# Histopathological Alterations in the Liver and Kidney of Alloxan-Induced Diabetic Rats and the Ameliorative Role of Olea europea, Rumex nervosus, and Ziziphus spina-christi Extracts

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***Abstract:***

*This study showed the ameliorative effects of Olea europea (O.) subsp. Africana, Rumex nervosus (R.), and Ziziphus spina-christi (Z.) plant extracts against alloxan on the liver and kidney tissues of male albino rats. Thirty male albino rats weighing between 300 and 320 grams were split into five groups, with six rats in each group. The diabetic rats infected by injecting them with a dose of 120 mg/kg body weight of sterile cold alloxan solution into the peritoneal cavity. The diabetic rats were given orally the aqueous extracts of the mentioned three plants (2 times/day) at 300 mg/kg body weight (for 22 days). Significant alterations like cytoplasmic vacuolization of hepatocytes, blood vessel obstruction, necrosis, and edema in the liver and kidney of alloxan-induced diabetic rats group appeared in the histopathological studies. The treated groups with the alloxan and plant extracts showed amelioration in the hepatic and renal tissues represented as follows: treatment with O. extract, showed normal tissue of the distal and proximal convoluted tubules, but some alterations still present like a wide renal space and degenerated glomeruli. On the other hand, treatment with R. and Z. extracts showed more ameliorations than O., represented by normal tissue of the distal and proximal convoluted tubules, well-formed Malpighian corpuscles, but hemorrhage and congested renal blood vessels still seen in some areas of the renal tissues. In the groups treated with the extracts of the three plants, an improvement in histopathological features of the liver was observed; hepatic tissues of the diabetic rats treated with Olea and Rumex appeared normal, with a normal hepatic portal vein and normal bile duct compared to alloxan-diabetic rats. In conclusion, an improvement of histopathology of the liver and kidney was observed in the groups treated with Olea, Rumex, and Ziziphus compared with the alloxan group.*

Keywords: Alloxan, Ziziphus, Olea, Rumex, liver, kidney.

## 1. INTRODUCTION

In traditional medicine, plants are used safely to some extent, but some may have toxic effects, lead to complicated problems, and even cause death (Okigbo et al. 2009). The use of plants in traditional medicine has been passed down from generation to generation (Klotoé et al. 2013; Bayaga et al. 2017). El and Karakaya (2009) noted that olive leaves have traditionally been used in Europe to treat diabetic hyperglycemia and various diseases. The wild olive plant (Olea europaea L.) is recognized as a medicinal plant, with its leaves and fruits utilized for the treatment of several ailments in numerous countries (Abd El-Rahman, 2016). Guex et al. (2019) mentioned that the olive (Olea europaea L.) has anti-inflammatory, antioxidant, and hypoglycemic activities. Ahmad et al. (2024) showed that Olea europea fruit extract has a beneficial effect against sodium fluoride toxicity in the testes of male mice. Soldo et al. (2024) found that oleuropein, derived from wild olive leaf extract, exhibited gastrointestinal instability. They recommended using microencapsulation techniques to improve stability, particularly after the gastric phase. Qadir et al. (2016) determined in their study that the extract from Olea europaea leaves has a protective effect on the pancreatic tissue of alloxan-diabetic rats. The rats treated with the extract displayed normal-sized pancreatic islets, with a regular distribution of alpha, beta, and other cells within the islets. Ibrahim et al. (2024) mentioned that using Rumex nervosus nanoparticles in high doses completely restored the normal structure of stomach mucosa in treated female Albino rats compared with Rumex nervosus extract. Sekhon-Loodu and Rupasinghe (2019) provided the first pharmacological prudence about Rumex's antioxidant and antidiabetic potential and some of the traditional Canadian medicinal plants. They mentioned that polyphenols in the extracts of these plants can reduce the increase in hyperglycemia by delaying the digestion of carbohydrates. The leaves extract of Rumex nervosus have a high antioxidant contents against bacteria and fungi, it may be considered attractive for manufacturing food preservation against microbial contamination due to its content of bioactive constituents, especially gallic acid, Al-Garadi et al. (2022). Al-Naqeb and Taj Al- Deen (2017) stated that the Rumex nervosus leaves extract reduced the effect of diabetes and hyperlipidemia in female Albino rats without having any toxic effects.

Faleh et al. (2024) explained that adding 40 g/kg of Ziziphus spina-christi leaves extract to the Iraqi buck goats' diet increased the concentration of testosterone hormone and enhanced the parameters of semen. Alharbi (2024), in a new study, stated that Ziziphus spina-christi leaves methanolic extract has an antioxidant and anticancer effect against human gastric cancer cells, increasing their late apoptosis. Ramadan et al. (2021) concluded that the extract of Ziziphus spina-christi leaves mitigated the effects of HgCl2 on liver tissues and offered protection against hepatotoxicity in male rats. They proposed that this effect might be attributed to the ability of Ziziphus spina-christi leaf extract to protect hepatic tissues from the oxidative stress induced by HgCl2. Kirtikar and Basu (1984) and Han and Park (1986) stated that Ziziphus species are utilized as traditional medicine for the treatment of gastrointestinal and urinary tract disorders, hyperglycemia, and many diseases, such as hepatic disorders. Abdel-Zaher et al. (2005) revealed that continuous oral administration of the Ziziphus spina-christi leaves butanol extract to rats can be used safely as an antidiabetic agent without causing histopathological alterations in the kidneys and liver of diabetic rats. Ikram et al. (1981), Higuchi et al. (1984), Nawwar et al. (1984), Han et al. (1990), Barboni et al. (1994), Zarga et al. (1995), Cheng et al. (2000), Shahat et al. (2001), and Tripathi et al. (2001) stated that Ziziphus has different species and many of its derivatives have been isolated and chemically identified and used as antidiabetic and antioxidant agents. Al-Zahrani (2021) demonstrated the protective effect of aqueous extracts of *Olea europea*, *Rumex nervosus, and Ziziphus spina-christi* on the pancreas tissue of diabetic male rats represented by appearance of the normal histological pattern of Langerhans islets and normal distribution of islets cells.

*Olea europea* (O.) subsp. *Africana, Rumex nervosus* (R.), and *Ziziphus spina-christi* (Z.) are famous locally plants at Al-Baha region, especially in Al-Mandaq Governorate, Saudi Arabia. They have used in the previous research to treat diabetes in male albino rats. The aim of this study was to investigate the ameliorative role of watery extract of the *O.*, *R. and Z.* leaves on the liver and kidney of rats with diabetes induced by alloxan.

## 2. SUPPLIES AND TECHNIQUES

### 2.1. Substances

1- *Olea europea*, *Rumex nervosus*, and *Ziziphus spina-christi* leaves extracts.
2- Male albino rats.
3- Alloxan.
4- Pelleted diet.

### 2.2. Preparing plants for extraction

The plants mentioned above (*Olea europea*, *Rumex nervosus*, and *Ziziphus spina-christi*) were plucked from their natural habitat (Al-Mandaq governorate) in the Kingdom of Saudi Arabia, Al-Baha region. After washing the leaves under running water from the faucet, we let them air dry at 25 to 30 °C for four weeks while stored in the shade. The dried leaves were then pulverized in an electric mill. A shaking incubator set to 50°C (150 rpm) was used to soak 100g of each plant powder for the entire night in 1000 ml of distilled water disinfected. After that, we used muslin fabric to filter our solutions and an electric dryer set to 50 °C to dry them (Bhuyan, et al. 2015 & Al-Zahrani 2021).

### 2.3. Animals used in the experiment

The thirty male albino rats used in this investigation, weighing between 300 and 320 grams, were obtained from King Abdulaziz University's King Fahd Center for Medical Research. Prior to the experiment, the animals underwent three weeks of environmental adaptation in controlled conditions, which included a temperature of 22 ± 1 °C, a relative humidity of 65%, and a light/dark cycle of 12:12 hours. They also had access to water and food at will. Their regular diet consisted of pelleted food provided ad libitum, which included 20 IU/g of vitamin A, 70 IU/kg of vitamin E, 2.2 IU/g of vitamin D, 6% ash, 4% crude fat, 3.5% crude fiber, 1% calcium, and 6% phosphorus. Additionally, trace elements such as iron, copper, manganese, cobalt, zinc, and selenium were incorporated into the diet (Al-Zahrani 2021). At the end of the experiment, the rats were dissected, and the liver and kidneys were obtained for histopathological examinations.

