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Role of *Salvia officinalis* Aqueous Leaf Extracts in Modulating Kidney Damage Caused by Deltamethrin-Induced Oxidative Stress and Inflammation

Función de los Extractos Acuosos de Hojas de *Salvia officinalis* en la Modulación del Daño Renal Causado por el Estrés Oxidativo y la Inflamación Inducidos por Deltametrina

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SUMMARY: *Salvia officinalis* extract (Sage), an adequate source of polyphenolic ingredients, have antioxidants, anti-inflammatory, and anti-apoptotic characteristics. Mammals and other non-targeted species may have pathophysiological consequences from pyrethroid pesticide deltamethrin (DM) due to oxidative stress. Oxidative stress is a key mechanism for pesticide toxicity, with many plants possessing high levels of antioxidants that absorb and neutralize free radicals. The objectives of the current study were to investigate Sage's possible nephroprotective and antioxidant benefits against DM-induced toxicity in rats. Four groups of twenty-four albino rats were randomly assigned: control, Sage, DM, and DM plus Sage, with six animals per group. The medication was extended for one month, and then all rats' kidney tissues were examined employing the proper test kits to measure glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and tumour necrosis factor a (TNF-α) levels. This study also examined histological and ultrastructural alterations. Rats intoxicated with DM showed significantly higher levels of urea and creatinine than control rats. Furthermore, DM-treated rats showed considerable changes in kidney lipid peroxidation, antioxidant enzymes such SOD and GSH, and TNF-α. The kidney tissues' histological and ultrastructural alterations validated these biochemical disruptions. On the other hand, Sage restored the usual levels of urea and creatinine in the blood. Additionally, Sage reduced inflammatory processes, oxidative stress, and lipid peroxidation induced by DM. Additionally, it decreased ultrastructural degeneration and histopathology brought on by DM. It can be concluded that the antioxidant properties of sage may be contributing to its protective effects.

KEY WORDS: *Salvia officinalis* **aqueous leaf extracts; Deltamethrin; Oxidative stress; Kidney injury; Albino rats.**

INTRODUCTION

Pesticides are vital in agricultural regions because they can considerably boost crop productivity. Because they pollute the environment, pesticides provide serious health dangers, including acute and chronic poisoning in both humans and animals (Kaur *et al*., 2024). In addition to occupational exposure through contaminated food, humans are subjected to insecticides through consumption, inhalation and skin contact (Scorza *et al*., 2023). Since pyrethroids supplanted organophosphorus pesticides, human exposure to them has grown (Meftaul *et al*., 2023). Pyrethrins, which are hazardous natural substances present in *Chrysanthemum cineraria* folium flowers, are the source of synthetic pyrethroids (Singh *et al*., 2022). Pyrethroid pesticides are poisonous to birds and mammals and less persistent in the environment (Singh *et al*., 2022).

Pyrethroids are relatively safe insecticides that have been classified as type I or type II based on their chemical

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composition and clinical manifestations of the exposure (Aznar-Alemany & Eljarrat, 2020). One of the main classes of insecticides used in agriculture globally is A class II synthetic pyrethroid insecticide with comparatively low toxicity to mammals is deltamethrin (DM) (Lu *et al*., 2019). According to studies, DM can be easily absorbed through tainted food and water (Wu *et al*., 2021) as well as being bioavailable in urine and faeces (Liu *et al*., 2023). Despite its quick metabolism and minimal toxicity, DM has been shown in multiple studies to cause certain adverse effects in organisms that are not its intended target, including neurotoxicity (Khalil *et al*., 2022), genotoxicity, hemolysis (Arif *et al*., 2020), reproductive damage, pulmonary disorders (Gasmi *et al*., 2022), and hepatotoxicity (Kong *et al*., 2021). Additionally, it has recently been revealed that DM exposure can result in nephrotoxicity and degenerative alterations to renal tissue (Jiang *et al*., 2021a). Non-targeted species experience DM toxicity through inflammation, apoptosis, lipid peroxidation, free radical production, and disruption of the body's antioxidant capacity (Isıldar *et al*., 2020).

The key mechanisms underlying DM-induced oxidative damage and, subsequently, damage to tissue is caused by the overproduction of reactive oxygen species (ROS) and the depletion of the body's natural antioxidant system (Jiang *et al*., 2021b ; Yang *et al*., 2022). The kidney is responsible for the majority of DM excretion, while intestine esterases, plasma carboxylesterases, and the hepatic microsomal enzyme system facilitate its metabolism (Deng *et al*., 2021). Due to the onset of oxidative damage, the hepatic and renal tissues may become overloaded with these metabolites (Lu *et al*., 2019). By triggering mitochondria-mediated apoptosis, DM can induce cellular death (Allam *et al*., 2022).

Antioxidants may be useful tools for scavenging ROS and maintaining the activity of antioxidant enzymes in addition to traditional DM poisoning therapies (Meitha *et al*., 2020).

The initial line of defence against oxidative damage is provided by the antioxidant enzymes SOD and GSH. The main defence mechanism in the antioxidant system against oxidative stress is SOD, which catalyses converting two superoxide radicals (O_2) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Saxena *et al*., 2022). GSH is a significant naturally occurring antioxidant that conjugates molecules to help with detoxification andprevent the harmful effects of free radicals. Furthermore, GSH serves as a crucial cofactor for antioxidant enzymes and is vital to the cellular antioxidant defences (Gupta *et al*., 2018).

Since the kidney is an organ of excretion and the liver is the primary site of DM metabolism, several investigations have revealed that the liver accumulates a higher concentration of metabolites.

It has been demonstrated that exposure to DM strongly affects the enzymatic antioxidant molecules, such as SOD and GSH antioxidant molecules, when ROS generation exceeds antioxidant capacity. The constant contact between humans and agricultural produce with pesticides increases the risk of health issues for individuals. Among the organs that most frequently intoxicate are the kidneys (Oliveira *et al*., 2018). Research indicates that the liver, as the primary site of DM metabolism, accumulates a higher concentration of metabolites compared to the kidney, which is an excretion organ.

One of the first cytokines to emerge during an inflammatory response is tumour necrosis factor a (TNF-α) (Kong *et al*., 2021). It promotes the synthesis of interleukin-1ß when oxidative stress is sustained and was elevated in the kidneys handled with DM (Kong *et al*., 2021).

Consequently, it appears that taking antioxidant supplements is necessary to lessen the negative consequences. A rapidly growing variety of naturally occurring polyphenols—secondary metabolites of plants—with anti-inflammatory, anti-apoptotic, and antioxidant activities have been identified in recent decades Sage leaf extract is one of the primary sources of these compounds.

Numerous studies have looked at the therapeutic potential to treat or prevent various diseases, especially the ingestion of phytonutrients derived from natural plants. Because of their many pharmacological characteristics as well as low toxicity, research on the hepato- and nephroprotective benefits of herbal nutraceuticals is currently attracting more interest (Silva *et al*., 2023).

As a medicinal plant, Sage has been widely utilised to cure a wide range of illnesses. The presence of flavonoids, phenolic substances including caffeic, rosmarinic, carnosic, and salvianolic acids, as well as other compounds based on phenolic structures, gives Sage leaves some medicinal properties (Vosoughi *et al*., 2018; Khiya *et al*., 2021). Numerous experimental investigations have shown antioxidant properties of Sage extract and some of its constituents.

Among its many biological benefits, Sage has antidiabetic (Alharbi *et al*., 2022), antibacterial (Mendes *et al*., 2020) antiviral and antiulcer (Jalalipour *et al*., 2022) activities. Furthermore, we have shown that the floral extract possesses hepatoprotective and nephroprotective properties specifically against alcohol intoxication (Jedidi *et al*., 2023).

Consequently, in this study, we looked at the preventive benefits of Sage ingestion against DM-induced nephrotoxicity.

MATERIAL AND METHOD

Material. DM (Butox®, 50 mg/mL) was purchased as a commercial product in clinical formulation for veterinary usage from Intervet Company (Paris, France).

