chemicals and drugs*

Alloxan monohydrate, Metformin (Glucophage 500mg/tablet), Zinc sulfate (Octozinc 110mg/capsule) and Vitamin D (Vi drop 2800 unit/ml) were obtained from Loba Chemie Pvt Ltd (India), Mina Pharm (Egypt), October Pharma S.A.E and Medical Union Pharmaceuticals (Egypt), respectively.

### *2.3. Induction of diabetes*

Except rats of control groups, all rats were fasted for 12 hours (overnight) and received a single intraperitoneal injection of freshly prepared alloxan (150 mg/kg.Bwt) dissolved in 0.9% normal saline solution using an insulin syringe. After the injection, they were provided free access to solution of glucose (5%) for 24 hours to counteract drug-induced hypoglycemia, as alloxan can cause fatal hypoglycemia due to the massive insulin leakage from pancreas following β-cell damage [16]. To confirm that diabetes mellitus was successfully induced, serum fasting blood glucose levels were assessed after 72 hours using a glucometer (PreciChek Autocode, AC-302, and Germany). For this investigation, only rats with fasting blood glucose levels exceeding 250 mg/dl were selected as diabetics [17].

### *2.4. Experimental Design*

The current study was performed in accordance of the International Guidelines regarding animal experiment. A total of 55 adult male albino rats were divided randomly into eleven groups (n=5 for each) as follow; group 1: healthy control receive normal saline only, group 2: diabetic control receive a solution of monohydrate, group 3: healthy control treated with zinc sulfate only, group 4: healthy control treated with vitamin D only, group 5: healthy control treated with metformin only, group 6: diabetic post-treated with zinc sulfate, group 7: diabetic post-treated with vitamin D, group 8: diabetic post-treated with metformin, group 9: diabetic pre-treated with zinc sulfate, group 10: diabetic pre-treated with vitamin D and group 11: diabetic pre-treated with metformin. Metformin, zinc sulfate and vitamin D were given orally to the rats once daily for 28 consecutive days at a dose of 120 mg/kg body weight, 100 mg/kg body weight and 10 IU/kg body weight respectively [18-20]. All measurements except body weights and fasting blood glucose were performed at the end of experiment.

### *2.5. Preparation and administration of drugs*

The administered drugs were freshly prepared daily for 28 days except for alloxan which freshly prepared once before and after the experiment. Each rat orally administered metformin, zinc and vitamin D solution via oral gavage feeding needle and intraperitoneally injected with alloxan solution according to animal grouping as reported above. The animals held tightly to avoid harming it during procedure.

### *2.6. Weight measurements*

The weights of healthy control rats, diabetic rats and post & pre-treated rats were recorded before and at the end of experiment using vibra HT scale.

### *2.7. Blood sample collection and dissection*

Four weeks after treatment, animals were sacrificed by decapitation after overnight fasting, and The blood was first collected in clean centrifuge tubes, allowed to clot for 30 minutes at room temperature, and then centrifuged at 4000 rpm for 10 minutes (Z 200 A, Hermle, Germany) to obtain the sera (supernatants) which preserved at -80 C immediately after separation for biochemical analysis. Eventually, the abdomen was dissected and the liver and pancreas were rapidly excised and weighed. Then saved in formalin solution for histopathological investigations.

### *2.8. Biochemical analysis*

#### *2.8.1. Blood glucose, Liver enzymes and some serum proteins determination*

Glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST). Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), albumin and total

proteins colorimetric assay kits were purchased from Spectrum-diagnostics, Cairo, Egypt.

#### *2.8.2. Oxidative stress markers determination*

Malondialdehyde (MDA) and Reduced glutathione (GSH) colorimetric assay kits were obtained from Biodiagnostic, Giza, Egypt.

#### *2.8.3. Inflammatory markers determination*

Tumor necrosis factor-alpha (TNF-α) and Interleukin (IL-6) ELISA kits were purchased from Elabscience, Texas, USA.

### *2.9. Histopathological examination*

Liver and pancreas were washed with normal saline solution and instantly fixed in solution of 10% formalin prepared in saline for 3 days. Then organs were washed, dehydrated in increasing ethanol grades from 70% to absolute, cleared in xylene, impregnated and embedded in paraffin wax. Serial sections (5 µm thick) were obtained using a microtome and stained with hematoxylin and eosin (H&E) for histopathological examination and photography by light microscope equipped with a digital camera [21].

### *2.10. Statistical analysis*

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) program version 26.0. Results were shown as mean ± standard deviation (SD). Differences with $P<0.05$ were accepted as significant, differences with $P<0.01$ or $P<0.001$ considered as moderately or highly significant respectively. The symbols (#) refer to insignificant, (*) refer to significant, (**) refers to moderately significant and (***) refers to highly significant.

## 3. Results and discussion

### *3.1. Results*

#### *3.1.1. Effect of zinc, vitamin D and metformin administration on body and organ weights*

Effect of administered drugs on body weights and organs weight of alloxan-induced diabetic rats was summarized in the Table 1. Figure 1(A-C) showed a normal body weights, liver weights and pancreas weights of the healthy control groups (1,3,4 and 5) respectively and highly significant ($P<0.001$) decrease (88, 3.98, 0.33 gm respectively) in the diabetic control group (2) following the administration of alloxan compared to healthy controls, whereas the body weight of the post and pre-treated groups (6, 7, 9 and 10) showed highly significant ($P<0.001$) increase, liver weights showed highly significant ($P<0.001$) increase in group (6), moderately significant ($P<0.01$) increase in groups (7&9) and significant ($P<0.05$) increase in group (10) and pancreas weights showed highly significant ($P<0.001$) increase in group (6), significant ($P<0.05$) increase in group (9) and insignificant ($P>0.05$) increase in groups (7&10) comparative to diabetic group. Administration of zinc sulfate and vitamin D attenuated the weight loss and tissues damage in alloxan induced-diabetic rats.

#### *3.1.2. Effect of zinc, vitamin D and metformin administration on blood glucose levels*

The effect of the oral administration of zinc sulfate and vitamin D on fasting blood glucose are presented in Table 2. The experimentally induced diabetes highly significant ($P<0.001$) increased the level of fasting glucose (310 mg/dl) of diabetic control group (2) compared to the control levels of healthy groups. However, post and pre-treatment of the alloxan-diabetic rats of groups (6, 7, 9 and 10) with zinc and vitamin D highly significant ($P<0.001$) reduced their fasting glucose levels, compared with the diabetic group (2) as showed in figure 1D. But rats of post treated groups (6 and 9) showed a significant reduction in blood glucose levels than levels of pre-treated groups (7 and 10)

#### *3.1.3. Effect of zinc, vitamin D and metformin administration on some liver enzymes*

Liver enzymes concentrations of rat's serum were measured and summarized in table 2. In alloxan-diabetic rats of group (2) the concentrations of serum ALT, AST, ALP and GGT were highly significant ($P<0.001$) increased (124, 209, 255 and 15 U/L) compared to healthy control groups as explained in figures 1(E, F). On the other hand, Post and pre-treatment of the diabetic rats with zinc sulfate and vitamin D caused a highly significant ($P<0.001$) reduction in the concentrations of ALT in rats serum of groups (6, 7 and 9) and a significant ($P<0.05$) reduction in ALT levels of group (10), while AST & ALP showed highly significant ($P<0.001$) decrease in their levels of groups (6, 7, 9 and 10) and levels of GGT showed a significant ($P<0.05$) reduction in group (6) & insignificant ($P>0.05$) reduction in groups (7, 9 and 10) compared to the mean values of diabetic control group (2).