The animals used in this research are those utilized in the project granted by Al Baha University, entitled "Antidiabetic Effect of Extracts of Some Plants Collected from Al-Mandaq Governorate" (Grant No. 1439/30), and all university requirements have been met.

### 2.4. Diabetes induction by alloxan

Diabetes in experimental animals was induced using single intraperitoneal injections of monohydrated alloxan dissolved in sterile cold 0.85% saline (120 mg/kg body weight). Before alloxan administration, rats were made to be fast. After twelve hours, we gave animals 10% glucose solution to prevent hypoglycemia. We took blood samples from the animals' tail veins after seven days so we could

use a glucometer to measure their blood sugar levels. We considered the rats diabetic when they had levels above 220 mg/dl of blood glucose (Ahmed and Saddam 2024).

### 2.5. Design of the experiment

The five experimental animal groups (six per cage) were split up as follows:

- First group: negative control (control group).
- The second group was the positive control, consisting of diabetic rats.
- Third group: for 22 days, the diabetic rats were given 300 mg/kg/day of O. extract.
- Fourth group: for 22 days, the diabetic rats were given 300 mg/kg/day of R. extract.
- Fifth group: for 22 days, the diabetic rats were given 300 mg/kg/day of Z. extract.

Two daily doses of 150 mg/kg/day (300 mg/kg/day) were given orally to the groups receiving the three plant extracts, Al-Zahrani (2021). We handled and treated the animals in this experiment following all ethical protocols.

### 2.6. Blood glucose levels

Blood samples were collected from the tail vein of the animals for evaluation of glucose levels at the 1st day using the glucometer (Contour TS). The examinations were repeated every 7 days to confirm maintenance of the glucose levels.

### 2.7. Histopathology

The liver and kidney were obtained from control and diabetic animals after the 22th day of the experiment, the rats dissected and the liver and kidneys obtained for histopathological examinations, Al-Zahrani (2021).

### 2.8. Data analysis

Evaluations of the significance among means of extracts diabetic rats; the diabetic control and the normal control were analyzed by one-way ANOVA online. The data were represented as means ± SEM. $P<0.05$ was considered statistically significant.

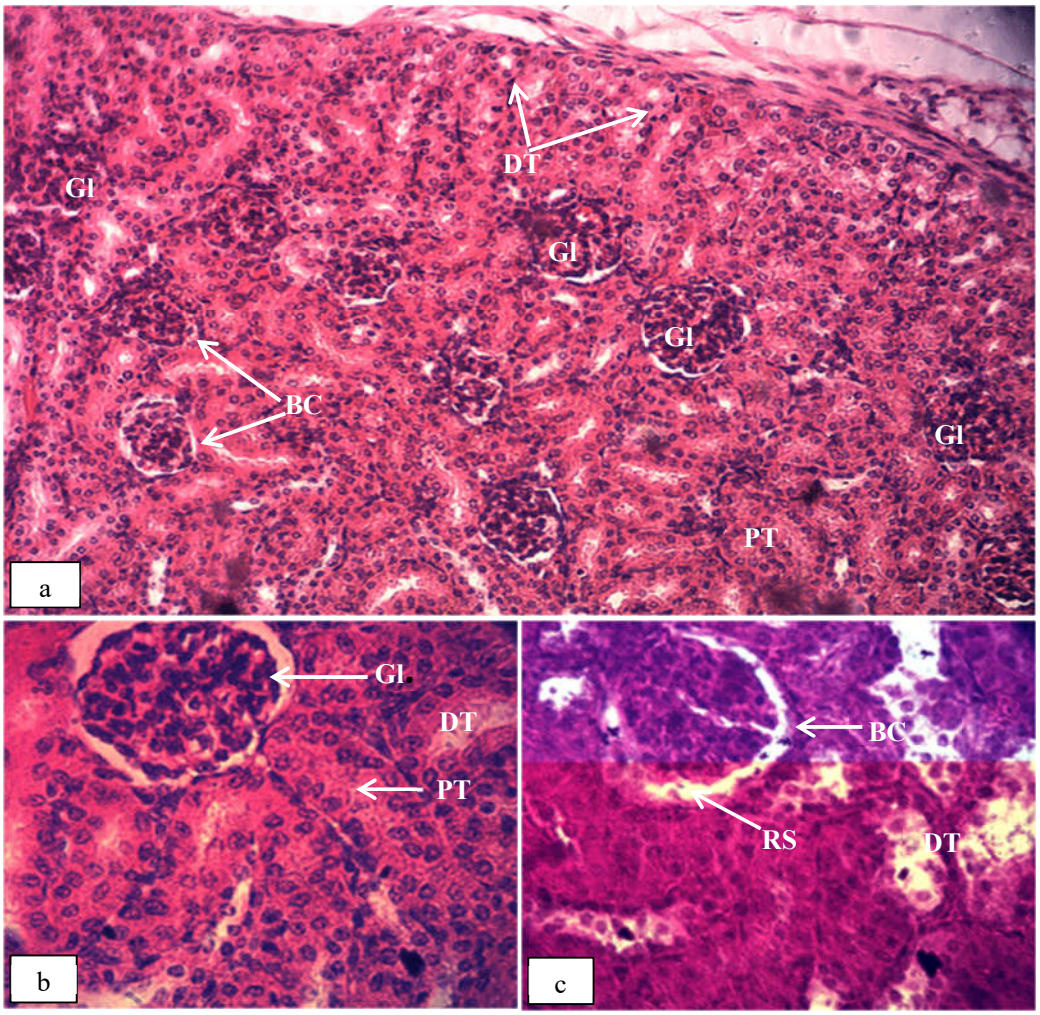
## 3. RESULTS

This study examined the potential ameliorative impact of aqueous extracts from the leaves of the specified plants (O., R., and Z.) on the kidney and liver tissues of alloxan-induced diabetic rats. The experimental animals were induced with diabetes by administering a single intraperitoneal injection of cold sterile saline solution containing 120 mg/kg body weight of monohydrated alloxan.

### 3.1. Histopathological effects on kidney

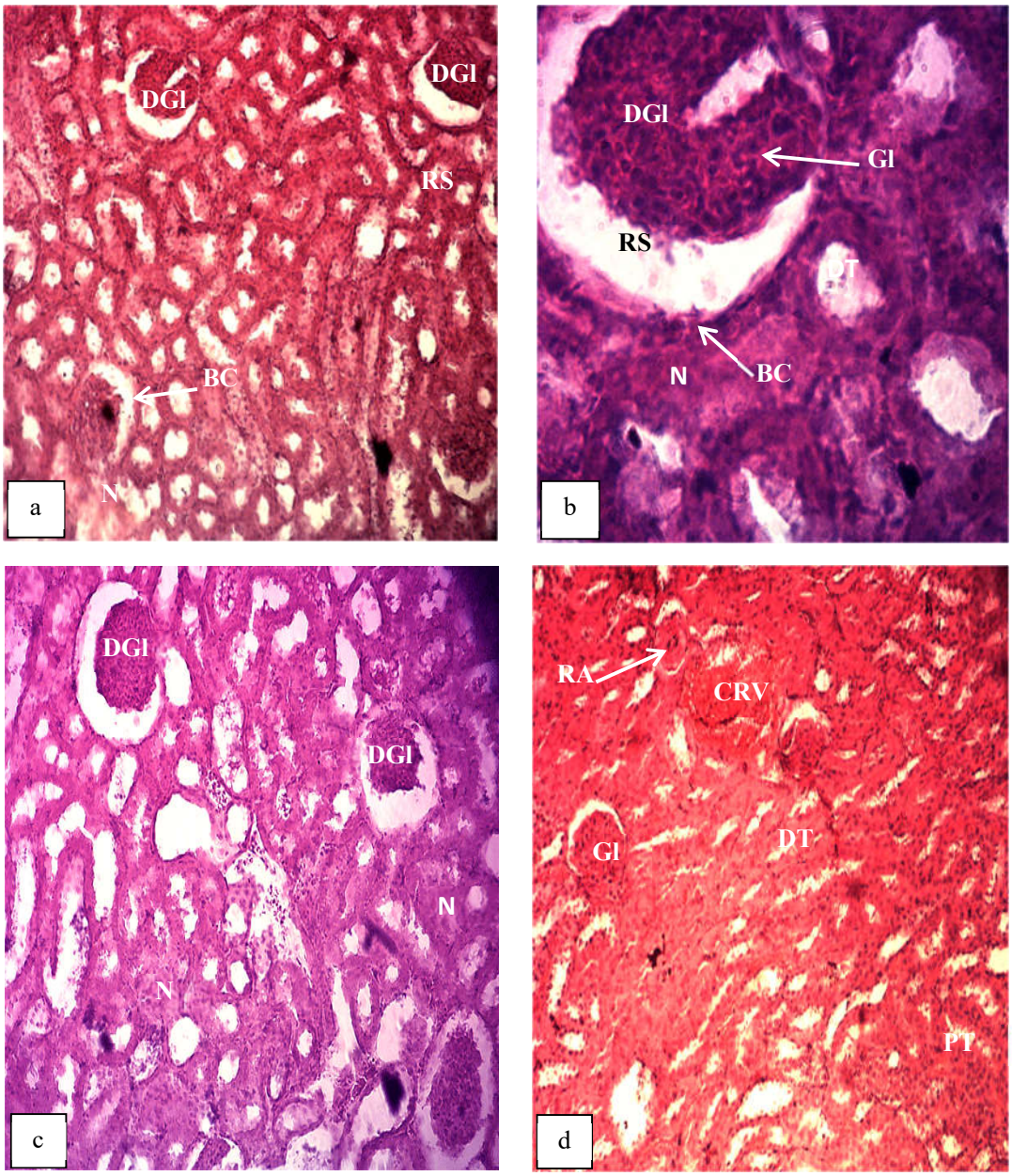
Figures 1 a, b, and c display the typical renal tissues of control rats. Each kidney has an external cortex and an internal medulla. The nephron is the primary functional component of the kidney, comprising the renal corpuscle (consisting of the glomerulus and glomerular capsule) and the renal tubule system (including the proximal tubule, nephron loop, distal tubule, and collecting ducts). Sections of the control rat kidney revealed normal tissue, the glomerulus surrounded by the Bowman's capsule