Plant preparation. Salvia officials was extracted aqueously using ultrasonication (Moldavanova *et al*., 2021). A glass beaker containing 50 g of powdered plant was filled with 1000 mL of distilled water, well mixed, and then sonicated for 2.5 h in an ultrasonic machine bath (Decon FS 200 Frequency Sweep) maintaining a constant temperature (25 °C). Simple filtering used to separate the extract, and any leftover material was cleaned with 20 mL of pure water. The soluble extracts were then concentrated ten times using a rotating vacuum evaporator at 45 to 50 °C and stored in a refrigerator for future research. The final drying was done for 24 h at 50 °C in an oven; the entire *S. officinalis* plant was sun-dried for 10 days. Both a manual and an electric grinder were used to powder the dried herbs. Before being filtered, 15 g of powdered *S. officinalis* were soaked in 350 mL within a beaker of distilled water and agitated on a lab bench for a full day. At 40-50 °C, the filtrate has evaporated using a hot-air oven. To get the concentration needed for the studies, distilled water was used to prepare the residue in the proper weights.

Animals and treatments. This study was carried out in compliance with the relevant national laws on the use of animals in research. In this investigation, twenty-four adult male albino rats in good physical condition were employed. The animals' initial average body weight was 150 + 2g (+SE). Laboratory Animal Breeding Colony at King Khalid University's Faculty of Medicine is where the animals were acquired. For a minimum of one week before the study, the animals were given time to acclimatise to a controlled temperature (22-25 °C), humidity, and light/dark cycle. A standard commercial pellet diet was given to the rats. They have unlimited access to food and drink.

According to the National Institutes of Health and the Animal Care and Use Committee of King Khalid University's Faculty of Medicine in Abha, Saudi Arabia, all animals were treated humanely (NIH publication 86-23 revised 1985).

The animals were divided into four groups of six rats each, and each group was given the following treatment:

- Group I control: A single dosage of 0.5 cc of corn oil was given to these rats.
- Group II: Injected by gavage with Sage (100 mg/kg) (Allaithi & Al-Azawi, 2019).
- Group III: The animals were fed 300 mg/kg of DM.
- Group IV: Obtained a diet of DM (300 mg/kg) and Sage (100 mg/kg).

For 30 days, rats received oral treatments of DM and Sage in successive dosages daily. The kidneys were removed for biochemical, histological, and ultrastructural evaluations at the conclusion of the experiment.

Biochemical Analysis

Preparation for kidneys' Homogenate. Kidney specimens were homogenised in 5 mmol/L of Tris-HCl buffer and 2 mmol/L of ethylene diamine tetra-acetic acid (EDTA), pH 7.4, to produce 10 % (w/v) kidney homogenates. After separating the supernatants from the homogenates by centrifuging for approximately 10 minutes at 4 °C and 1000 rpm, the oxidant-antioxidant status was promptly examined.

Renal Function Tests. Utilising colorimetric kits that were acquired from Abcam, USA, in accordance with the manufacturer's instructions, kidney function tests such as creatinine and urea were measured.

Determination of Malondialdehyde (MDA) and Oxidative Stress Markers. After being properly cleaned of blood, the obtained kidney samples were promptly frozen and kept in a freezer at -80 °C for tissue malondialdehyde (MDA) (Cat. No. MD 2529) testing. MDA is a byproduct of lipid peroxidation.

SOD activity (Cat. No. SD 2521) and glutathione (GSH) levels (Cat. No. GR 2511). Using spectrophotometry, the absorbance of the supernatant was determined.

Kidney tissue malondialdehyde (MDA) level. The double heating method was used to measure the level of MDA in renal tissue in order to evaluate the lipid peroxidation (Draper & Hadley, 1990). The procedure involved mixing homogenates with a solution of butylated hydroxytoluene (BHT)-trichloroacetic acid (TCA), which contained 1 % BHT dissolved in 20 % (w/v) TCA. At 4°C, for five minutes, the product was centrifuged at 1000 g. After mixing the supernatant with a solution that contained 120 mM TBA buffer buffered in 26 mM Tris and 0.5 N HCl, it was heated to 80 °C for 10 min. MDA levels were determined using the MDA-TBA complex's molar extinction coefficient, which is 1.56×105 M/cm.

Assessment of superoxide dismutase (SOD) activity. Using the epinephrine/adenochrome combination, the spectrophotometric approach employed for assessment the renal SOD activity (Misra & Fridovich, 1972). The autoxidation of adrenaline to adenochrome is induced by the superoxide anion (O2•-) at basic pH. SOD competes in this mechanism by causing O2•- to produce hydrogen peroxide (H2O2) molecules. At 480 nm, variations in absorbance were measured.

Glutathione (GSH) estimation. Rat kidney tissues' glutathione content was calculated using 5,5-dithiobis-2-nitrobenzoic acid (DTNB) as a substrate (Ellman, 1959). The yellow colour development was measured at 412 nm right away and molar extinction coefficient of DTNB (13.6 $\times 10^{-6}$ nM-1 cm^{-1}) was used to represent the results as mmol/g wet tissue.

Kidney TNFα assays. Following the manufacturer's instructions, pre-made kits from R&D Systems (Minneapolis, MN) used to quantify renal TNF-α levels. The assay diluent was essentially added to each microplate well in an amount of 50 µL. The plate was covered and left to stand at room temperature for two hours after 50 µL of the diluted renal tissue homogenate supernatant samples were added to each well. After a minute of tapping the plate frame, the samples were carefully combined. After incubation, the kit's washing buffer was used to aspirate and rinse each well on the plate five times. The plate was then incubated for 30 minutes at room temperature in the dark after 100 µL of substrate solutions had been added. The optical density at 450 nm was then measured using a 96-well microtiter plate ELISA reader. The TNF-α level was determined using a standard curve and multiplied by the dilution factor.

Histopathological examinations. Each rat kidney was quickly sliced into little pieces and fixed in a 10 % formalin-saline for a full day. Following that, the samples were washed and dehydrated in 70 %, 90 %, and 100 % ethanol concentrations; samples had to spend two hours cleared of xylene. Three hours were spent embedding and impregnating soft paraffin wax at 45-50 °C, whereas one hour was spent at 60 °C embedding firm paraffin wax. Haematoxylin and eosin (H&E) staining was applied to 5 µm sections of paraffin blocks (Bancroft & Gamble, 2008). Histopathological investigation carried out with an Olympus BX50 light microscope (Tokyo, Japan). The samples were analysed at the pathology department of King Khalid University College of Medicine.

Transmission electron microscopy. Fresh kidney sections were fixed into one mm^3 pieces using 0.1 M phosphate buffer (pH 7.4) and 2.5 % glutaraldehyde for the TEM investigation. 1% osmium tetroxide was then used to post-fix these sections. Following that, the specimens were put in an oven set to 60 °C and embedded in spur's resin. Ultrathin sections on copper grids were counterstained with uranyl acetate and lead citrate (Hayat, 2012). The pathology department of King Khalid University College of Medicine employed TEM (JEOL, 1011 Tokyo, Japan) for analysis.

Statistical analysis. Version 29 of the SPSS software (IBM SPSS Statistics for Windows, Armonk, New York, USA: IBM Corp.) used to analyse the data. The data's normality was evaluated using the Shapiro test. The mean ± SD (standard deviation) is employed to symbolise continuous variables. The one-way ANOVA test was used to compare means, and then Tukey's *post hoc* test was used. The findings were deemed significant when the likelihood of error was less than 5% ($p \leq 0.05$) and nonsignificant when the probability of error was greater than 5 % ($p > 0.05$) (Tello & Crewson, 2003).