#### *3.1.4. Effect of zinc, vitamin D and metformin administration on some serum proteins*

The effect of zinc and vitamin D on total proteins and albumin of alloxan diabetic rats serum was illustrated in Table 3 & Figure 1G, A highly significant ($P<0.001$) reduction in serum albumin levels (2.81 gm/dl) and insignificant ($P>0.05$) reduction in serum total proteins level (5.59 gm/dl) of diabetic rats in group (2) was observed compared to healthy control groups (1, 3, 4 and 5). After post & pre-treatment with test drugs, a significant ($P<0.05$) increase in albumin was observed in rats of groups (6&7), while no significant ($P>0.05$) change observed in albumin levels of groups (9&10). And insignificant ($P>0.05$) increase in total proteins levels of groups (6, 7, 9&10) was observed compared to diabetic group.

#### *3.1.5. Effect of zinc, vitamin D and metformin administration on oxidative stress markers*

In Table 3 & Figures 1(H, I), the diabetic control group (2) showed a highly significant ($P<0.001$) increased levels of MDA (2.9 nmol/ml) and decreased levels of GSH (1.1 mmol/ml) compared to the healthy control groups (1, 3, 4 and 5). While Post and pre-treatment with zinc sulfate and vitamin D reversed the levels of oxidative stress and inflammatory markers. Thus, after administration of Zn and Vit D, a highly significant ($P<0.001$) decreased MDA levels and highly significant ($P<0.001$) increased GSH levels observed in groups (6, 7, 9 and 10) compared to diabetic control group (2).

#### *3.1.6. Effect of zinc, vitamin D and metformin administration on inflammatory markers*

The inflammatory markers were impacted before and after alloxan injection in rats, as shown in Table 3 & Fig. 1J. The diabetic group (2) exhibited a highly significant

(P<0.001) elevated serum levels of TNF-α and IL-6 (157.5 and 80.8 pg/ml respectively) compared to the healthy groups (1, 3, 4 and 5). But administration of Zinc sulfate & vitamin D indicated a highly significant (P<0.001) decrease in the levels of TNF-α and IL-6 in diabetic rats of groups (6, 7, 9 and 10) compared to diabetic control group (2).

#### *3.1.7. Effect of zinc, vitamin D and metformin administration on histopathological parameters*

##### *3.1.7.1. Hepatic Tissues*

Our study also showed that alloxan-induced diabetes in rats of group (2) produced alterations in the hepatic structures as well as functions while the healthy control groups (1, 3, 4 and 5) showed no significant pathological changes. This was evident from the histological sections as in Fig. 2B of diabetic group (2) which showed congested central vein, dilated congested sinusoid, steatosis, kpuffer cell hyperplasia, chronic inflammatory cells, proliferated bile ducts and liver cells necrosis (karyolysis) and Fig. 2A of healthy group (1) which showed normal central vein and radiating trabeculae of hepatocytes with intervening sinusoids. While post and pre-treated diabetic rats of groups (6, 7, 9 and 10) with zinc and vit D showed decreased central vein and sinusoidal congestion, minimal periportal inflammation, normal looking hepatocytes and mild steatosis as showed in Fig. 2F, 2G, 2I and 2J compared to diabetic control group (2).

##### *3.1.7.2. Pancreatic Tissues*

The histological studies on pancreatic tissues showed that pancreas of diabetic control rats of group (2) has atrophic islets with signs of vacuolation and degeneration of islet cells surrounded by pancreatic acini with sighns of signs of acinar cell necrosis noted by absence of nuclei and congested blood vessels and interstitial haemorrhage as in Fig. 3B compared to healthy control groups (1, 3, 4 and 5) as shown in Fig. 3A, 3C, 3D and 3E. While post and pre-treated rats of groups (6, 7, 9 and 10) showed increased number of islets, surrounded by normal pancreatic acini, vacuolated cytoplasm and diminished interstitial hemorrhage or edema as showed in Fig. 3F, 3G, 3I and 3J.

### *3.2. Discussion*

In a healthy individual, the pancreatic ß-cell mass can adapt to varying insulin demands when faced with different blood glucose levels. However, this ability is significantly impaired in DM. In T1DM, the ß-cells themselves are either partially or completely destroyed, resulting in a decreased insulin secretion. In T2DM, there is either a problem with the insulin molecule or its receptors, or a reduction in ß-cell mass. These factors indicate that endocrine pancreas is unable to maintain sufficient ß-cell mass in DM [22]. The experimental diabetic rats showed an elevated blood glucose levels and altered histological pancreatic tissues. This is a result of alloxan monohydrate, which induces diabetes by damaging the β-cells in the islets of Langerhans in the pancreas. This damage leads to a reduction in insulin production and subsequent hyperglycemia. Alloxan exerts its cytotoxic effects through ROS, particularly hydrogen peroxide with simultaneous significant rise in cytosolic $Ca^{2+}$ concentration, leading to the rapid destruction of β-cells [23]. The ROS generated by cyclic redox reactions of dialuric acid (the reduction product of alloxan), that acts as a free radical generator and damages the liver and pancreas [24].

The pancreas is particularly vulnerable to the free radical damage induced by alloxan, resulting in a reduction of endogenous insulin. This disruption impairs the body's ability to utilize glucose effectively. Consequently, blood glucose levels rise, protein content decreases, and levels of other biochemical parameters increase [25]. Alloxan is the most widely used agent for inducing diabetes in the experimental rat models. There is growing evidence that alloxan induces diabetes by rapidly depleting β-cells through alkylation of DNA and accumulation of harmful cytotoxic free radicals. This process is believed to be triggered by inflammation of islet which followed by the infiltration of activated macrophages and lymphocytes into the inflamed area. As a result, plasma insulin levels decrease, leading to a persistent state of hyperglycemia. [23]. in diabetes, chronic elevation of blood glucose ultimately leads to destruction of both non-enzymatic and enzymatic antioxidants through various mechanisms, including protein glycation and glucose auto-oxidation. These processes result in a rise in free radicals generation, particularly ROS [26]. The elevated levels of ROS contribute to serious conditions like diabetes and tissue damage in various organs and systems.

While insulin is an effective treatment for diabetes but it has drawbacks, such as promoting oxidative stress. As a result, there is a growing need to identify alternative anti-diabetic and antioxidant molecules for managing the disease. Several nutritional molecules have been identified as potential options for diabetes management. [27]. Despite consuming more food and fluids, the diabetic rats' body weight significantly decreased. Dehydration and increased protein and lipid breakdown could be the cause of this weight loss as compared to the rats without diabetes. Because cells are unable to efficiently metabolize glucose, gluconeogenesis from amino acids and body proteins results, causing these metabolic disturbances. Consequently, there is finally a breakdown of muscle and tissue [28]. The reduction in organ weight observed in this experiment, caused by the alloxan, may be attributed to atrophy, a common consequence of diabetes. Specifically, the reduction in pancreatic weight could be due to a decrease in secretory granules, which is consistent with the results of Kumar et al., who found that diabetic rats had less secretory granules of the pancreatic beta cells [29]. In our study, rats with diabetes weighed less than healthy control rats, which is in line with a number of the earlier studies [30].

However, beneficial effects of zinc supplementation on weight may be attributed to its role in regulating appetite via modifications in the hypothalamic neurotransmitter metabolism of the leptin system and its receptors. In other words, zinc may stimulate leptin synthesis [31]. Additionally, zinc might increase levels of insulin-like growth factor I (IGF-I) and potentially raise serum testosterone levels. Testosterone and IGF-I are both anabolic agents. That could contribute to increased body weight [32]. In the current investigation, the rats with diabetes induced by alloxan showed an elevated fasting blood glucose levels in contrast to the healthy control group. This observation aligns with earlier studies [33]. This is due to a notably decrease in β-cells number caused by pancreatic cell damage, resulting in a drastic decrease in insulin hormone secretion and an increase in blood glucose levels. [34-35]. the liver is a key organ

involved in metabolism, biotransformation, gluconeogenesis, and the glycogenolysis. Glucose homeostasis is regulated by balance b/w glucose production in liver and its utilization by the peripheral tissues. Alloxan monohydrate is toxic not only to pancreas but also to liver, impairing its function and integrity. This damage is reflected by leakage of the liver enzymes into the bloodstream, resulting in elevated enzyme levels [36].