with normal renal space, convoluted tubules proximal (PT) and distal (DT) appeared normal without inflammatory changes as seen in Figures 1 a, b, and c.



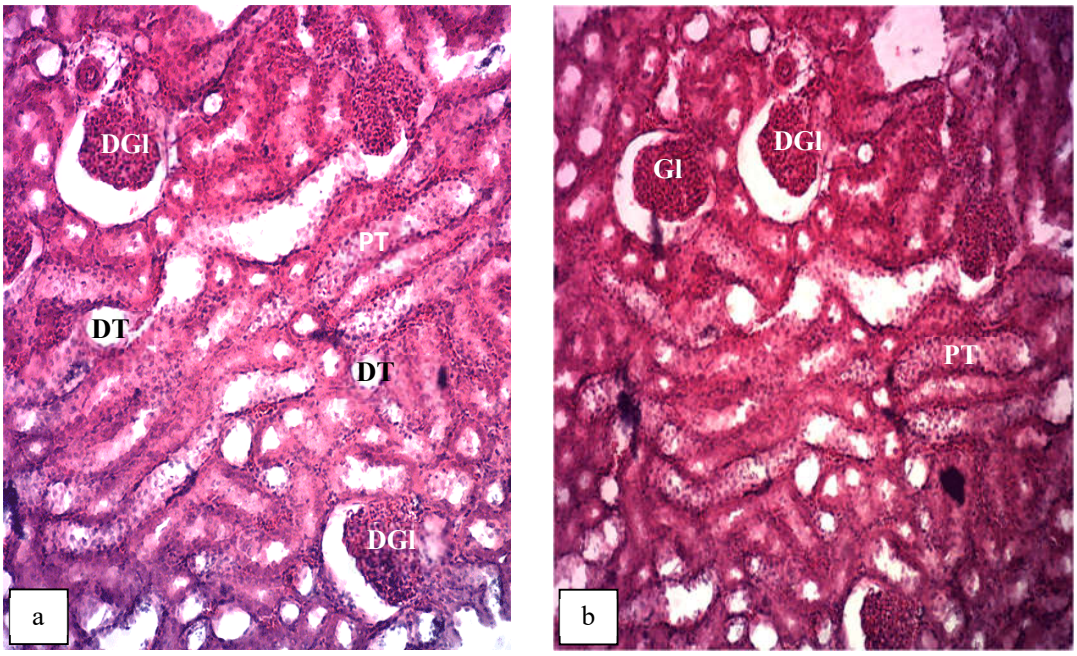

Figures 1. a (H&E: 100x), b and c (H&E: 400x): Photomicrographs of the kidney sections from the negative control group demonstrating the normal renal tissues in the form of normal glomeruli (Gl), bowman's capsule (BC), distal convoluted tubules (DT), proximal convoluted tubules (PT) and normal renal space (RS).

Sections of the alloxan-treated male rats' kidneys revealed histopathological alterations represented by degenerated glomeruli, necrosis in the uriniferous tubules, increasing renal space, renal vein congestion, obstructive renal artery, and thickening of the basement membrane (Figures 2 a, b, c, and d).



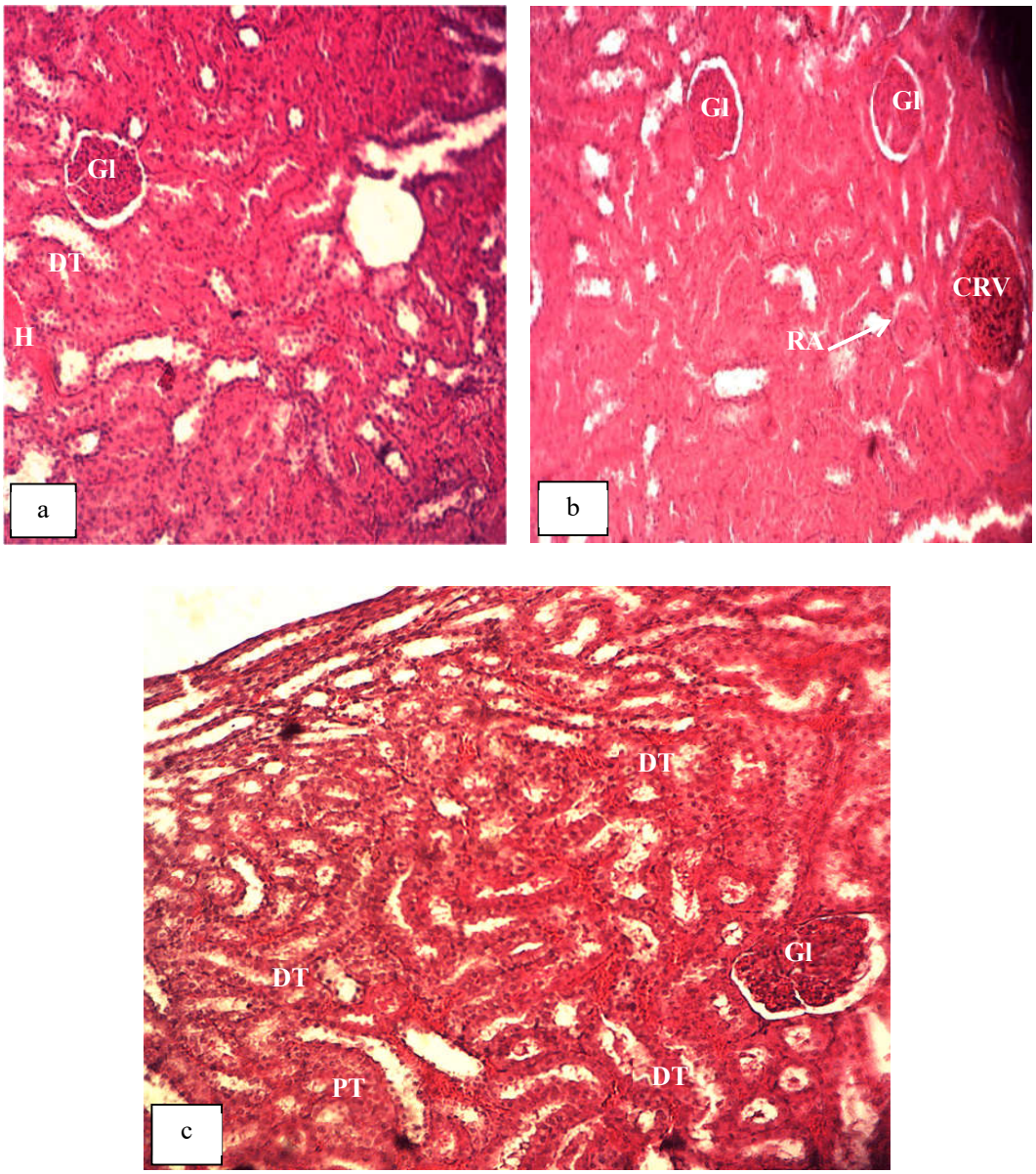

Figures 2. a, c, and d (H&E: 100x), and b (H&E: 400x): Photomicrographs of the kidney sections from the positive control group (Alloxan-treated rats) demonstrating histopathological alterations represented by degenerated glomeruli (DG), necrosis in the uriniferous tubules (N), increasing the renal space (RS), congested renal vein (CRV) and obstructive renal artery (RA).