RESULTS

The Effect of DM and/or Sage on Renal Functional Tests. Serum urea (Fig. 1A) and creatinine (Fig. 1B) increase significantly in response to DM treatment in contrast to the

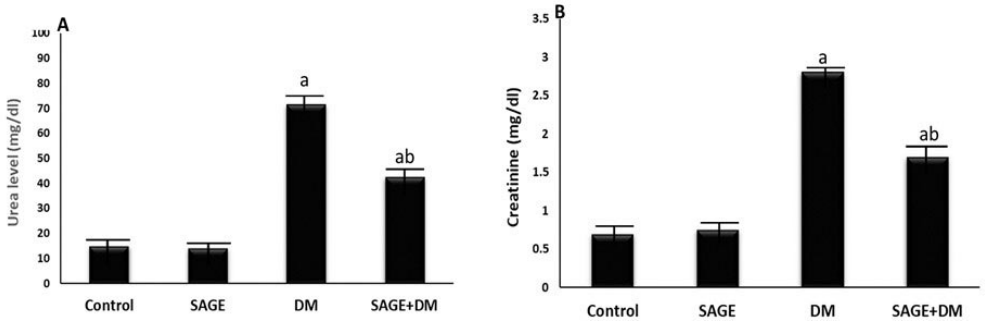

Fig. 1. The effects of Sage administration on renal functional parameters including urea (A) and creatinine (B) in DM nephrotoxicity. Data are represented as Mean ± SD (n=6). One-way ANOVA followed by a Tukey's post hoc test was used for comparison between different groups. aSignificant change in comparison with the control group at $P < 0.05$; ab Significant change in comparison with the DM group at $P < 0.05$

control group. Sage-treated group's serum, however, these tests did not exhibit any appreciable alterations in these parameters. However, there was a noticeable improvement in the kidney function tests when DM-intoxicated rats were given Sage at the same time.

Sage and/or DM effects on the Oxidative Cascade in Kidney Tissue. Oxidative Cascade in Kidney Tissue: Figure 3 shows that neither the control nor the Sage groups showed any changes in the oxidative stress indices. In contrast to controls, the kidney tissue homogenate rats treated with DM showed that MDA had significantly increased (Fig. 2) and a significant decrease in SOD activity (Fig. 3A) and GSH content (Fig. 3B). Interestingly, when compared to the DM group, Sage treatment in addition to DM may significantly reduce the DM-induced changes in the oxidative stress markers.

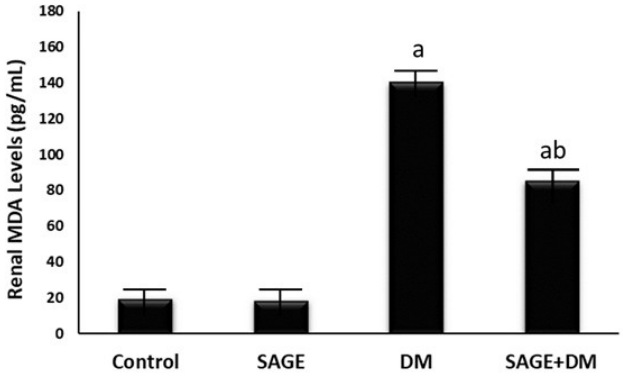

Fig. 2. The effects of Sage administration on MDA activity in DM nephrotoxicity. Data are represented as Mean ± SD (n=6). One-way ANOVA followed by a Tukey's post hoc test was used for comparison between different groups. a Significant change in comparison with the control group at $P < 0.05$; ab Significant change in comparison with the DM group at $P < 0.05$.

Effect of DM and/or Sage on the release of TNF-α. The DM-treated group's TNF-a levels were noticeably greater than the control groups. Treatment with Sage considerably reduced TNF-α levels in comparison to the DM group (Fig. 4).

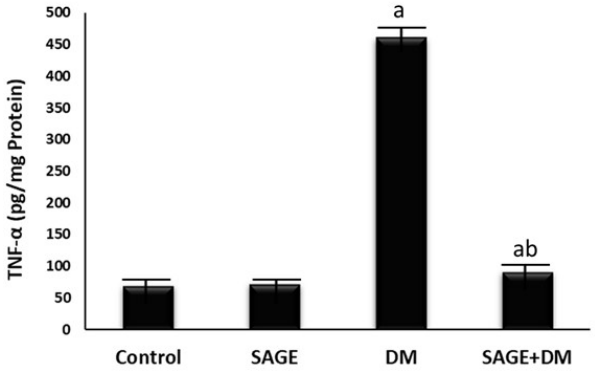

Fig. 4. Levels of pro-inflammatory biomarker TNF-a in the studded groups. The DM group's TNF-α activity significantly reduced after taking Sage. Data are represented as Mean ± SD (n=6). One-way ANOVA followed by Tukey's post hoc test was used for comparison between different groups. a Significant change in comparison with the control group at $P < 0.05$; ab Significant change in comparison with the DM group at $P < 0.05$; TNF-α: Tumour necrosis factor-α

Histological results

Haematoxylin and eosin-stained photomicrographs of rat kidney sections from the control and experimental groups were shown in Figures 5A, 5B, 5C and 5D.

Rats with normal renal cortex architecture, including a renal corpuscle enclosed by proximal and distal convoluted tubules, were shown in sections of kidney tissue from the control and Sage groups that did not demonstrate any

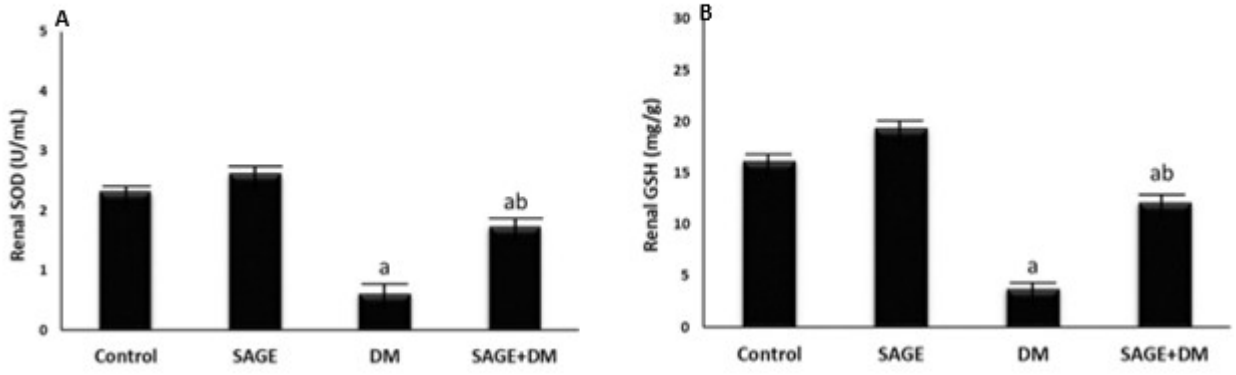

Fig. 3. The effects of Sage administration on oxidative markers including SOD (A) and GSH (B) activities in DM nephrotoxicity. Data are represented as Mean ± SD (n=6). One-way ANOVA followed by a Tukey's post hoc test was used for comparison between different groups. aSignificant change in comparison with the control group at $P < 0.05$; abSignificant change in comparison with the DM group at $P < 0.05$

pathogenic alterations. The renal corpuscle, which includes the glomerulus, has been enclosed by glomerular capsule (Bowman's capsule), which was made up of a parietal layer of simple squamous epithelium, a visceral layer of podocytes (Fig. 5A).

A damaged renal cortex architecture, comprising a smaller glomerulus, a broad Bowman's space, and a discontinuity in glomerular capsule, were among the pathological changes observed in the DM-exposed animals (Figs. 5B and 5C). The glomerulus formed capsular adhesions with some renal corpuscles. Additionally, there was extravasation, vacuolation, and tubular epithelium loss that sloughed into the lumen. Additionally, extravasation and sloughing into the lumen were observed, along with some inflammatory cells.

Rats treated with DM and Sage showed significantly reduced renal tissue degenerative changes with fewer adhesions than rats treated with DM alone. (Fig. 5D).