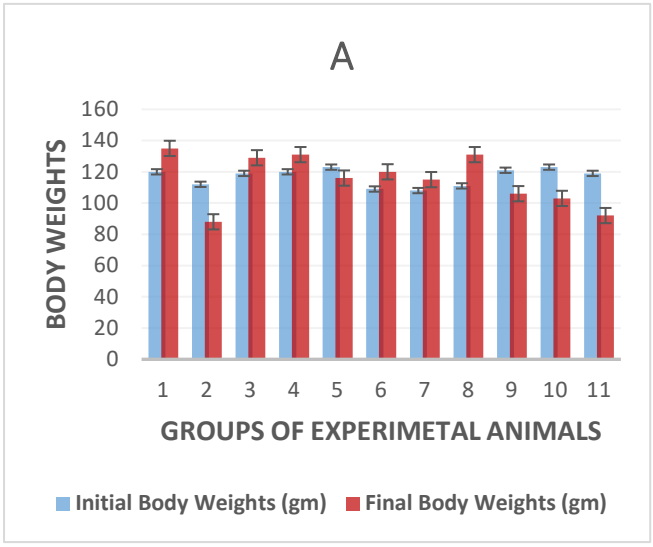
These alterations may be related to the hepatocytes' changed architecture. This membrane alterations are enough to permit intracellular enzymes to leak or pass through into the blood, which explains why serum levels of these enzymes are higher. Additionally, cytosolic isoenzymes leak into the interstitium and then into the peripheral circulation due to increased permeability caused by cell injury. Thus, according to the study by Navarro et al., which provides evidence of the hepatotoxic action of alloxan, the increase in activities of ALT, AST, ALP, and GGT in serum is mostly caused by the leakage of these enzymes from the liver cytosol into the blood stream. Total proteins and albumin levels decreased following alloxan injection. Increased protein catabolism or microproteinurea and albuminurea, two significant clinical indicators of diabetic nephropathy, could be the cause of this drop in the concentrations of total proteins and albumin [37]. A significant increase in MDA levels and a significant decrease in GSH, which indicate development of oxidative stress and a decline in antioxidant enzyme defense mechanisms, were observed in rats given alloxan in our study. This could be because alloxan is chemically reduced to dialuric acid, which causes oxidative damage by producing free radicals [23]. Our results were in line with research done on animals that were given alloxan to cause diabetes [38-39].

Additionally, a number of studies have demonstrated that a reduction in the activity of the antioxidants GSH and an excess of MDA are important factors in the development of diabetes [40]. Since hyperglycemia is always associated with the release of pro-inflammatory cytokines, inflammation (like oxidative stress) plays a significant role in diabetes mellitus [41-42]. It is well recognized that inflammation serves as the host's defense mechanism against infections and tissue damage, either promoting tissue repair or stopping the spread of pathogens. A transcriptional factor called NF-κB regulates the production of cytokines, such as TNF-α and IL-6, and inflammation process. Cytokines in diabetes trigger an autoimmune reaction by activating NF-κB, which leads to the death of pancreatic β-cells. The present investigation found that after receiving an injection of alloxan monohydrate, the expression levels of the inflammatory cytokines TNF-α and IL-6 in the serum of diabetic rats were significantly greater than those of normal healthy rats. This suggested that an excess of ROS, generated by alloxan, caused the inflammation in rats. Our results corroborated earlier research showing that chronic inflammation contributes significantly to the development of diabetes [41-43].

When compared to diabetic rats, the diabetic rats' levels of the inflammatory markers TNF-α and IL-6 were much lower after receiving zinc sulfate and vitamin D for 28 days. Zinc is the most prevalent trace elements in the human body [44]. It included in hundreds of enzymes and even more protein domains involved in various cellular functions, such as apoptosis, differentiation, and proliferation. It is a common element in subcellular metabolism and is a crucial part of at least one enzyme's catalytic site or sites in all categories of enzymes. [45]. Zinc's antioxidant properties have been extensively studied [46-47]. In addition to being a crucial part of antioxidant enzymes like superoxide dismutase, zinc also inhibits catalytic capabilities of iron and copper, two redox active transition metals, which encourages the fenton reaction to produce hydroxyl from hydrogen peroxide and superoxide. Zinc is crucial for integrity and function of biological membranes. Zinc's ability to stabilize biomembranes and biostructures without need of enzymes has also demonstrated its antioxidant potential. Zinc's protective effects may be explained by its capacity to lessen accumulation of collagen in liver and by its vital physiological role in controlling cell structure and function [48].

Additionally, zinc stimulates the production of the antioxidant protein metallothionein, which is high in cystein. Metallothionein stabilizes membranes and aids in heavy metal detoxification. An essential part of the body's antioxidant defenses, zinc helps slow down oxidative processes, especially those associated with diabetes mellitus. In particular, zinc is necessary for the proper synthesis and operation of several metallothioneins and the antioxidant enzyme copper-zinc superoxide dismutase [49]. According to the study's findings, vitamin D supplements control blood sugar levels. Vitamin D helps lower insulin resistance and the inflammatory processes that cause both type 1 and type 2 diabetes [50]. Furthermore, Boucher showed that vitamin D's mechanism of action in T2DM involves both direct action on pancreatic β-cell function, which is mediated by the binding of the active form of 1,25(OH)2D to its receptor (VDR), which is expressed in pancreatic β-cells, and the regulation of calcium trafficking in these cells, which controls insulin synthesis, secretion, and sensitivity [51]. 1, 25(OH) 2D activates the human insulin gene's promoter and transcription, which supports the direct effects of vitamin D on insulin production and secretion. Petramala found that excessive synthesis of glucocorticoids (GCs) and aldosterone is linked to a decrease in vitamin D levels.