Sections of the alloxan and Olea leaves extract treated male rats kidney showed relative improvement in the renal tissues represented by normal tissues of the distal and proximal convoluted tubules, but some Malpighian corpuscles still affected by alloxan and appeared vast renal space and degenerated glomeruli as seen in the Figures 3 a and b.



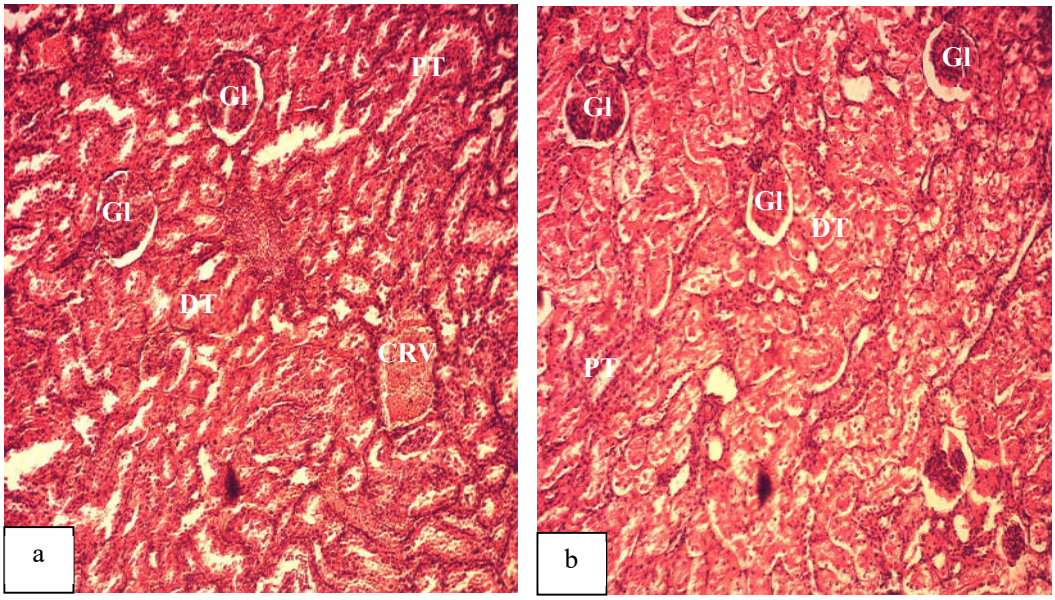

Figures 3. a and b (H&E: 100x): Photomicrographs of the kidney sections from Alloxan and Olea leaves extract treated male rats; Kidney showed relative improvement in the renal tissues represented by normal tissues of the distal (DT) and proximal convoluted tubules (PT), but some Malpighian corpuscles still affected by alloxan and appeared vast renal space (RS) and degenerated glomeruli (DGl).

Sections of the alloxan and Rumex leaves extract-treated male rats' kidneys showed relative improvement in the renal tissues represented by normal tissues of the distal and proximal convoluted tubules and well-formed Malpighian corpuscles, but the effect of alloxan is still clear on the renal blood vessels, causing hemorrhage in some areas, as seen in Figures 4 a, b, and c.




Figures 4. a, b, and c (H&E: 100x): Photomicrographs of the kidney sections from Alloxan and Rumex leaves extract treated male rats; Kidney showed relative improvement in the renal tissues represented by normal tissues of the distal (DT) and proximal convoluted tubules (PT), well-formed Malpighian corpuscles, but the effect of alloxan is still apparent on the renal blood vessels, causing hemorrhage (H) in some areas and congested renal vein (CRV).

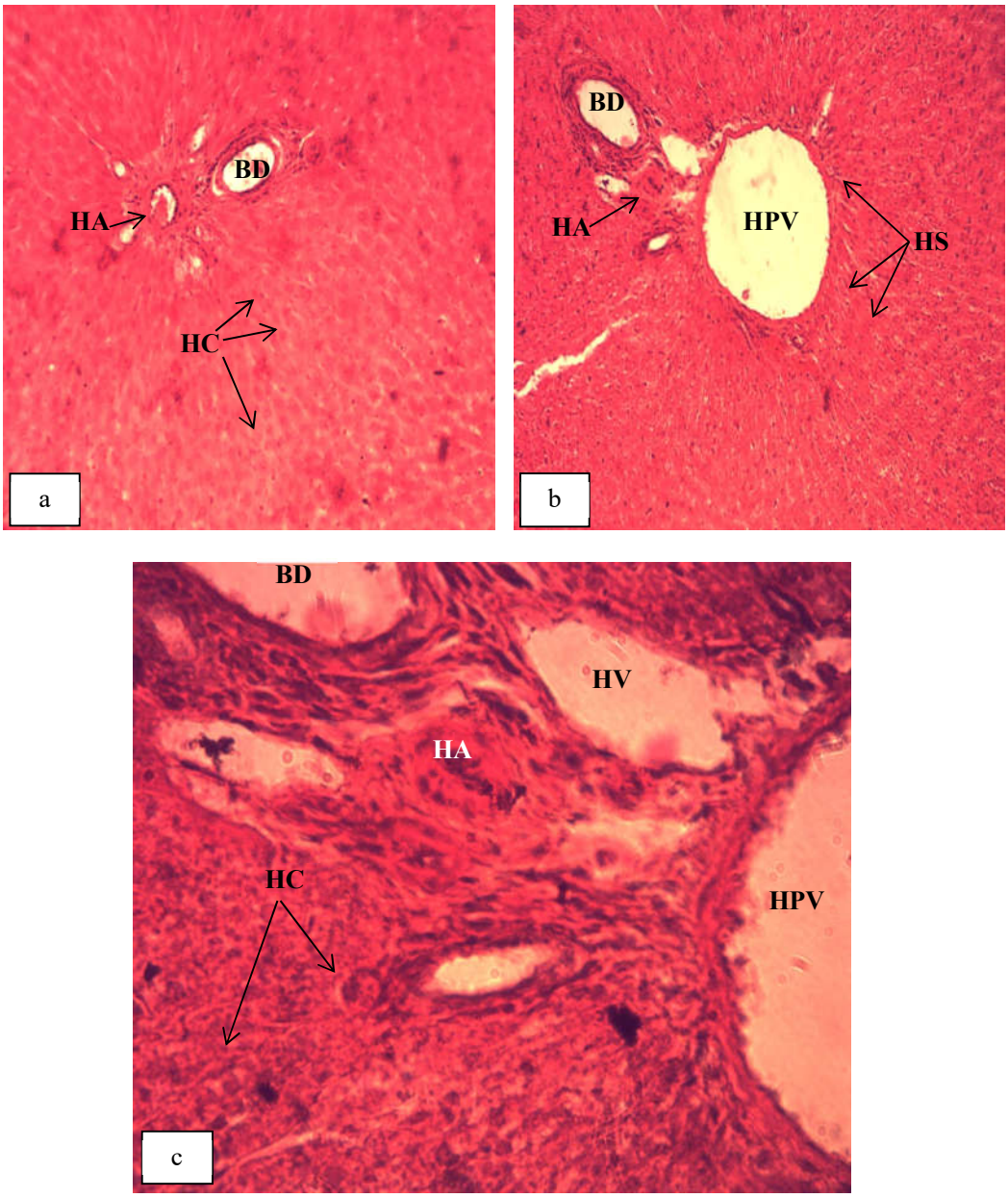
The diabetic rats' kidney sections treated with Z. exhibited significant improvement in the renal tissues, as evidenced by the presence of normal tissues in the distal and proximal convoluted tubules, well-developed Malpighian corpuscles, and persistent congestion in the renal blood vessels, as seen in the Figures 5 a and b.