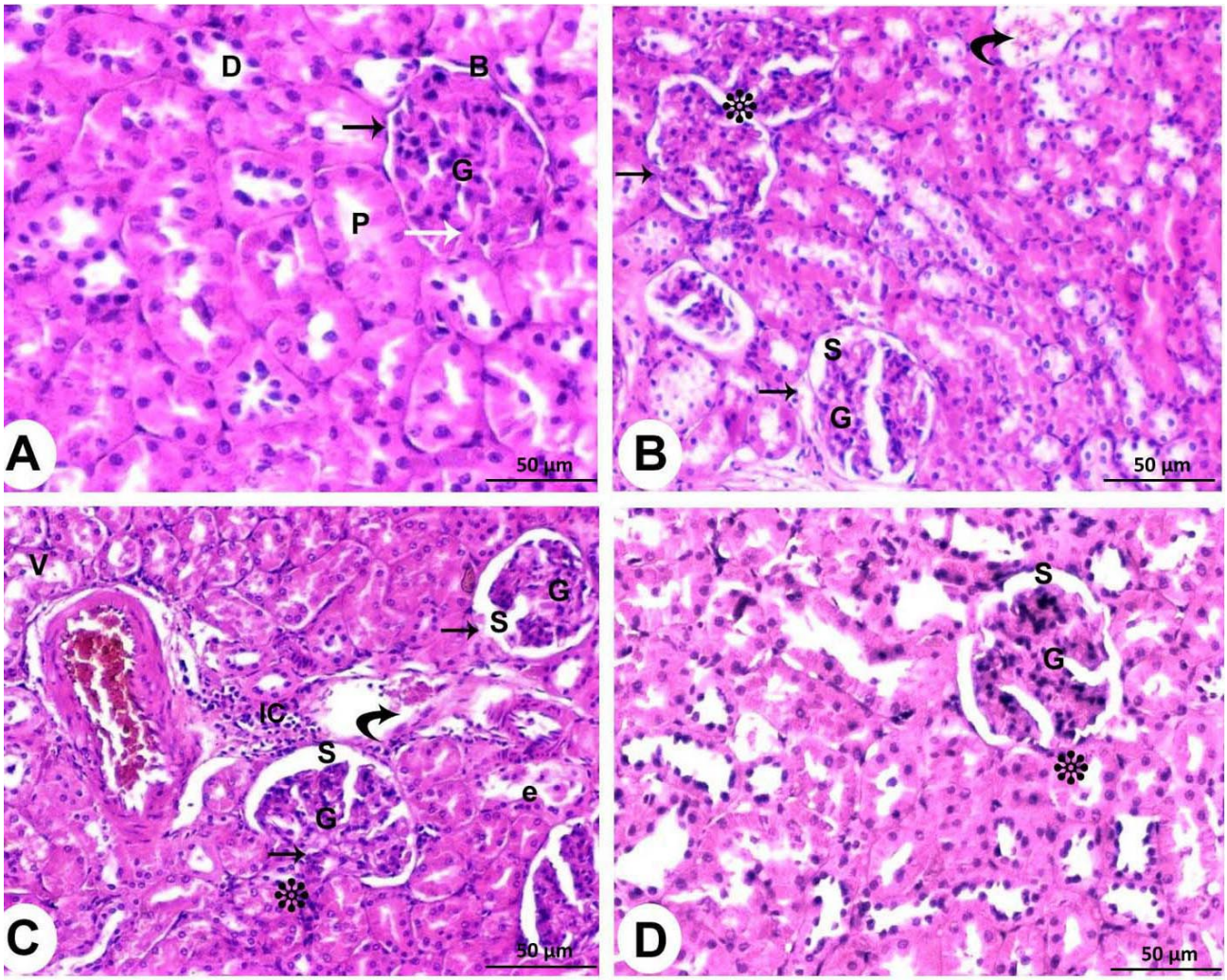

Fig. 5. Photomicrographs of all groups of rat kidneys stained with H& E: Bar = 50 µm. **A.** (Groups control & Sage): A normal renal cortex architecture includes a renal corpuscle surrounded by proximal (P) and distal (D) convoluted tubules. Bowman's capsule (B) envelopes the renal corpuscle, which consists of the glomerulus (G). Glomerular capsule has a parietal layer made of simple squamous epithelium (black arrow), a visceral layer made of podocytes (White arrow), and Bowman's space in between. **B.** (DM group): A damaged renal cortex architecture, including a shrunken glomerulus (G), a wide Bowman's space (s), and discontinuity in Bowman's capsule (black arrow). Some renal corpuscles constitute capsular adhesions with the glomerulus (asterisk). There is also tubular epithelium loss (curved arrow), sloughing into the lumen, vacuolation, and extravasation. **C.** (DM group): The renal cortex's architecture is deformed, with a narrower glomerulus (G), a wider Bowman's space (s), and a discontinuous Bowman's capsule (black arrow). There are capsular adhesions between the glomerulus and certain renal corpuscles (asterisks). Also, some inflammatory cells (IC) are seen. Moreover, there is extravasation (e), sloughing into the lumen, vacuolation (V), and loss of tubular epithelium (curved arrow). **D.** (Sage plus DM group): In comparison to the control group, the glomerulus (G) and Bowman's space (s) are essentially normal, with minimal adhesions (asterisks).

Transmission electron microscopic observations.

Rat kidney tissue ultrastructural alterations in the control and experimental groups were displayed in Figs. 6 to 10.

The control and Sage rats' renal glomeruli were shown to have a normal ultrastructure in the electron micrographs (Figs. 6A and 6B). The capillary lumina, basement membranes, endothelial cells, visceral epithelial cells of podocytes and urinary spaces were all normal in a glomerulus. The glomerular basement membrane's three layers were visible: the dense centre layer (lamina densa), the outside layer (lamina rara externa), and the innermost one (lamina rara interna). Multiple fenestrae and thin diaphragms were also observed in the glomerular capillary lumina.

In a proximal convoluted tubule, epithelial cells with a round nucleus and many mitochondria were resting on the basement membrane. The brush boundary is formed by the long microvilli on the apical surface of the epithelial cell (Fig. 6C).