By raising the cellular concentrations of enzymes and substrates that promote the hepatic glucose synthesis process, GCs promote gluconeogenesis [52]. Pancreatic GCs negatively impact the cells and reduce insulin release. Additionally, GCs in peripheral tissues attenuate skeletal muscle and adipose tissue's ability to absorb glucose [53]. According to earlier research, an increase in pro-inflammatory cytokines promotes glucose toxicity associated with diabetes, which results in oxidative stress, mitochondrial malfunction, and hepatocellular death. Since blood vitamin D repletion reduces IL-6 expression, we might hypothesize that a vitamin D shortage may promote the activity of these pro-inflammatory factors. Vitamin D supplementation markedly improved MDA concentrations. Depending on the kind of cell, vitamin D can either attach to VDR in the nucleus or use its hydrophobic components to minimize oxidative stress and limit the production of free radicals. By attaching itself to the VDR in the cell nucleus, vitamin D helps regulate the production of free radicals in the liver cells of diabetic rats [54]. This vitamin can modulate antioxidant enzymes to produce its antioxidant effects and safe gaurd the cell membrane by preventing lipid peroxidation. In the early phases, it scavenges free radicals prior to the activation of defense mechanisms against oxidative stress [55].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160
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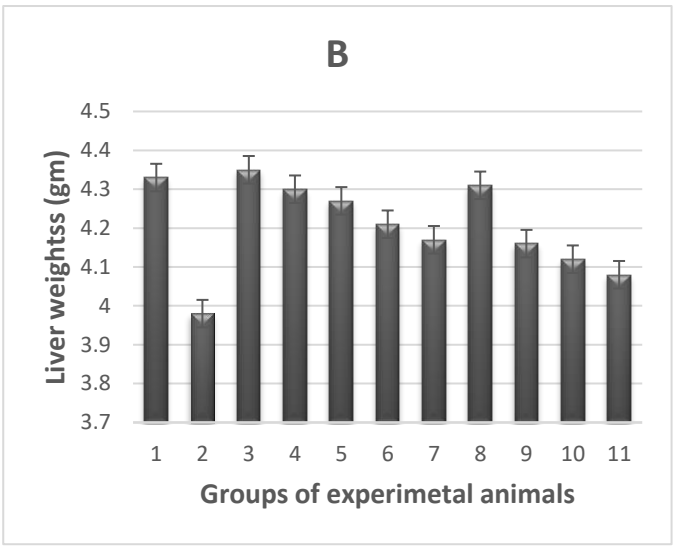
GROUPS OF EXPERIMETAL ANIMALS
Initial Body Weights (gm)
Final Body Weights (gm)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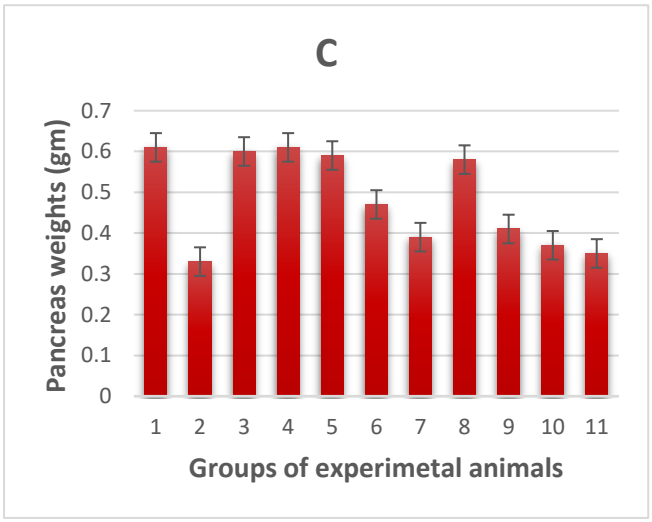
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Pancreas weights (gm)
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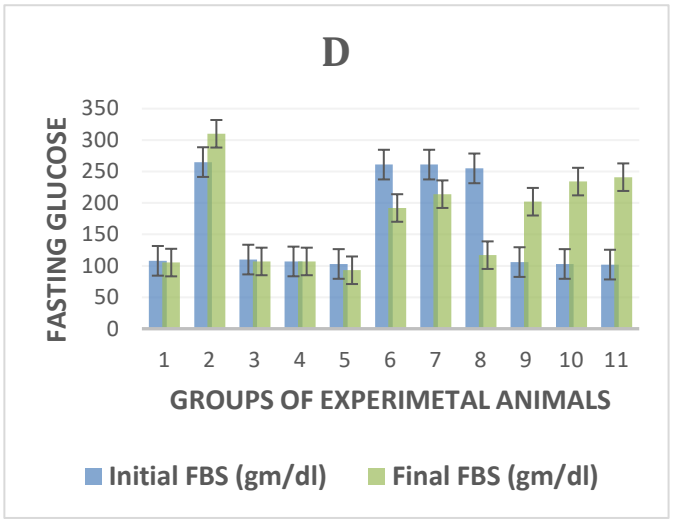
Groups of experimetal animals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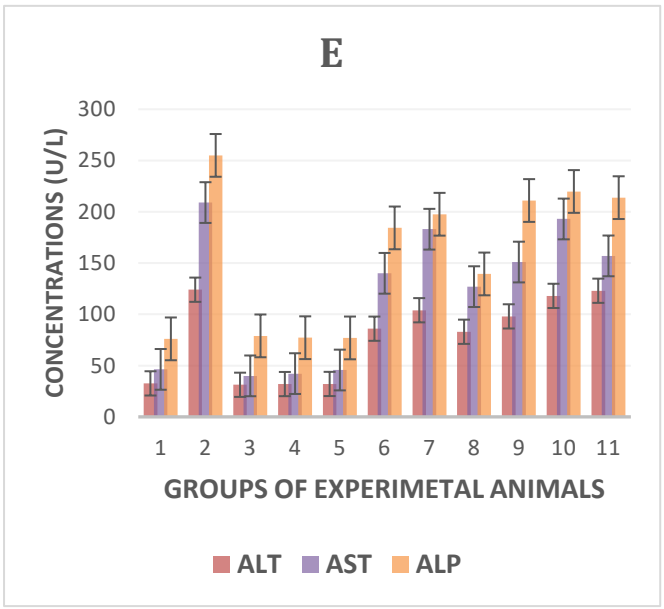
GROUPS OF EXPERIMETAL ANIMALS
Initial FBS (gm/dl)
Final FBS (gm/dl)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GROUPS OF EXPERIMETAL ANIMALS
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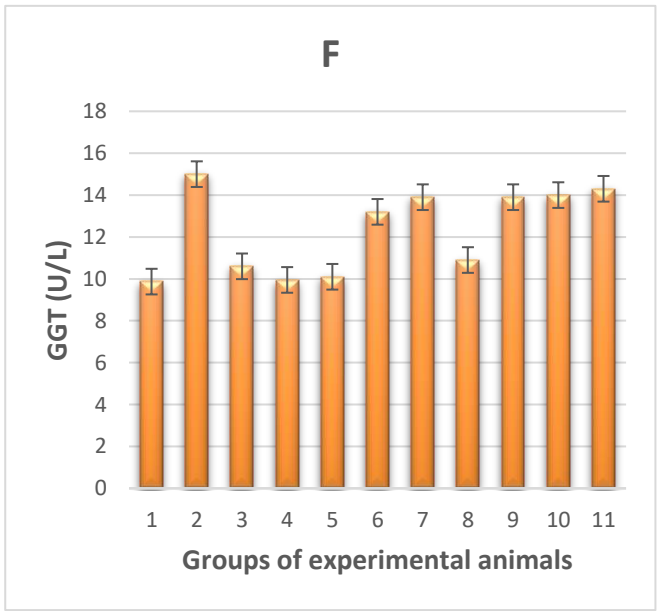
F
GGT (U/L)
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Groups of experimental animals