Figures 5. a and b (H&E: 100x): Photomicrographs of the kidney sections from Alloxan and Ziziphus leaves extract treated male rats kidney showed relative improvement in the renal tissues, but the effect of alloxan is still represented by congestion in renal vein (CRV).

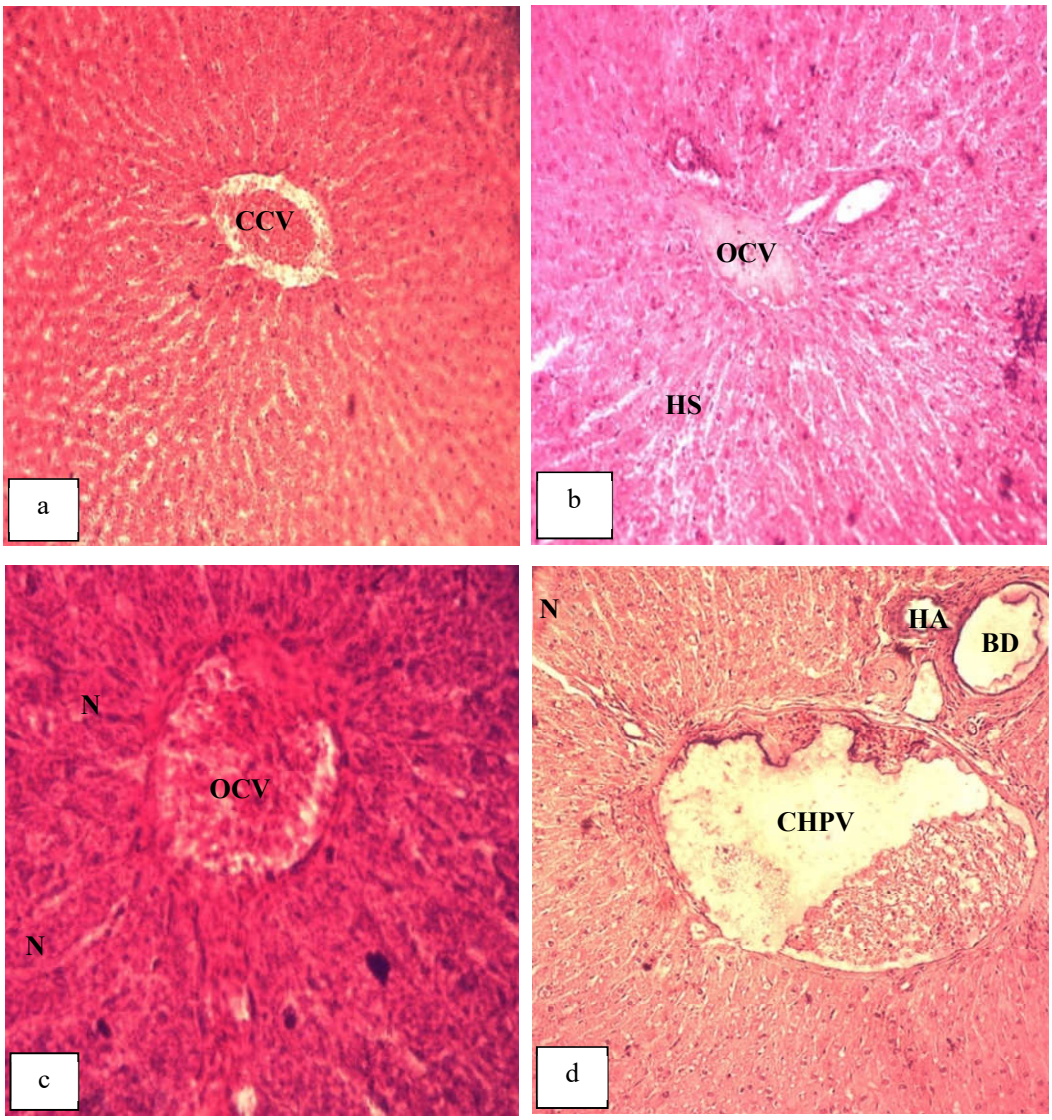
### 3.2. Histopathological effects on liver

Histological sections of the control liver showed that each lobule consists of a central vein and a portal space that includes the portal hepatic vein, hepatic artery, bile duct, and the strands of hepatocytes around it, as shown in Figures 6 a, b, and c.



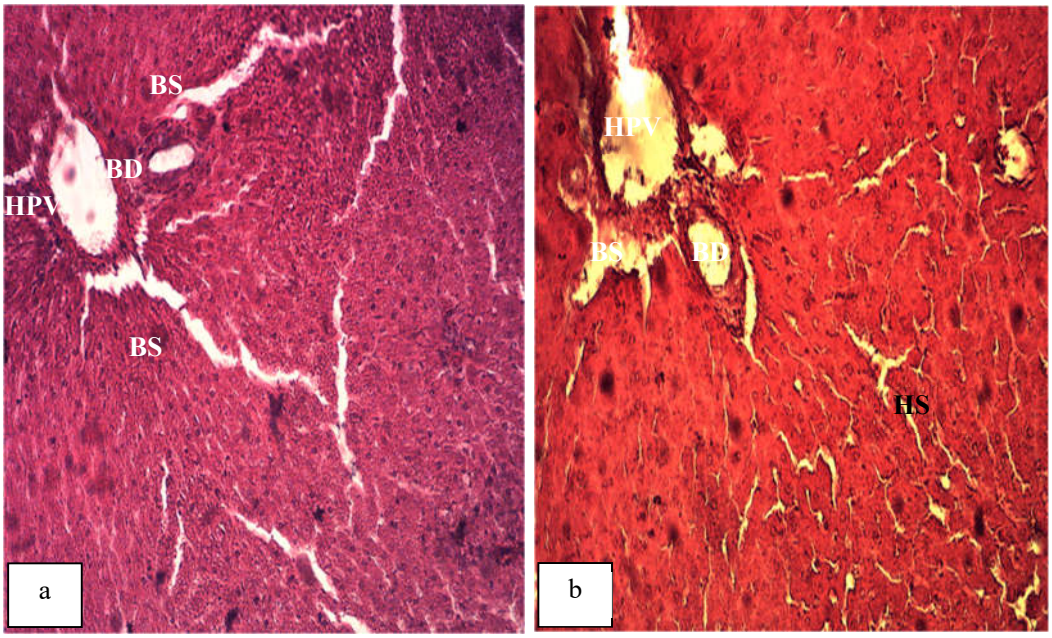

Figures 6. a and b (H&E: 100x), and c (H&E: 400x): Photomicrographs of the liver sections from negative control group demonstrating the usual hepatic tissues in the form of normal central vein (HV) and a portal space that includes the portal hepatic vein (HPV), hepatic artery (HA), bile duct (BD), and the strands (HS) of hepatocytes (HC) around it.

Sections of the alloxan-treated male rats' livers revealed histopathological alterations represented by congested blood vessels, disarray in the hepatic strands, necrotic areas, and hepatocyte vacuolation (Figures 7 a, b, c, and d).