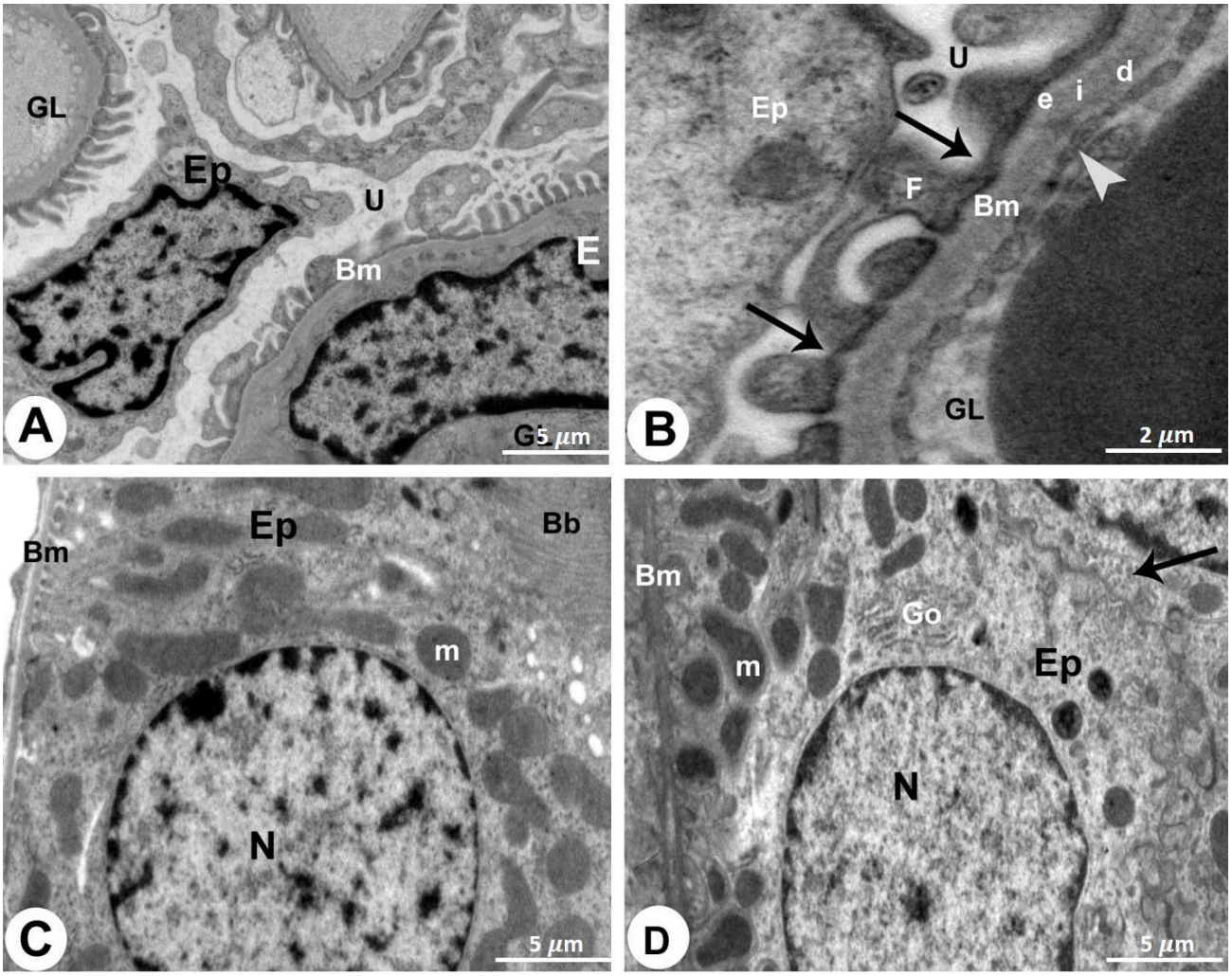

Fig. 6. Ultrastructure of control and SAGE rat kidney stained with Uranyl acetate & lead citrate: Bar = 5 µm & 2 µm. **A.** A glomerulus showing some capillary lumina (GL), basement membranes (Bm), endothelial cells (E) and visceral epithelial cells of podocytes (Ep). Urinary spaces (U) are also seen. **B.** Higher magnification of the above figure displaying the three layers of the glomerular basement membrane (Bm); an inner layer (lamina rara interna, i), an outer layer (lamina rara externa, e), and a dense central layer (lamina densa, d). Thin diaphragms (arrow) and multiple fenestrae (white arrowhead) are seen in the glomerular capillary lumina (GL). **C.** A proximal convoluted tubule showing epithelial cells (Ep) resting on the basement membrane (Bm) and containing numerous mitochondria (m) and a round nucleus (N). The apical surface of the epithelial cell containing long microvilli to form the brush margin (Bb). **D.** A distal convoluted tubule showing some epithelial cells (Ep) resting on the basement membrane (Bm), short microvilli (arrow), Golgi apparatus (Go) and mitochondria (m).

Figure 6D showed a distal convoluted tubule with mitochondria, small microvilli, the Golgi apparatus (*Complexus golgiensis*; *Apparatus golgiensis*), and some epithelial cells lying on the basement membrane.

Figures 7 to 9 of the DM rats displayed tubular degraded epithelial cells, a damaged glomerular capillary, and a disturbed Bowman's capsule.

A damaged glomerular tuft and thinning Bowman's capsule were seen in a glomerulus (Fig. 7), which also showed a few erythrocytes in the large urinary space. Additionally, disruption of glomerular capsule, glomerular capillary occlusion by proteinaceous materials, hump-like immune deposits, subendothelial immune deposits, and glomerular capillary obliteration by hypertrophied endothelium were observed in a damaged glomerular tuft. Additionally, mesangial cell proliferation and localised hypertrophy were observed in the glomeruli, together with degenerated podocytes and their endothelial cells (Fig. 8A).

The DM rats demonstrated signs of pyknosis in the proximal tubules, nuclei that shrank with chromatin clumping and chromatin margination, pale cytoplasm, slightly enlarged mitochondria, and irregularly organised microvilli. The apical cytoplasm contained several vacuoles and a greater number of lysosomes (Figs. 8B, 8C, 8D, 9A and 9B).

The DM rats' distal tubule displayed cytoplasmic organelles of districted epithelial cells that were injured inside the tubular lumen, along with a few organelles found in certain inflated epithelial cells where the microvilli were

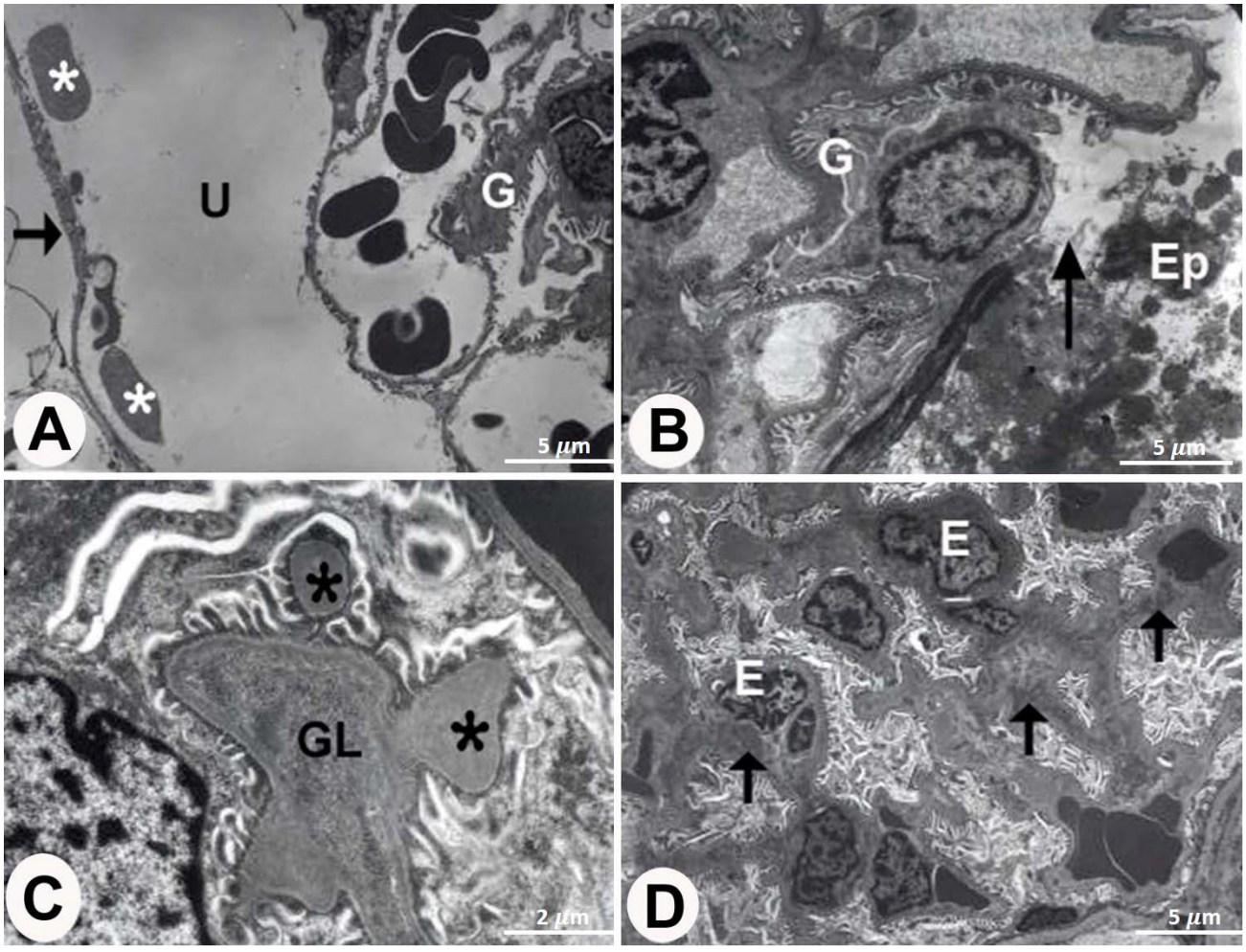

Fig. 7. Ultrastructure of the DM-treated rat kidneys stained with Uranyl acetate & lead citrate: Bar = 5 μm & 2 μm. **A.** A glomerulus demonstrates a few erythrocytes (asterisks) in the huge urinary space (U) and damaged glomerular tuft (G) and thinning Bowman's capsule (arrow). **B.** A damaged glomerular tuft (G) showing disrupted Bowman's capsule (arrow) and tubular degenerated epithelial cells (Ep). **C.** A glomerulus showing abnormal glomerular capillary (GL) occluded by proteinaceous-like materials and hump-like immune deposits (asterisks). **D.** Abnormal glomerulus displaying subendothelial immune deposits (arrows) and glomerular capillary obliteration by hypertrophied endothelium (E).

either destroyed or disappeared. Patchy tubular dropouts of the brush margin, mitochondria, and nucleus were visible in the interstitial region (Fig. 9C).