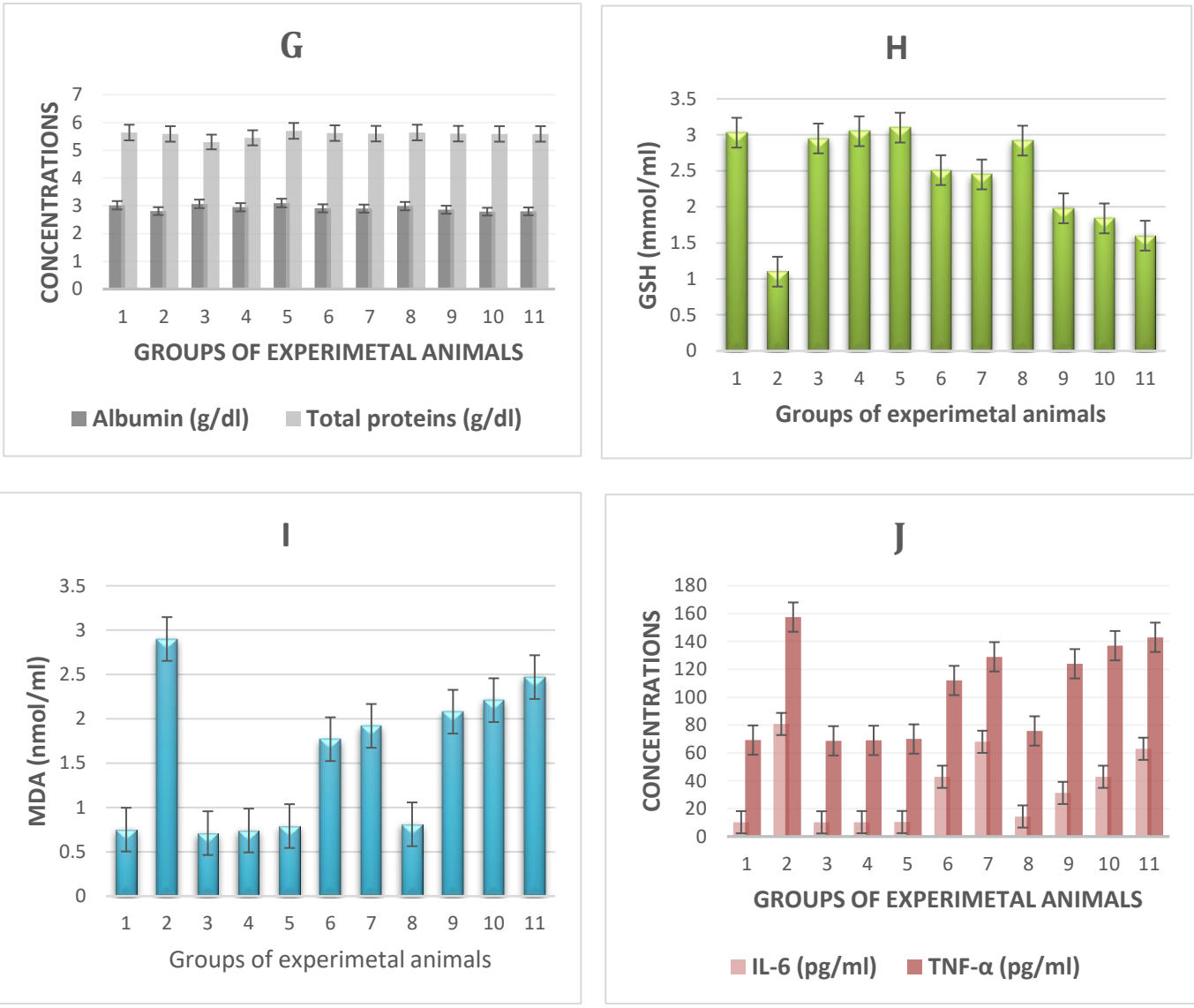
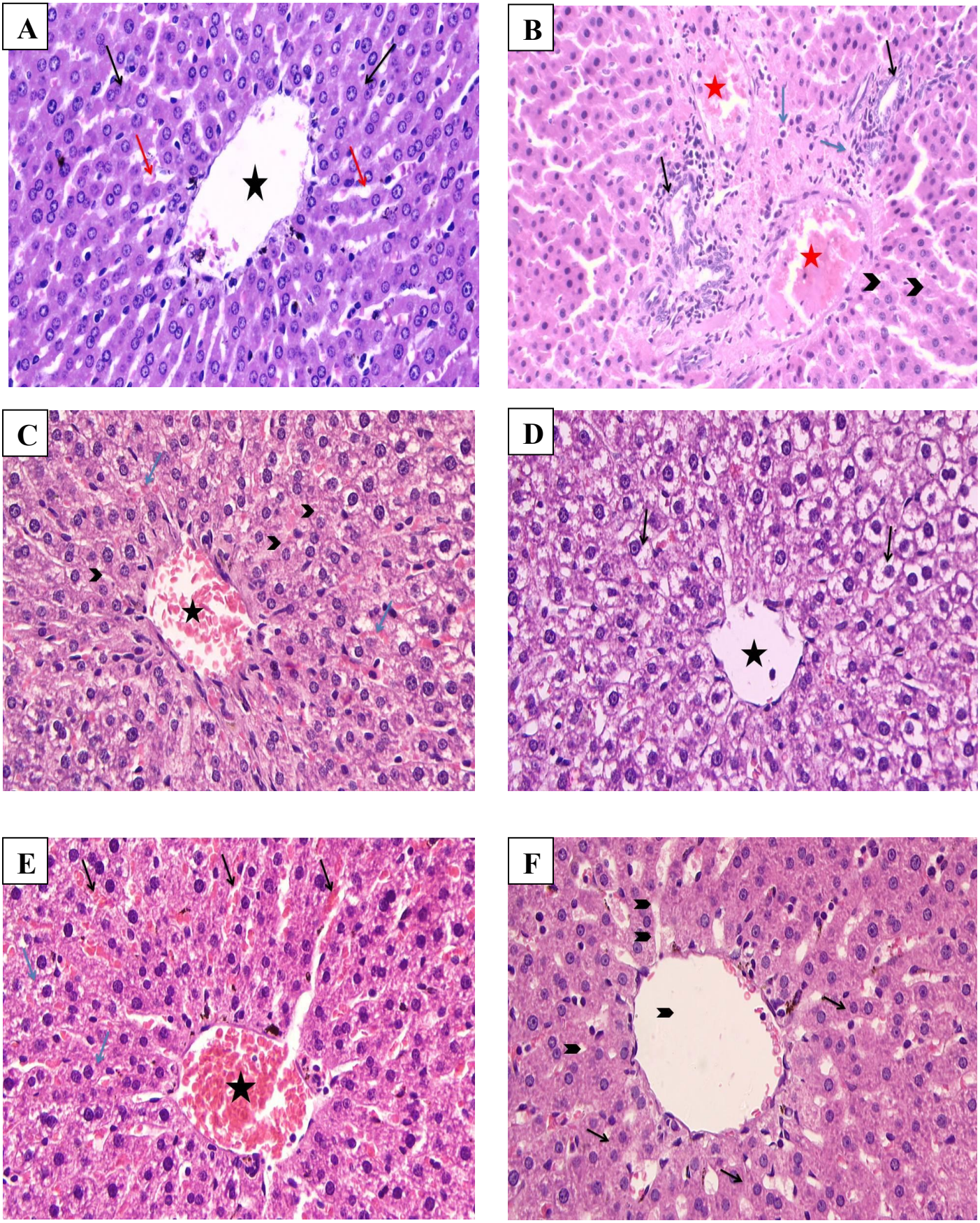