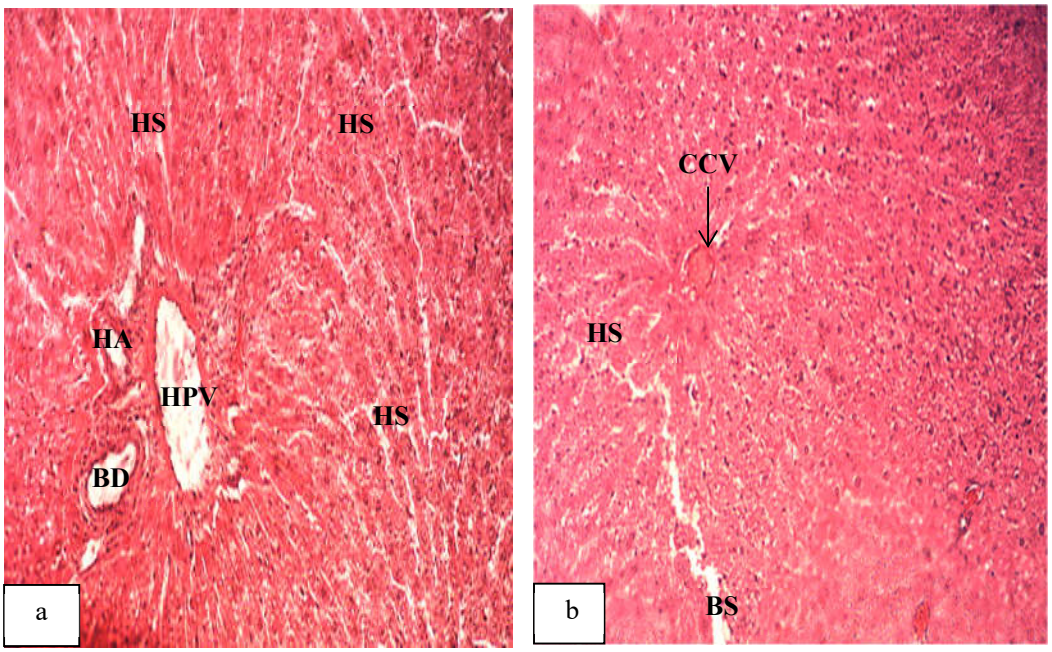

Figures 7. a, b, c, and d (H&E: 100x): Photomicrographs of the liver sections from alloxan-treated male rats demonstrating alterations represented by congested central vein (CCV), congested hepatic portal vein (CHPV) disarray in the hepatic strands (HS), necrotic areas (N) and normal hepatic artery (HA) & bile duct (BD).

Hepatic tissues of the diabetic rats treated with Olea leaves, as seen in Figures 8 a and b, appeared to have normal hepatic portal veins with normal bile ducts compared to alloxan-diabetic rats; however, the expansion of blood sinusoids and disarray of the hepatic strands were observed.



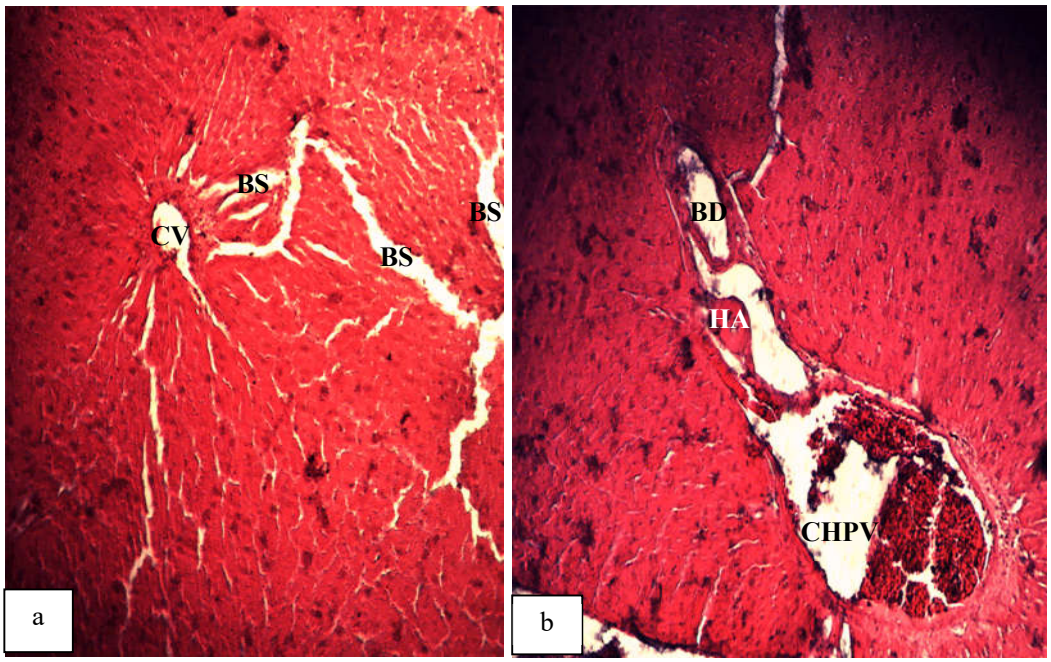

Figures 8. a and b (H&E: 100x): Photomicrographs of hepatic tissues of the diabetic rats treated with Olea leaves revealed normal hepatic portal vein (HPV), normal bile duct (BD), expansion of blood sinusoids (BS) and disarray of the hepatic strands (HS).

Sections of the diabetic rats' liver treated with R. showed relatively normal structure-like sections in the liver of the control group represented by the normal hepatic portal vein, hepatic artery, bile duct, and the strands of hepatocytes around it; however, some lesions still present as congested central vein and disarray in hepatic strands in some areas as shown in the Figures 9 a and b.




Figures 9. a and b (H&E: 100x): Photomicrographs of hepatic tissues of the diabetic rats treated with Rumex leaves revealed normal hepatic portal vein (HPV), normal hepatic artery (HA), normal bile duct (BD), Normal hepatic strands (HS) and blood sinusoids (BS), but congestion in the central vein (CCV) still present.

The hepatic tissues of the diabetic rats treated with Z. were relatively improved, represented by normal tissues of the central vein, bile duct, and hepatic artery, while congested hepatic portal vein and disarray in hepatic strands were still shown in Figures 10 a and b.




Figures 10 a and b (H&E: 100x): Photomicrographs of hepatic tissues of the diabetic rats treated with Ziziphus leaves revealed normal central vein (CV), normal bile duct (BD), normal blood sinusoids (BS), but congestion in the hepatic portal vein (CHPV) and hepatic artery (HA) still present.

### 3.3. Effect on the serum glucose concentration

Table 1 shows significant increases in glucose levels ($P<0.05$) in alloxan-diabetic rats compared to control and *Olea europea (O.), Rumex nervosus (R.), and Ziziphus spina-christi (Z.),* treated groups. The blood glucose levels were significantly decreased in the groups treated with Olea (174.6 mg/dl) and Ziziphus (162.8 mg/dl), while an insignificant decrease is observed in the group treated with Rumex. (256.1 mg/dl).

Table 1. Effects of leaves aqueous extract of *Olea europea (O.), Rumex nervosus (R.), and Ziziphus spina-christi (Z.),* on serum glucose concentrations (mg/dl) in diabetic rats.

| No. Treatments | Day 1 | Day 8 | Day 15 | Day 22 |
|---|---|---|---|---|
| 1. Control | 107.1±11.4 | 101.1±4.6 | 99.1±8.01 | 103.3±17.2 |
| 2. Diabetic rats | 246.1±16.3 | 253.3±14.4 | 254.8±10.2 | 278.3±8.3 |
| 3. Diabetic rats + O. | 243.5±23.2 | 128.3±14.6 | 188.1±6.9 | 175.6±9.1 |
| 4. Diabetic rats + R. | 252.5±13.9 | 227±41.01 | 252.6±4.9 | 257.2±7.1 |
| 5. Diabetic rats + Z. | 256.8±13.3 | 190.8±9.57 | 203.5±12.4 | 163.7±6.1 |

## 4. DISCUSSION

Kumari et al. (2021) showed that alloxan administration induced harm to the liver, kidney, and pancreatic tissues in Swiss albino mice, aligning with our investigation's findings on the liver and kidney of male albino rats. A study conducted by Pochhi (2019) found that rats with diabetes induced by alloxan exhibited notable histopathological alterations in their livers, including extensive degeneration and necrosis of hepatocytes, as well as cell swelling accompanied by vacuolar degeneration. However, treatment with Cinnamonum tamala showed a positive histopathological improvement in the liver tissues. The findings of our investigation, which are consistent with the results observed in both R. and Z.-treated rats but contradict the results obtained from the Olea-treated rats (Al-Zahrani 2021) who showed significant hypoglycemic activity of the aqueous extracts of Z. and O. in diabetic rats in his work on pancreas histology, underscore the unique contribution of our work. The findings of our investigation, which are consistent with the results observed in both R. and Z.-treated rats but contradict the results obtained from the Olea-treated rats, underscore the unique contribution of our work. Our research revealed the presence of central venous congestion and hepatocyte necrosis with vacuolization in liver sections of rats treated with alloxan, which is consistent with the findings of Elangovan et al. (2019). Elangovan et al. (2019) also reported a reduction in the detrimental effects of alloxan when treated with the ethanolic extract of the skin and the methanolic extract of the seed of M. cymbalaria. This finding aligns with the results of the current investigation. Additionally, the histological sections of renal tissues from diabetic rats showed an aberrant glomerular architecture and expanded Bowman's space, which was consistent with the results observed in the male rats treated in this study. Treatment with the ethanolic extract of the skin and the methanolic extract of the seed of M. cymbalaria resulted in the regeneration of glomeruli and the restoration of normal renal space, similar to the treatments with R. and Z. However, there were still some histological changes observed, including congested renal blood vessels and hemorrhage in certain areas of the renal tissues. The findings of Elangovan et al. (2019) were consistent with our own results about the effects of O. treatment on male rats' renal tissues. Our results showed that there was improvement specifically in the convoluted tubules, whereas the degraded glomeruli and expanded renal space remained unchanged.