Patchy tubular dropouts of the brush margin, mitochondria, and nucleus were seen in the interstitial area (Fig. 9D).

Rats in the Sage plus DM group had slight structural alterations in the glomeruli, proximal tubules, and distal tubule tissue, according to electron microscopy analysis (Fig. 10).

DISCUSSION

Pesticides' widespread use in agriculture and public health initiatives poses biological risks to human and animal health, with their toxicity facilitated by free radicals. Oxidative damage occurs when the balance among free radical generation and the antioxidative profile is disrupted. As per this investigation, oxidative stress may be brought on by the renal damage brought on by DM because of the generation of free radicals. Kidney injury markers, such as creatinine and urea, were markedly elevated by DM intoxication. DM therapy dramatically reduced kidney enzymatic SOD and GSH antioxidant levels and dramatically exacerbated lipid peroxidation by raising kidney MDA levels. Additionally, when compared to the control group, it markedly elevated TNF-α in renal tissue.

This study stated that, rats administered an aqueous extract of Sage leaves were protected from DM-induced nephrotoxicity, however animals treated to DM alone showed

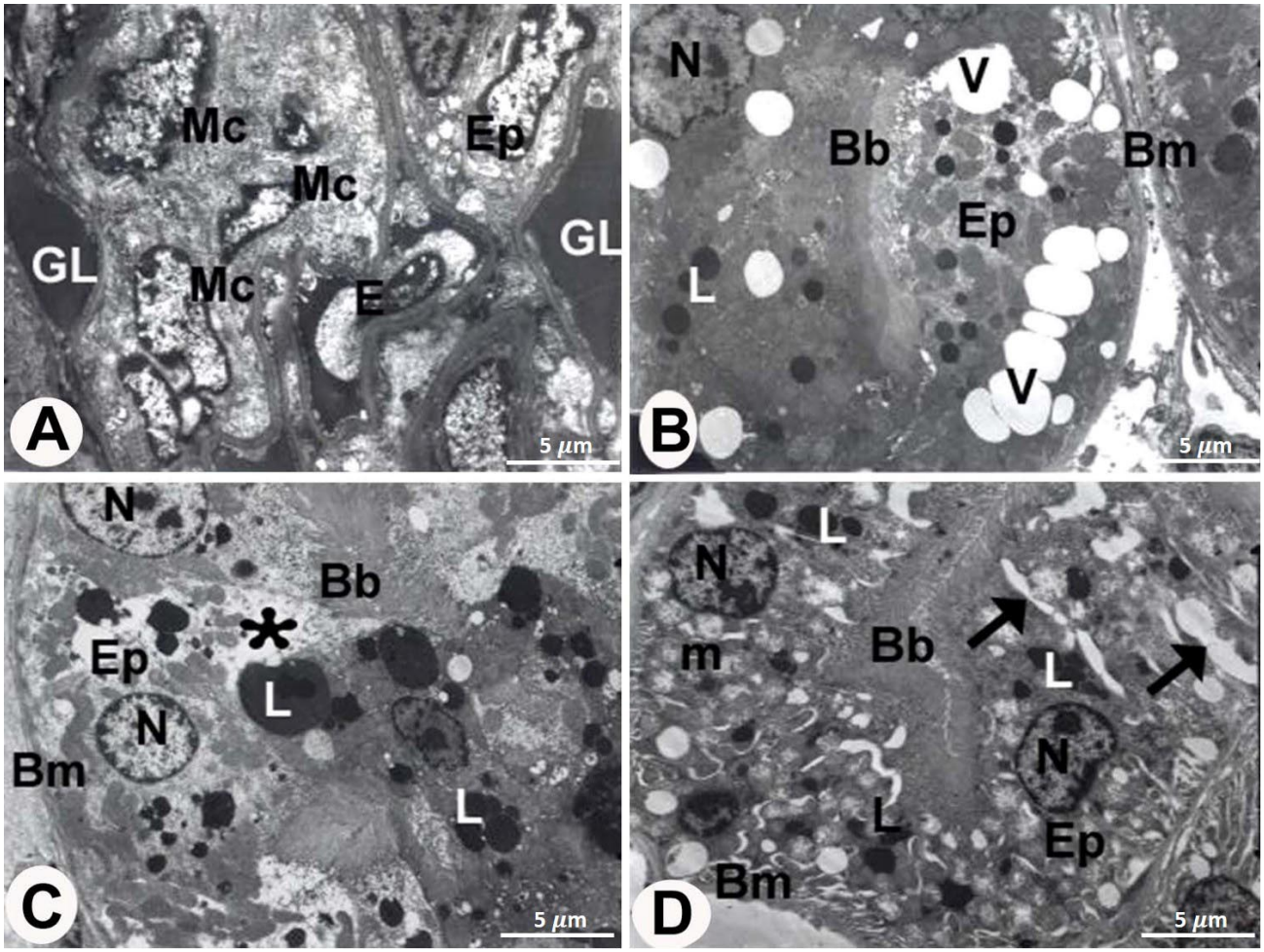

Fig. 8. Ultrastructure of the DM-treated rat kidneys stained with Uranyl acetate & lead citrate: Bar = 5μm. **A.** A glomerulus showing focal hypertrophied and proliferation of mesangial cells (Mc), degenerated podocytes (Ep) and degenerated endothelial cells (E). **B.** A proximal tubule showing vacuolar (V) degeneration of epithelial cells (Ep) and increase amounts of lysosomes (L). N, nuclei; Bm, basement membrane; Bb, brush margin. **C.** A proximal tubule displaying degenerated epithelial cells (Ep) with damaged cytoplasm (asterisk) and increase amounts of different sizes of lysosomes (L). N, nuclei; Bm, basement membrane; Bb, brush margin. **D.** A proximal tubule showing more degeneration of epithelial cells (Ep) with dilatation of infolding membranes (arrows), damaged mitochondria (m) and increase amounts of irregular-shaped lysosomes (L). N, nuclei; Bb, Brush margin.

increased serum urea and creatinine levels. After DM treatment, elevated serum levels of creatinine and urea indicated a possible deterioration in renal function. DM-induced nephrotoxicity, shown mainly as direct tubular injury and a glomerular filtration rate reduction, could also be reason of these effects (Thind *et al*., 2022a,b). Rats with DM showed a marked reduction in blood urea and creatinine levels following the administration of Sage extracts. Its ability to reduce renal impairment seems to be the reason for this research's lowering in blood urea (Dawood *et al*., 2020).

In this context, research has demonstrated that as compared to the control group, DM administration dramatically raised the kidney MDA level in rats, a measure of lipid peroxidation. Free radicals cause lipid peroxidation, a significant pathologic event that involves the degradation of polyunsaturated fatty acids (Tekeli *et al*., 2021).