**Figure 1:** Effect of pre and post administration of metformin, zinc and vitamin D on body weights, organs weight and other biochemical parameters of alloxan-induced diabetic rats. **A**: Rats' body weights, **B**: liver weights, **C**: pancreas weights, **D**: blood glucose levels before and after the experiment period, **E & F**: liver enzymes, **G**: serum proteins, **H & I**: oxidative stress markers and **J**: inflammatory markers.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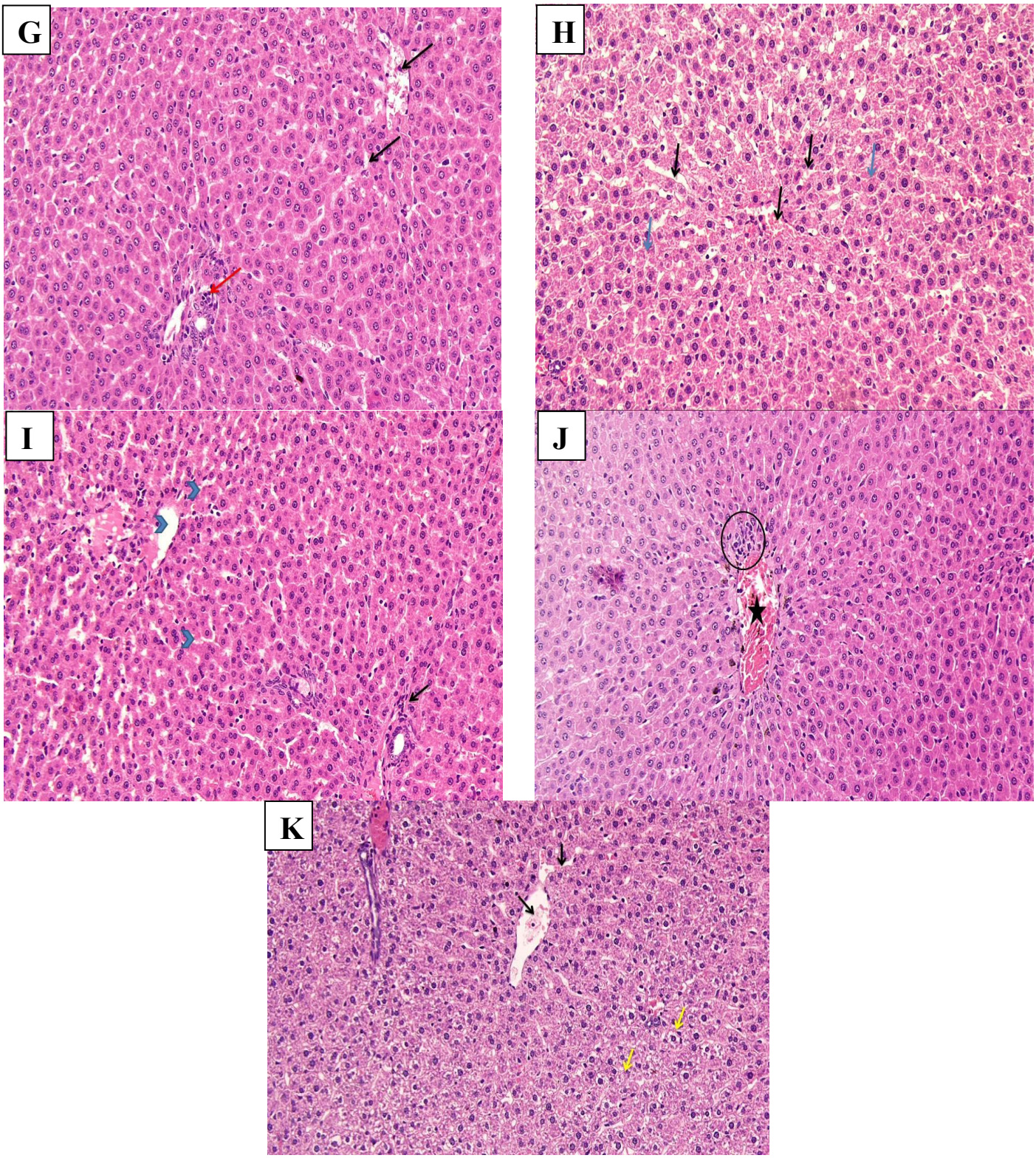
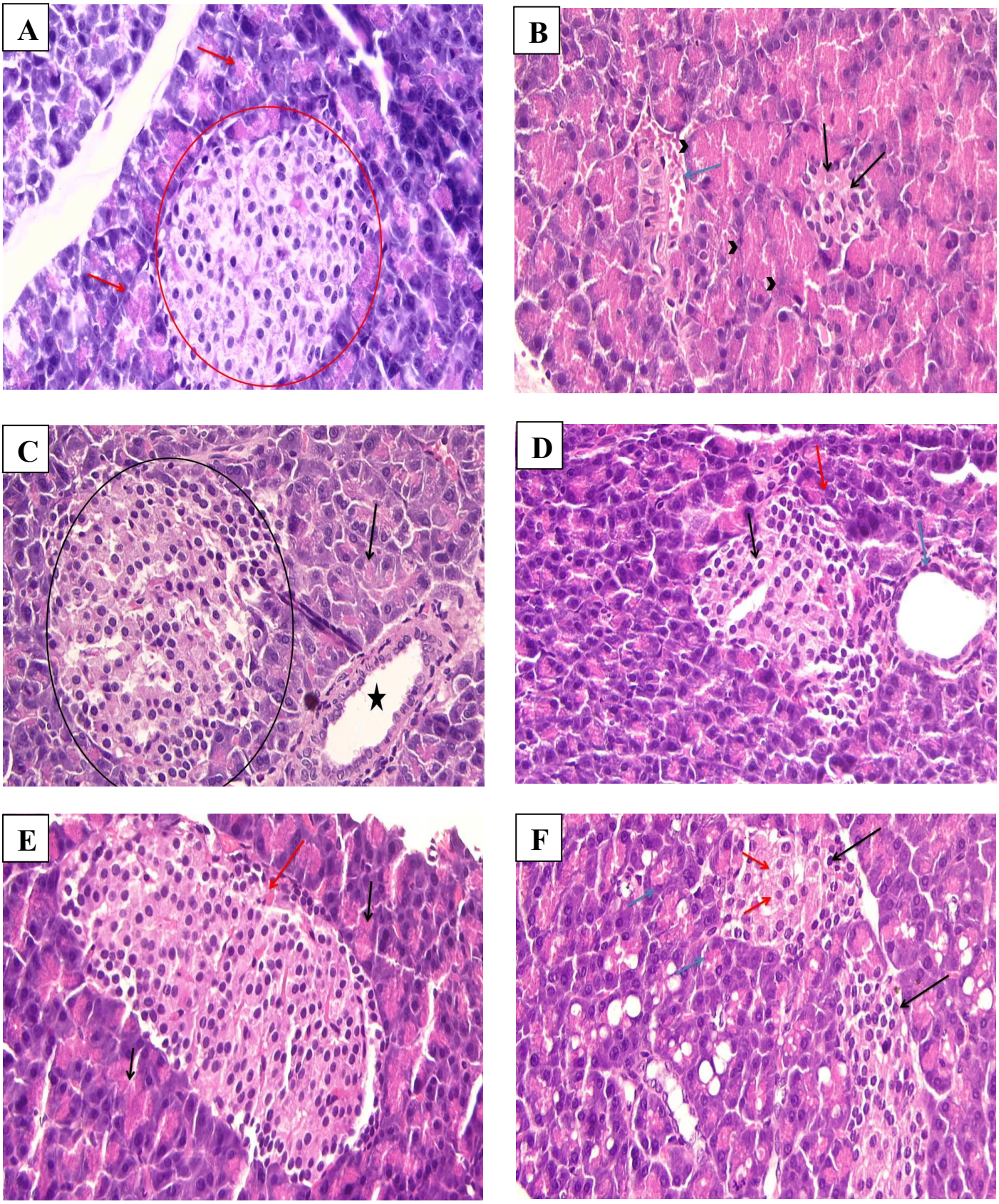