In their study, Mohammed et al. (2020) found that the liver sections of diabetic rats treated with streptozotocin (STZ) exhibited various histological changes, including vacuolar and fatty degradation, bile duct growth, and necrosis. The rats given encapsulated cinnamon oil emulsion exhibited central vein dilation and blood sinusoid with a moderate increase in Kupffer cells. However, no pathological changes were observed in the liver sections. These findings are consistent with the results obtained from both R. and Z.-treated rats. However, there is a discrepancy between our results and those obtained from O.-treated rats. The diabetic rats who received a high dose of cinnamon oil emulsion exhibited significant improvement in liver damage. However, there were also indications of blood vessel expansion, congestion, and necrosis, similar to what was observed in the rats treated with O. in this investigation.

Singh et al. (2022) identified a bioactive phytochemical compound called 4a-methyl-5-(6-methylhept-5-en-1-yl) octahydro-1H-cyclopenta[a] Pyridazine (MOCP) as an alkaloid secondary metabolite. This compound was isolated from the leaves of L. racemosa. The study found that MOCP exhibited a modest improvement in liver tissue at a low dose. However, certain lesions, such as dilated central veins and blood sinusoids, were still present. The central vein showed slight dilation in rats treated with glibenclamide. The current study's results align with those of previous studies across all classes of plant extracts, similar to those of Ahmed and Saddam's (2024) study on grape seed extract, which had a hypoglycemic effect in alloxan diabetic mice.

The kidney sections of rats given a high dose of MOCP and rats treated with glibenclamide showed normal tubules and glomeruli in the renal parenchyma, which is consistent with our investigation's findings. In their study, Jdir et al. (2017) found that the extract from the flowers of Diplotaxis simplex, known as DSFE, contains bioactive chemicals that can safeguard the pancreas, liver, and kidneys from damage caused by hyperglycemia in rats with alloxan-induced diabetes.
The livers of diabetic rats exhibited a buildup of lipids within the cytoplasm of hepatocytes. The kidney sections of the diabetic rats showed an augmentation in the renal space and deteriorated glomeruli. The administration of DSFE to diabetic rats resulted in a ameliorative effect on the kidney and liver, as evidenced by a reduction in histological damage. These findings are consistent with the results of the present investigation. Alloxan-diabetic rats' hepatic and renal tissues demonstrated enhanced improvement when treated with aqueous extracts of both R. and Z., compared to O. aqueous extracts.
Muruganandan et al. (2005), Miyake et al. (2006), and Kumar et al. (2012) stated that antidiabetic, antihyperlipidemic, and antioxidant agents are found in medicinal plants and can be used as alternative drugs for diabetes. According to Marles and Farnsworth (1995), scientific studies of medicinal plants used in traditional diabetes treatment could provide helpful information for creating alternative medications and therapeutic approaches. Similar to the results of the current study, Abd El Latif et al. (2014), Tang et al. (2017), Bamagous et al. (2018), Villarruel-López et al. (2018), Al-Zahrani (2021), and Kumari et al. (2021) reported the antidiabetic effects of medicinal plants like *Olea europea*, *Ziziphus spina-christi*, *Rumex nervosus*, and *M. oleifera*.

## 5. Conclusion

The present investigation demonstrated significant alterations in the liver and kidney of the alloxan-induced diabetic rats group that appeared in the histopathological studies. Remarkable relief in the hepatic and renal tissues of diabetic rats administered with the aqueous extracts of Rumex nervosus and Ziziphus spina-christi, as opposed to Olea europea. The extracts' potential to restore the liver and kidney of diabetic rats to their normal histological form may be because they contain bioactive substances that can be included as alternative therapies to routine chemical treatments, which could be an acceptable justification for these findings. Further research is necessary to determine the effects of the active ingredients in the utilized extracts on different organs.

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## References


Abd El Latif, A., El Bialy, B. E. S., Mahboub, H. D., & Abd Eldaim, M. A. (2014). Moringa oleifera leaf extract ameliorates alloxan-induced diabetes in rats by regeneration of β cells and reduction of pyruvate carboxylase expression. Biochemistry and cell biology, 92(5), 413-419.

Abd El-Rahman, H. S. M. (2016). The effect of olive leaf extract and A-Tocopherol on nephroprotective activity in rats. J. Nutr. Food Sci, 6, 479.

Ahmed, H. M., & Saddam, A. C. (2024). IMPACT OF GRAPE SEEDS EXTRACT AGAINST ALLOXAN INDUCED DIABETES IN MICE. Iraqi Journal of Agricultural Sciences, 55(6), 2085-2093.

Abdel-Zaher, A. O., Salim, S. Y., Assaf, M. H., & Abdel-Hady, R. H. (2005). Antidiabetic activity and toxicity of Zizyphus spina-christi leaves. Journal of ethnopharmacology, 101(1-3), 129-138.

Ahmad, S. N., Bilal, M. A., Kanwal, M. A., Inayat, I., Younis, A., Suleman, S., & Ahmad, K. R. (2024). Remedial Potential of Olea europea and Punica granatum Fruit Extracts on Testicular Histopathology in Fluoride Exposed Mice. Fluoride, 57(11).

Al-Garadi, M. A., Qaid, M. M., Alqhtani, A. H., Pokoo-Aikins, A., & Al-Mufarrej, S. I. (2022). In vitro phytochemical analysis and antibacterial and antifungal efficacy assessment of ethanolic and aqueous extracts of Rumex nervosus leaves against selected bacteria and fungi. Veterinary World, 15(11), 2725.

Alharbi, S. A. (2024). Anticancer and Antioxidant Activities of Methanolic Extract of Ziziphus Spina-Christi Leaves against Human Gastric Carcinoma Cells. Journal of Pharmacy and Bioallied Sciences, 16(Suppl 4), S3354-S3357.

Al-Naqeb, G., & Deen, A. (2017). The effect of Rumex nervosus Vahl leaves on high fat diet-induced hyperglycemia and hyperlipidemia in albino rats. Int J Chem, 1, 80-83.

Al-Zahrani Khalid S. (2021). Antidiabetic Effect of Leaves Aqueous Extracts of Three Endemic Plants in Al-Mandaq Governorate, Al-Baha, KSA on Diabetic Rats. Albaha University Journal of Basic and Applied Sciences, 5(1), 3–9.

Bamagous, G. A., Al Ghamdi, S. S., Ibrahim, I. A. A., Mahfoz, A. M., Afify, M. A., Alsugoor, M. H., ... & Rengarajan, T. (2018). Antidiabetic and antioxidant activity of ethyl acetate extract fraction of Moringa oleifera leaves in streptozotocin-induced diabetes rats via inhibition of inflammatory mediators. Asian Pacific journal of tropical biomedicine, 8(6), 320.

Barboni, L., Gariboldi, P., Torregiani, E., & Verotta, L. (1994). Cyclopeptide alkaloids from Ziziphus mucronata. Phytochemistry, 35(6), 1579-1582.

Bayaga, H. N., Guedje, N. M., & Biye, E. H. (2017). Approche ethnobotanique et ethnopharmacologique des plantes utilisées dans le traitement traditionnel de l'ulcère de Buruli à Akonolinga (Cameroun). International Journal of Biological and Chemical Sciences, 11(4), 1523-1541.

Bhuyan, T., Mishra, K., Khanuja, M., Prasad, R., & Varma, A. (2015). Biosynthesis of zinc oxide nanoparticles from Azadirachta indica for antibacterial and photocatalytic applications. Materials Science in Semiconductor Processing, 32, 55-61.