In the present investigation, a significant decrease in the antioxidants (SOD and GSH) in rats given DM provides compelling evidence that oxidative damage to the kidneys contributes to nephrotoxicity. SOD and GSH are two crucial antioxidant systems that shield cells from free radical damage. It is commonly recognised that oxidative damage caused by free radicals is prevented and neutralised by endogenous non-enzymatic antioxidants and antioxidant enzymes (Lu *et al*., 2019).

However, inflammation is one of the primary mechanisms via which DM poisoning occurs in non-targeted organisms. Recent research has shown that oral

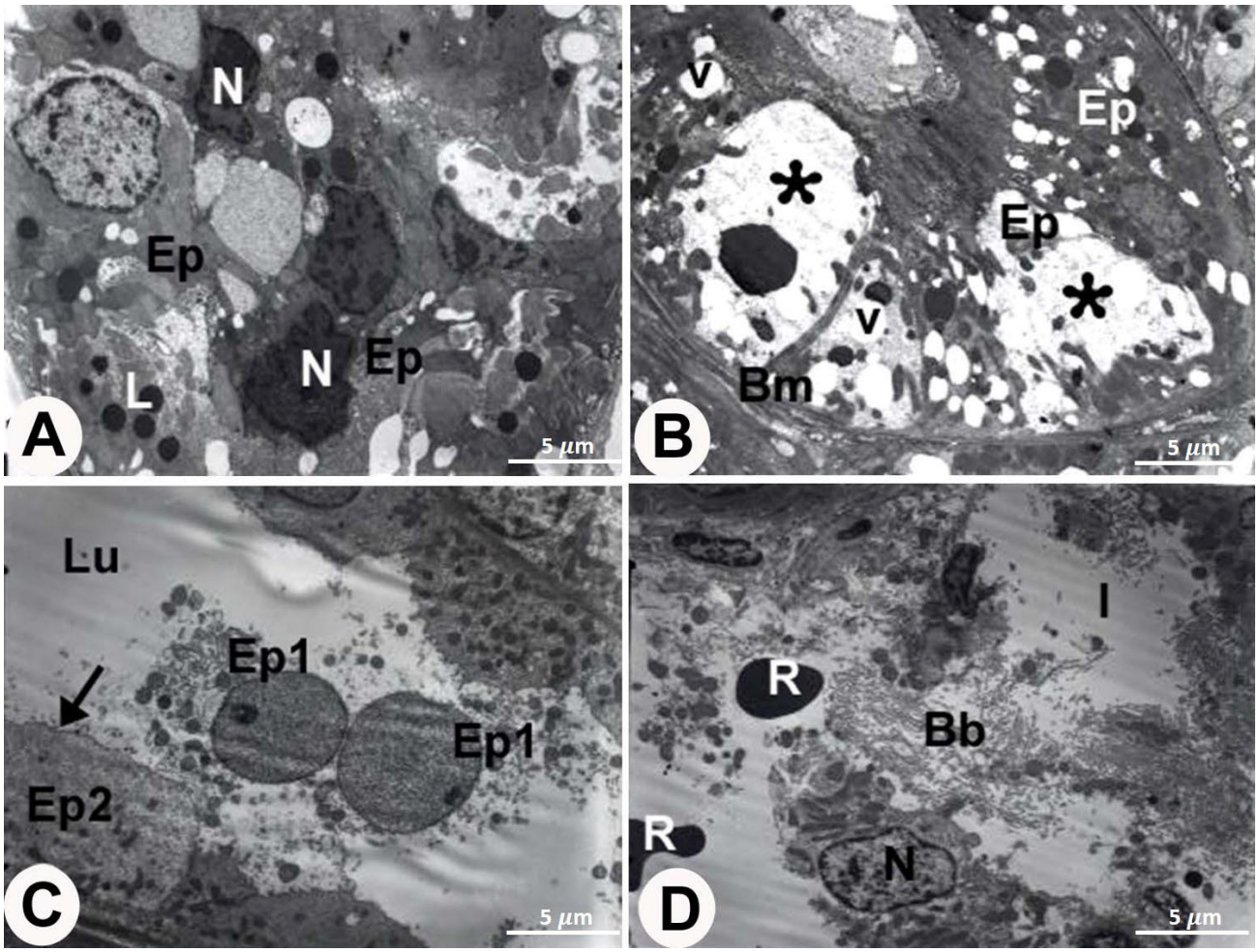

Fig. 9. Ultrastructure of the DM-treated rat kidneys stained with Uranyl acetate & lead citrate: Bar = 5 μm. **A.** A proximal tubule showing irregularity in the arrangement of the degenerated epithelial cells (Ep) with dense irregular-shaped pyknotic nuclei (N). Increase amounts of lysosomes (L) were also seen. **B.** A proximal tubule demonstrating more destruction (asterisks) of basal part of the vacuolar (V) degenerated epithelial cells (Ep). Bm, basement membrane. **C.** A distal tubule showing damaged cytoplasmic organelles of districted epithelial cells (Ep1) inside the tubular lumen (Lu), and a few organelles present in some swollen epithelial cells (Ep2) with damaging or disappearance of the microvilli (arrow). **D.** Interstitial area (I) showing patchy tubular dropout such as brush margin (Bb), mitochondria (m) and nucleus (N). R, erythrocytes.

administration of DM causes degenerative changes in kidney tissue and dramatically increases the tumour necrosis factor (TNF-α).

Significant alterations, such as glomerular congestion, tubular degeneration, necrosis, inflammatory cell infiltration, renal haemorrhage, and vacuolization, were found in the rat kidneys treated with DM. Furthermore, a significant infiltration of interstitial mononuclear cells had also been noted (Azzam *et al*., 2024). In order to identify morphologic alterations following DM therapy, the study performed histologic investigations on kidneys from rats who survived in order to identify tubular necrosis (Deng *et al*., 2021). In comparison to the control group, the rats given DM showed thicker basement membranes in the glomerular capillaries and renal tubules (Yumnam *et al*., 2017).

This present investigation analysed kidneys of rats treated with MD at the ultrastructural level, revealing increased lysosomes, vacuolated cytoplasm, mitochondrial vacuolation, tubular casts, damaged mitochondria, irregular nucleus, myeloid bodies, and nuclear condensation. These results were consistent with previous MD research involving rats (Nady Ouais *et al*., 2024) who documented thicker glomerular basement membrane, deformed foot processes, and cytoplasmic vacuolation.