**Figure 2**: Photomicrographs of rat liver tissues. **A** (the healthy control group): showing central vein (star) and radiating trabeculae of hepatocytes (black arrows) with intervening sinusoids (red arrows) X200, **B** (the diabetic control group) showing proliferated bile ducts (black arrows), congested vessels (red star), chronic inflammatory cells (blue arrows) and signs of karyolysis (arrow head) X200, **C** (healthy control treated with zinc sulfate only) showing mild congested central vein (star) and sinusoids(blue arrow), mild steatosis (arrow head) X400, **D** (healthy control treated with vitamin D only) showing congested central vein (star), moderate fatty change (black arrows) X400, **E** (healthy control treated with metformin only) showing congested central vein (star), markedly congested sinusoids (black arrows), mild to moderate steatosis (blue arrows) X400.**F** (diabetic post-treated with zinc sulfate): showing congested central vein and sinusoids (arrows head), normal looking hepatocytes arranged in trabeculae (black arrows) X400, **G** (diabetic post-treated with vitamin D): showing decreased central vein and sinusoidal congestion (black arrows), minimal periportal inflammation (red arrows) X200, **H** (diabetic post-treated with metformin): showing mild congestion of central vein and sinusoids (black arrows) and mild steatosis (blue arrows) X200, **I** (diabetic pre-treated with zinc sulfate): showing marked restoration of hepatic architecture, decreased central vein and sinusoidal congestion (arrow head), minimal periportal inflammation (black arrows) X200, **J** (diabetic pre-treated with vitamin D): showing increased central vein congestion (star), centrilobular inflammation and necrosis (black circle) X200 and **K** (diabetic pre-treated with metformin): showing mild congestion of central vein and sinusoids (black arrows) and moderate steatosis (yellow arrows) X200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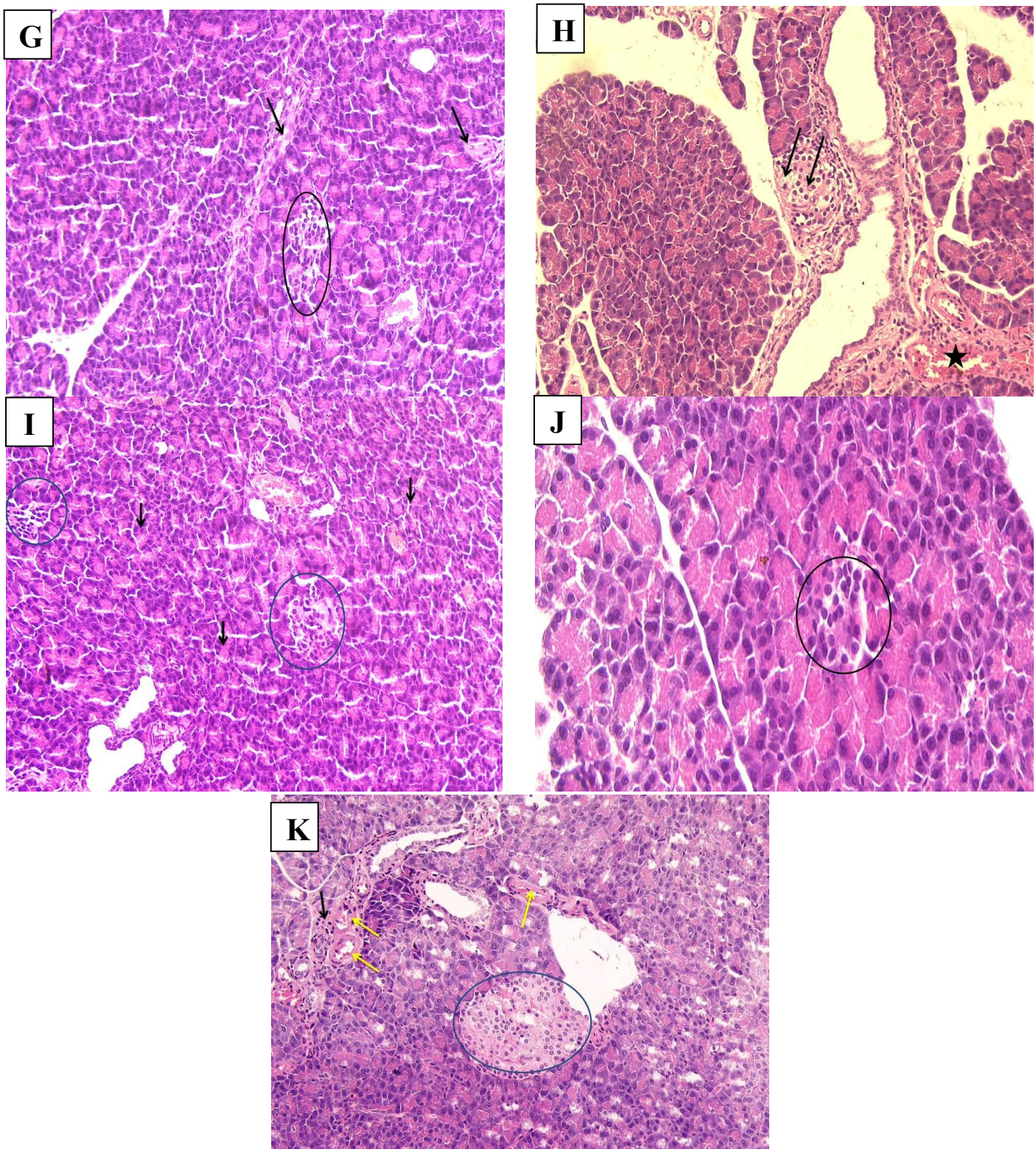


**Figure 3:** Photomicrographs of rat pancreatic tissues. **A** (the healthy control group): showing normal islets of Langerhans (red circle) and pancreatic acini (red arrows) X200, **B** (the diabetic control group) showing atrophic islets with signs of vacuolation and degeneration of islet cells (black arrow), interstitial haemorrhage (blue arrow), signs of acinar cell necrosis noted by absence of nuclei (arrow head) X400, **C** (healthy control treated with zinc sulfate only) showing normal pancreatic islets with increased number if islet cells (red circle), normal pancreatic acini (black arrows) and ducts (star) X400, **D** (healthy control treated with vitamin D only) showing increased density and number of islet cells, islet cells show slight vacuolation (black arrow) and normal pancreatic acini (red arrow) and normal ducts (blue arrows) X400, **E** (healthy control treated with metformin only) showing normal pancreatic islets (red arrow) and acini (black arrows) X400. **F** (diabetic post-treated with zinc sulfate): showing increased number of islets (black arrows), surrounded by normal pancreatic acini (blue arrows), few islet cells show vacuolated cytoplasm (red arrows) X400, **G** (diabetic post-treated with vitamin D): showing decreased number and size of pancreatic islets (black circle), interstitial fibrosis and inflammation (red arrows) X200, **H** (diabetic post-treated with metformin): showing vaculated and degenerated islet cells (blac arrow) and congested blood vessels (star) X200, **I** (diabetic pre-treated with zinc sulfate): showing normal pancreatic islets (circles) surrounded by lobules of pancreatic ducts and acini (black arrows) and diminished interstitial haemorrhage or edema X200, **J** (diabetic pre-treated with vitamin D): showing decreased number of pancreatic islets and signs of atrophy(circle) X400 and **K** (diabetic pre-treated with metformin): showing Some islets are atrophic (black circles) while others show vacuolated islet cells (blue circle) , congested blood vessels (yellow arrow) and perivascular mononuclear inflammatory cell infiltrate (black arrow) X200.

**Table 1:** Represents mean of body weights before and after the experiment and Rat organs weights. All Values Are Expressed As Mean ± Standard Deviation.

| Groups | Body Weights | | Organs Weights | |
|---|---|---|---|---|
| | Initial B.Wt (gm) | Final B.Wt (gm) | Pancreas Wt (gm) | Liver Wt (gm) |
| Group 1 | 120 ± *2.19* | 135 ± *2.44* | 0.61 ± *0.02* | 4.33 ± *0.09* |
| Group 2 | 112 ± *2-28* | 88$^{***}$ ± *1.46* | 0.33$^{***}$ ± *0.02* | 3.98$^{***}$ ± *0.03* |
| Group 3 | 119 ± *0.63* | 129 ± *1.26* | 0.6 ± *0.04* | 4.35 ± *0.03* |
| Group 4 | 120 ± *2.00* | 131 ± *1.41* | 0.61 ± *0.03* | 4.3 ± *0.14* |
| Group 5 | 123 ± *1.41* | 116 ± *1.09* | 0.59 ± *0.02* | 4.27 ± *0.01* |
| Group 6 | 109 ± *0.63* | 120$^{***}$ ± *2.00* | 0.47$^{***}$ ± *0.02* | 4.21$^{***}$ ± *0.05* |
| Group 7 | 108 ± *1.41* | 115$^{***}$ ± *1.41* | 0.39$^{\#}$ ± *0.01* | 4.17$^{**}$ ± *0.02* |
| Group 8 | 111 ± *1.26* | 131 ± *2.00* | 0.58 ± *0.03* | 4.31 ± *0.02* |
| Group 9 | 121 ± *1.41* | 106$^{***}$ ± *1.14* | 0.41$^{*}$ ± *0.02* | 4.16$^{**}$ ± *0.02* |
| Group 10 | 123 ± *0.63* | 103$^{***}$ ± *1.27* | 0.37$^{\#}$ ± *0.03* | 4.12$^{*}$ ± *0.01* |
| Group 11 | 119 ± *2.00* | 92 ± *0.70* | 0.35 ± *0.03* | 4.08 ± *0.02* |