Cheng, G., Bai, Y., Zhao, Y., Tao, J., Liu, Y., Tu, G., ... & Xu, X. (2000). Flavonoids from Ziziphus jujuba Mill var. spinosa. Tetrahedron, 56(45), 8915-8920.

El, S. N., & Karakaya, S. (2009). Olive tree (Olea europaea) leaves: potential beneficial effects on human health. Nutrition reviews, 67(11), 632-638.

Elangovan, A., Subramanian, A., Durairaj, S., Ramachandran, J., Lakshmanan, D. K., Ravichandran, G., ... & Thilagar, S. (2019). Antidiabetic and hypolipidemic efficacy of skin and seed extracts of Momordica cymbalaria on alloxan induced diabetic model in rats. Journal of ethnopharmacology, 241, 111989.

Faleh, H., Dakheel, M., & Quijada, J. (2024). Effect of Ziziphus Spina-Christi Leaves on the Level of Testosterone Hormone and Semen Parameters of Iraqi Bucks Semen. The Iraqi Journal of Veterinary Medicine, 48(1), 9-14.

Guex, C. G., Reginato, F. Z., de Jesus, P. R., Brondani, J. C., Lopes, G. H. H., & de Freitas Bauermann, L. (2019). Antidiabetic effects of Olea europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin. Journal of ethnopharmacology, 235, 1-7.

Han, B. H., & Park, M. H. (1986). Folk medicine: The art and science. Am. Chem. Soc. Pub., Washington, DC, p205.

Han, B. H., Park, M. H., & Han, Y. N. (1990). Cyclic peptide and peptide alkaloids from seeds of Zizyphus vulgaris. Phytochemistry, 29(10), 3315-3319.

Higuchi, R., Kubota, S., Komori, T., Kawasaki, T., Pandey, V. B., Singh, J. P., & Shah, A. H. (1984). Triterpenoid saponins from the bark of Zizyphus joazeiro. Phytochemistry, 23(11), 2597-2600.

Ibrahim, B. M., Awad, H. M., Shaffie, N. M., Youness, E. R., & Mahmoud, A. H. (2024). Rumex nervosus nanoparticles versus aqueous extract: introduction of novel immunomodulatory anti-inflammatory nano-preparation for management of paw oedema and gastritis in rats. Bulletin of the National Research Centre, 48(1), 12.

Jdir, H., Kolsi, R. B. A., Zouari, S., Hamden, K., Zouari, N., & Fakhfakh, N. (2017). The cruciferous Diplotaxis simplex: Phytochemistry analysis and its protective effect on liver and kidney toxicities, and lipid profile disorders in alloxan-induced diabetic rats. Lipids in health and disease, 16, 1-9.

Pochhi, M. L. (2019). An antioxidant activity of Cinnamonum tamala improves histopathological alterations and biochemical parameters in alloxan induced diabetic rats. Asian Journal of Medical Sciences, 10(6), 50-56.

Kirtikar, K. R., & Basu, B. D. (1984). Indian Medicinal Plants (II nd ed.). Vol. I, 826-827.

Klotoé, J. R., Dougnon, T. V., Koudouvo, K., Atègbo, J. M., Loko, F., Akoègninou, A. & Gbeassor, M. (2013). Ethnopharmacological survey on antihemorrhagic medicinal plants in South of Benin. European Journal of Medicinal Plants, 3(1), 40.

Kumar, B. A., Lakshman, K., Jayaveea, K. N., Shekar, D. S., Khan, S., Thippeswamy, B. S., & Veerapur, V. P. (2012). Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of Amaranthus viridis Linn in alloxan induced diabetic rats. Experimental and toxicologic pathology, 64(1-2), 75-79.

Kumari, R., Singh, A. K., Kumar, R., & Kumar, A. (2021). Phytoremedial effect of fruit extract of Moringa oleifera on alloxan induced diabetic model in Swiss albino mice: Phytoremedial effect of fruit extract of Moringa oleifera on Alloxan induced Diabetes. Journal of Applied and Natural Science, 13(4), 1420-1429.

Marles, R. J., & Farnsworth, N. R. (1995). Antidiabetic plants and their active constituents. Phytomedicine, 2(2), 137-189.

Miyake, Y., Suzuki, E., Ohya, S., Fukumoto, S., Hiramitsu, M., Sakaida, K., ... & Furuichi, Y. (2006). Lipid-lowering effect of eriocitrin, the main flavonoid in lemon fruit, in rats on a high-fat and high-cholesterol diet. Journal of Food Science, 71(9), S633-S637.

Mohammed, K. A., Ahmed, H. M., Sharaf, H. A., El-Nekeety, A. A., Abdel-Aziem, S. H., Mehaya, F. M., & Abdel-Wahhab, M. A. (2020). Encapsulation of cinnamon oil in whey protein counteracts the disturbances in biochemical parameters, gene expression, and histological picture of the liver and pancreas of diabetic rats. Environmental Science and Pollution Research, 27, 2829-2843.

Muruganandan, S., Srinivasan, K., Gupta, S., Gupta, P. K., & Lal, J. (2005). Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. Journal of ethnopharmacology, 97(3), 497-501.

Nawwar, M. A. M., Ishak, M. S., Michael, H. N., & Buddrust, J. (1984). Leaf flavonoids of Ziziphus spina-christi. Phytochemistry, 23(9), 2110-2111.

Okigbo, R. N., Anuagasi, C. L., & Amadi, J. E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. Journal of medicinal plants Research, 3(2), 86-95.

Qadir, N. M., Ali, K. A., & Qader, S. W. (2016). Antidiabetic effect of oleuropein from olea europaea leaf against alloxan induced type 1 diabetic in rats. Brazilian archives of biology and technology, 59.

Ramadan, S. S., Almeer, R. S., Alkahtani, S., Alarifi, S., Albasher, G., & Abdel Moneim, A. E. (2021). Ziziphus spina-christi leaf extract attenuates mercuric chloride-induced liver injury in male rats via inhibition of oxidative damage. Environmental Science and Pollution Research, 28, 17482-17494.

Sekhon-Loodu, S., & Rupasinghe, H. V. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. Frontiers in nutrition, 6, 53.

Shahat, A. A., Pieters, L., Apers, S., Nazeif, N. M., Abdel-Azim, N. S., Berghe, D. V., & Vlietinck, A. J. (2001). Chemical and biological investigations on Zizyphus spina-christi L. Phytotherapy Research, 15(7), 593-597.

Singh, D. K., & Jadhav, B. L. (2022). Antidiabetic Activity of an Alkaloid (4a-Methyl-5-(6-Methylhept-5-En-1-Yl) Octahydro-1H-Cyclopenta [A] Pyridazine) Isolated from Lumnitzera racemosa in Streptozotocin-Induced Diabetic Wistar Rats. International Journal of Pharmaceutical Investigation, 12(3).

Soldo, B., Bilušić, T., Giacometti, J., Ljubenkov, I., Čikeš Čulić, V., Bratanić, A., ... & Ilić, K. (2024). A Comparative Study of Oleuropein Extraction from Wild Olive Leaves (Olea europea subsp. oleaster, Hoffmanns. & Link), Its Gastrointestinal Stability, and Biological Potential. Applied Sciences, 14(2), 869.

Tang, Y., Choi, E. J., Han, W. C., Oh, M., Kim, J., Hwang, J. Y., ... & Kim, E. K. (2017). Moringa oleifera from Cambodia ameliorates oxidative stress, hyperglycemia, and kidney dysfunction in type 2 diabetic mice. Journal of Medicinal Food, 20(5), 502-510.

Tripathi, M., Pandey, M. B., Jha, R. N., Pandey, V. B., Tripathi, P. N., & Singh, J. P. (2001). Cyclopeptide alkaloids from Zizyphus jujuba. Fitoterapia, 72(5), 507-510.

Villarruel-López, A., López-De La Mora, D. A., Vázquez-Paulino, O. D., Puebla-Mora, A. G., Torres-Vitela, M. R., Guerrero-Quiroz, L. A., &

Nuño, K. (2018). Effect of Moringa oleifera consumption on diabetic rats. BMC complementary and alternative medicine, 18(1), 1-10.

Zarga, M. A., Sabri, S., Al-Aboudi, A., Ajaz, M. S., & Sultana, N. (1995). New cyclopeptide alkaloids from Zizyphus lotus. Journal of natural products, 58(4), 504-511.