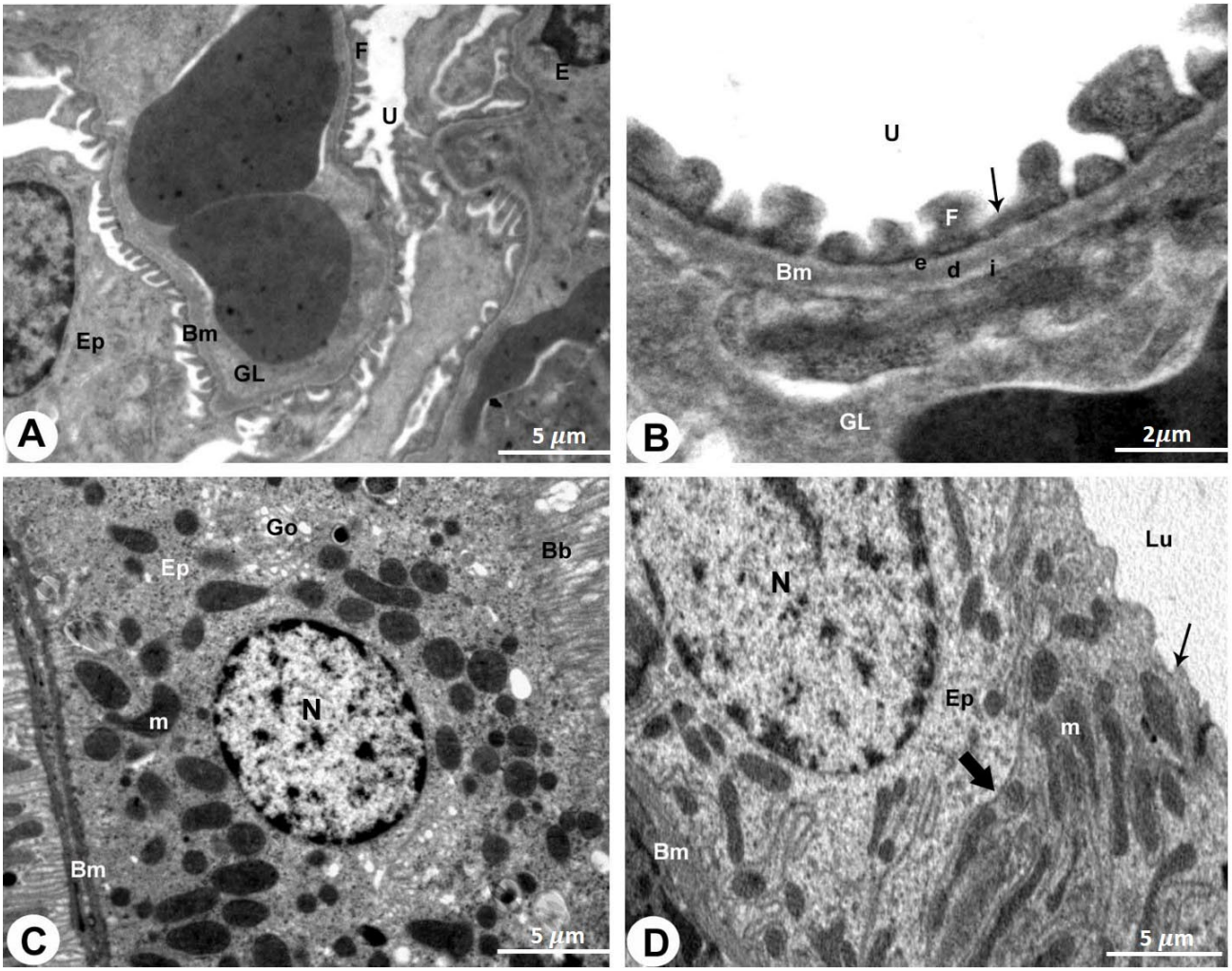

Fig. 10. Ultrastructure of SAGE+DM rat kidney stained with Uranyl acetate & lead citrate: Bar = 5 μm & 2 μm. **A.** A glomerulus showing improvement of capillary lumina (GL), basement membranes (Bm), endothelial cells (E) and visceral epithelial cells of podocytes (Ep). Urinary spaces (U) are also seen. **B.** Higher magnification showing the three layers of the glomerular basement membrane (Bm); an inner layer (lamina rara interna, i), an outer layer (lamina rara externa, e), and a dense central layer (lamina densa, d). Glomerular capillary lumina (GL) and urinary space (U) are also seen. **C.** A proximal convoluted tubule showing intact epithelial cells (Ep) resting on the basement membrane (Bm) and containing numerous mitochondria (m) and a round nucleus (N). The apical surface of the epithelial cell containing long microvilli to form the brush margin (Bb). **D.** A distal convoluted tubule showing improvement of epithelial cells (Ep) resting on the basement membrane (Bm), short microvilli (arrow), Golgi apparatus (Go) and mitochondria (m).

The current study's key findings demonstrated that Sage supplementation reduces inflammation, oxidative stress, lipid peroxidation, renal function tests, histopathology, and ultrastructural alterations. Sage, on the other hand, increased antioxidant enzyme activities that DM had decreased, thus minimising kidney damage triggered by DM. Endogenous antioxidants guard against the oxidative damage that free radicals convey to cells. Research has demonstrated that after DM treatment, kidney tissues' total antioxidant capacity, SOD, and GSH activities were significantly lower than in control groups (Mansour *et al*., 2018).

In our work, we demonstrated that Sage helps reduce ROS-generated inflammation by lowering TNF-a, which in turn helps prevent kidney damage from DM. The activation of several transcription factors by reactive oxygen species (ROS) results in the differential expression of certain genes involved in inflammatory pathways (Blaser *et al*., 2016).

The current study's findings demonstrated that plant extracts restore the progression of kidney damage in rats with DM to levels that are almost normal. This suggests that the flavonoid-based plant extracts have a protective impact and can repair organ tissue. The Sage aqueous extract significantly improved kidney function by stabilizing histological and histochemical alterations, including decreased glomerular and tubular basement membrane thickness, indicating improved kidney function (Ranasinghe *et al*., 2023). The biochemical and histological changes in the kidney caused by DM were significantly avoided by concurrent Sage supplementation (Aioub *et al*., 2024). Sage treatment in rats caused minor fibroplasia and renal blood vessel congestion, while some showed improvements in histopathological architecture of glomeruli, Bowman's corpuscle, and renal tubules (Abdel-Gawad *et al*., 2021).

In this work, Sage was found to have a protective effect against DM-induced changes to the kidney tissues of rats in terms of histology and ultrastructure. The improvement was observed in the less noticeable cytoplasmic vacuolation and distortion of glomerular and tubular cells, with no significant morphological damage, decreased inflammatory cell infiltration, and preserved microarchitecture. These results were consistent with the investigation that contrasted with other investigations that used other antioxidants (El-Gerbed, 2014).

CONCLUSION

The results of this study show that Sage may offer some protection against kidney impairment brought on by DM. Therefore, these extracts' beneficial benefits might be mediated via enhancing the antioxidant defence system and reducing oxidative stress. It is crucial to decrease the toxicity from pesticide exposure and minimise nephrotoxicity effects while evaluating the potential use of Sage, which is high in this antioxidant, for men in agricultural practices. This positions it as a promising candidate for further research into protective agents against pesticide-induced nephrotoxicity.

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RESUMEN: El extracto de *Salvia officinalis* (salvia), una fuente adecuada de ingredientes polifenólicos, posee propiedades antioxidantes, antiinflamatorias y antiapoptóticas. Los mamíferos y otras especies pueden sufrir consecuencias fisiopatológicas derivadas del plaguicida piretroide deltametrina (DM) debido al estrés oxidativo. El estrés oxidativo es un mecanismo clave de la toxicidad de los plaguicidas, y muchas plantas poseen altos niveles de antioxidantes que absorben y neutralizan los radicales libres. Los objetivos del presente estudio fueron investigar los posibles beneficios nefroprotectores y antioxidantes de la salvia contra la toxicidad inducida por DM en ratas. Se asignaron aleatoriamente cuatro grupos de veinticuatro ratas albinas: control, salvia, DM y DM más salvia, con seis animales por grupo. El tratamiento farmacológico se prolongó durante un mes, tras lo cual se examinaron los tejidos renales de todas las ratas utilizando los kits de prueba adecuados para medir los niveles de glutatión (GSH), malondialdehído (MDA), superóxido dismutasa (SOD) y factor de necrosis tumoral a (TNF-a). Este estudio también examinó las alteraciones histológicas y ultraestructurales. Las ratas intoxicadas con DM mostraron niveles significativamente más altos de urea y creatinina que las ratas control. Además, las ratas tratadas con DM presentaron cambios considerables en la peroxidación lipídica renal, en enzimas antioxidantes como la SOD y el GSH, y en el TNF-α. Las alteraciones histológicas y ultraestructurales de los tejidos renales confirmaron estas alteraciones bioquímicas. Por otro lado, la salvia restableció los niveles normales de urea y creatinina en la sangre. Adicionalmente, la salvia redujo los procesos inflamatorios, el estrés oxidativo y la peroxidación lipídica inducidos por la DM. Asimismo, disminuyó la degeneración ultraestructural y la histopatología provocadas por la DM. Se puede concluir que las propiedades antioxidantes de la salvia podrían contribuir a sus efectos protectores.

PALABRAS CLAVE: Extractos acuosos de hojas de *Salvia officinalis*; Deltametrina; Estrés oxidativo; Lesión renal; Ratas albinas.

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