**Table 2:** Represents mean of blood glucose before and after the experiment & mean of some liver enzymes concentrations in experimental rats after administration of alloxan and test drugs. All Values Are Expressed As Mean ± Standard Deviation.

| Groups | Pancreatic Functions | | Liver Functions | | | |
|---|---|---|---|---|---|---|
| | Initial FBS (mg/dl) | Final FBS (mg/dl) | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) |
| Group 1 | 108 ± 1.79 | 105 ± 0.59 | 33 ± 0.32 | 46 ± 1.11 | 76 ± 0.67 | 9.87 ± 0.47 |
| Group 2 | 265 ±1.67 | 310$^{***}$ ± 1.67 | 124$^{***}$ ± 0.79 | 209$^{***}$ ± 1.58 | 255$^{***}$ ± 1.90 | 15$^{***}$ ± 0.57 |
| Group 3 | 110 ± 1.41 | 107 ± 1.67 | 31 ± 0.32 | 40 ± 1.79 | 79 ± 1.01 | 10.6 ± 0.74 |
| Group 4 | 107 ± 1.09 | 107 ± 2.61 | 32 ± 0.49 | 42 ± 0.62 | 77 ± 1.33 | 9.95 ± 0.54 |
| Group 5 | 103 ± 1.27 | 93 ± 1.67 | 32 ± 0.32 | 46 ± 0.36 | 77 ± 1.27 | 10.1 ± 0.36 |
| Group 6 | 261 ± 1.67 | 192$^{***}$ ± 2.00 | 86$^{***}$ ± 1.16 | 140$^{***}$ ± 1.41 | 184$^{***}$ ± 1.38 | 13.2$^{*}$ ± 0.75 |
| Group 7 | 261 ± 1.67 | 214$^{***}$ ± 2.28 | 104$^{***}$ ± 1.58 | 183$^{***}$ ± 2.37 | 198$^{***}$ ±1.02 | 13.9$^{\#}$ ± 1.05 |
| Group 8 | 255 ± 2.28 | 117 ± 1.01 | 83 ± 1.09 | 127 ± 1.67 | 139 ± 1.27 | 10.9 ± 0.62 |
| Group 9 | 106 ± 1.67 | 202$^{***}$ ± 1.67 | 98$^{***}$ ± 1.79 | 151$^{***}$ ± 1.79 | 211$^{***}$ ± 0.80 | 13.9$^{\#}$ ± 0.86 |
| Group 10 | 103 ± 1.67 | 234$^{***}$ ± 1.86 | 118$^{*}$ ± 1.90 | 193$^{***}$ ± 1.10 | 220$^{***}$ ± 0.88 | 14$^{\#}$ ± 0.60 |
| Group 11 | 102 ± 2.10 | 241 ± 1.67 | 123 ± 2.97 | 157 ± 2.19 | 214 ± 0.62 | 14.3 ± 0.91 |

**Table 3:** Represents mean of some serum proteins concentration and mean of some serum oxidative stress and inflammatory markers concentration in experimental rats after administration of alloxan and test drugs. All Values Are Expressed As Mean ± Standard Deviation.

| Groups | Serum Proteins | | Oxidative Stress Markers | | Inflammatory Markers | |
|---|---|---|---|---|---|---|
| | Albumin (g/dl) | Total protein (g/dl) | GSH (mmol/ml) | MDA (nmol/ml) | IL-6 (pg/ml) | TNF-α (pg/ml) |
| Group 1 | 3.02 ± 0.04 | 5.64 ± 0.03 | 3.03 ± 0.07 | 0.75 ± 0.05 | 10.3 ± 1.34 | 69.3 ± 1.68 |
| Group 2 | 2.81$^{***}$ ± 0.05 | 5.59$^{\#}$ ± 0.05 | 1.10$^{***}$ ± 0.08 | 2.90$^{***}$ ± 0.08 | 80.8$^{***}$ ± 1.70 | 157.5$^{***}$ ± 1.64 |
| Group 3 | 3.07 ± 0.04 | 5.30 ± 0.08 | 2.95 ± 0.06 | 0.71 ± 0.04 | 10.2 ± 0.50 | 68.7 ± 3.51 |
| Group 4 | 2.95 ± 0.04 | 5.45 ± 0.06 | 3.05 ± 0.06 | 0.74 ± 0.05 | 10.3 ± 0.75 | 69.0 ± 0.81 |
| Group 5 | 3.10 ± 0.03 | 5.70 ± 0.02 | 3.10 ± 0.07 | 0.79 ± 0.03 | 10.4 ± 1.30 | 70.0 ± 2.76 |
| Group 6 | 2.91$^{*}$ ± 0.03 | 5.62$^{\#}$ ± 0.02 | 2.51$^{***}$ ± 0.06 | 1.77$^{***}$ ± 0.10 | 42.9$^{***}$ ± 1.48 | 112$^{***}$ ± 1.58 |
| Group 7 | 2.90$^{*}$ ± 0.05 | 5.60$^{\#}$ ± 0.06 | 2.45$^{***}$ ± 0.06 | 1.92$^{***}$ ± 0.09 | 68.0$^{***}$ ± 0.82 | 129$^{***}$ ± 1.56 |
| Group 8 | 2.99 ± 0.03 | 5.64 ± 0.03 | 2.92 ± 0.05 | 0.81 ± 0.10 | 14.4 ± 0.64 | 75.8 ± 0.68 |
| Group 9 | 2.86$^{\#}$ ± 0.03 | 5.60$^{\#}$ ± 0.04 | 1.98$^{***}$ ± 0.05 | 2.08$^{***}$ ± 0.09 | 31.3$^{***}$ ± 1.58 | 124$^{***}$ ± 2.10 |
| Group 10 | 2.79$^{\#}$ ± 0.02 | 5.59$^{\#}$ ± 0.03 | 1.84$^{***}$ ± 0.11 | 2.21$^{***}$ ± 0.70 | 42.9$^{***}$ ± 2.03 | 137$^{***}$ ± 2.60 |
| Group 11 | 2.80 ± 0.04 | 5.59 ± 0.04 | 1.60 ± 0.05 | 2.47 ± 0.08 | 63.0 ± 1.14 | 143 1.66 |

## 4. Conclusions

This study provides evidence that zinc and vitamin D may be helpful in the managing of diabetes because of their ability to reduce blood sugar levels. With their capacity to suppress oxidative stress, boost antioxidant defense system, and preventing hepatic and pancreatic tissues damage, zinc and vitamin D are therefore possible antidiabetic substances that may help avoid complications from diabetes.

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**Conflicts of Interest**

Authors declare that there is no conflicts of interest.

**Ethical approval**

The experiment and rats handling were carried out as stated by the guidelines of the Committee of Research Ethics of Faculty of Pharmacy, Minia University, Egypt (MPEC-230202), and Follow the principles outlined in the Guide for the Care and Use of Lab Animals. Every attempt was made to reduce animal suffering and use as few animals as possible.

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