Evaluation of the Possible Protective Effect of Citronellol Against Folic Acid-Induced Acute Kidney Injury in Mice

Presented by

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Introduction

Kidneys

- **The kidneys are complex organs, and they are vital in maintaining normal body functions.**
- **The most important renal functions:**

1. Regulation of water, inorganic ion balance, and acid-base balance
2. Removal of metabolic waste products from the blood and their excretion in the urine
3. Removal of foreign chemicals from the blood and their excretion in the Urine
4. Gluconeogenesis
5. Production of hormones/enzymes:
   - A. Erythropoietin, which controls erythrocyte production
   - B. Renin, an enzyme that controls the formation of angiotensin, which influences blood pressure and sodium balance.
   - C. Conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, which influences calcium balance

Acute kidney injury

Acute kidney injury (AKI) is defined by a sudden decline in kidney function, evaluated through elevated serum creatinine levels, which serve as a marker of kidney excretory capability, as well as increased blood urea nitrogen levels and decreased urine output, known as oliguria, indicating reduced urine production .

- Ranges from mild cases to severe AKI causing permanent kidney damage (Chronic Kidney Disease).
- Primarily affects elderly patients in hospitals.
- Associated with sepsis, certain medications, and invasive procedures.
- Management of AKI include volume control, nephrotoxic drug management, fluid/electrolyte balance.

Etiology

- **Pre-renal** (caused by decreased renal blood perfusion, typically as a result of volume depletion)
- **Intrinsic renal (intrarenal)** according to the damaged kidney structures, intrinsic renal causes are categorized acute tubular necrosis (ATN) and acute glomerulonephritis.
- **Post-renal** (related to inadequate urine drainage because of obstruction in the urinary tract due to kidney stones, prostate enlargement)

Risk factors

Volume depletion, hypotension, anemia, hypoxia, and usage of nephrotoxic medications

Chronic kidney, heart, liver, or gastrointestinal diseases, diabetes, severe infections, and sepsis

Genetic predispositions to myoglobinuria, hemoglobinuria, and urolithiasis may be implicated

Folic Acid

- FA or vitamin B9 in low doses is an essential component of one-carbon metabolism that is crucial for the development of cells, and they are required for the synthesis of DNA and RNA and, consequently, for the proliferation of cells

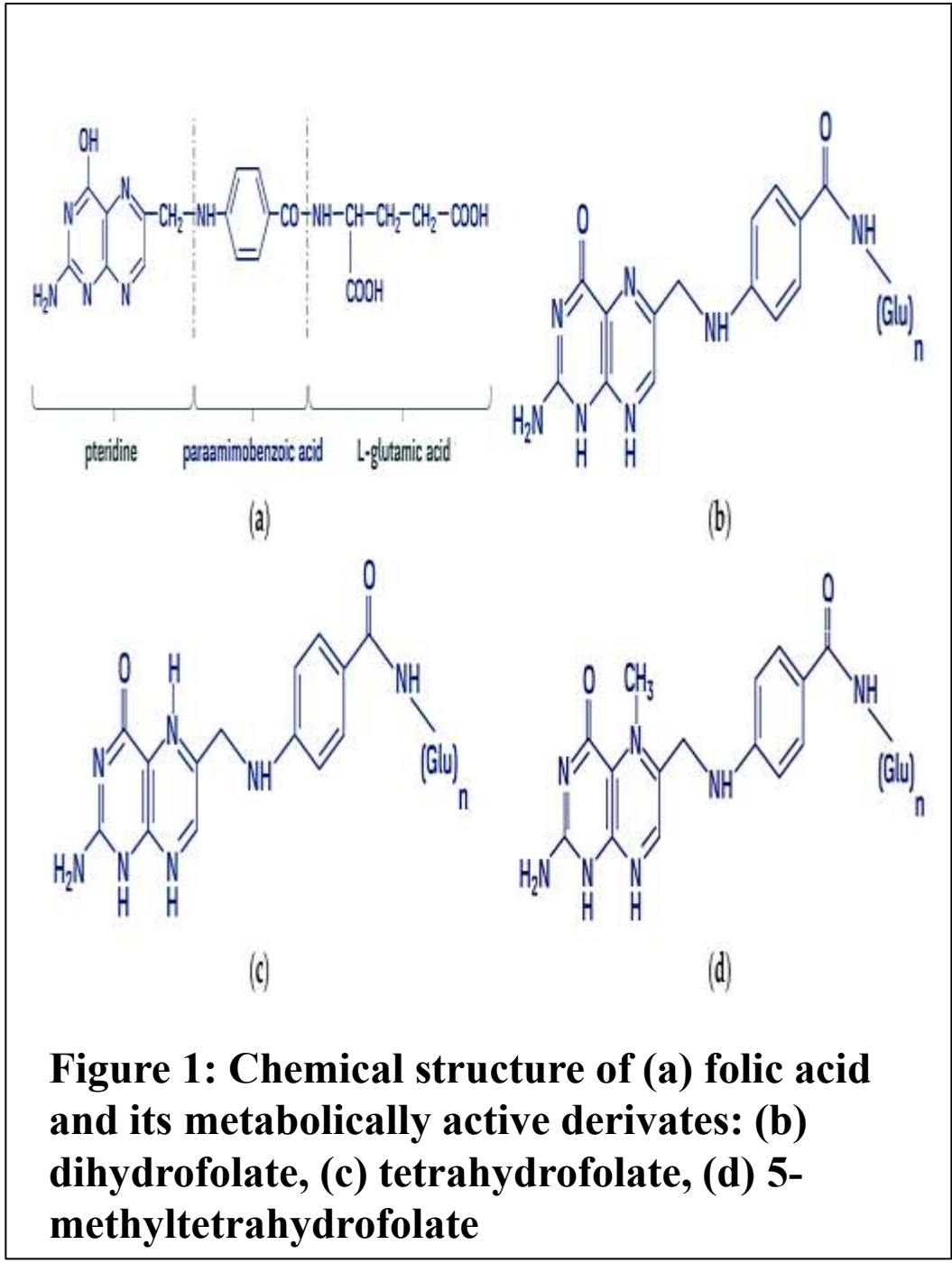
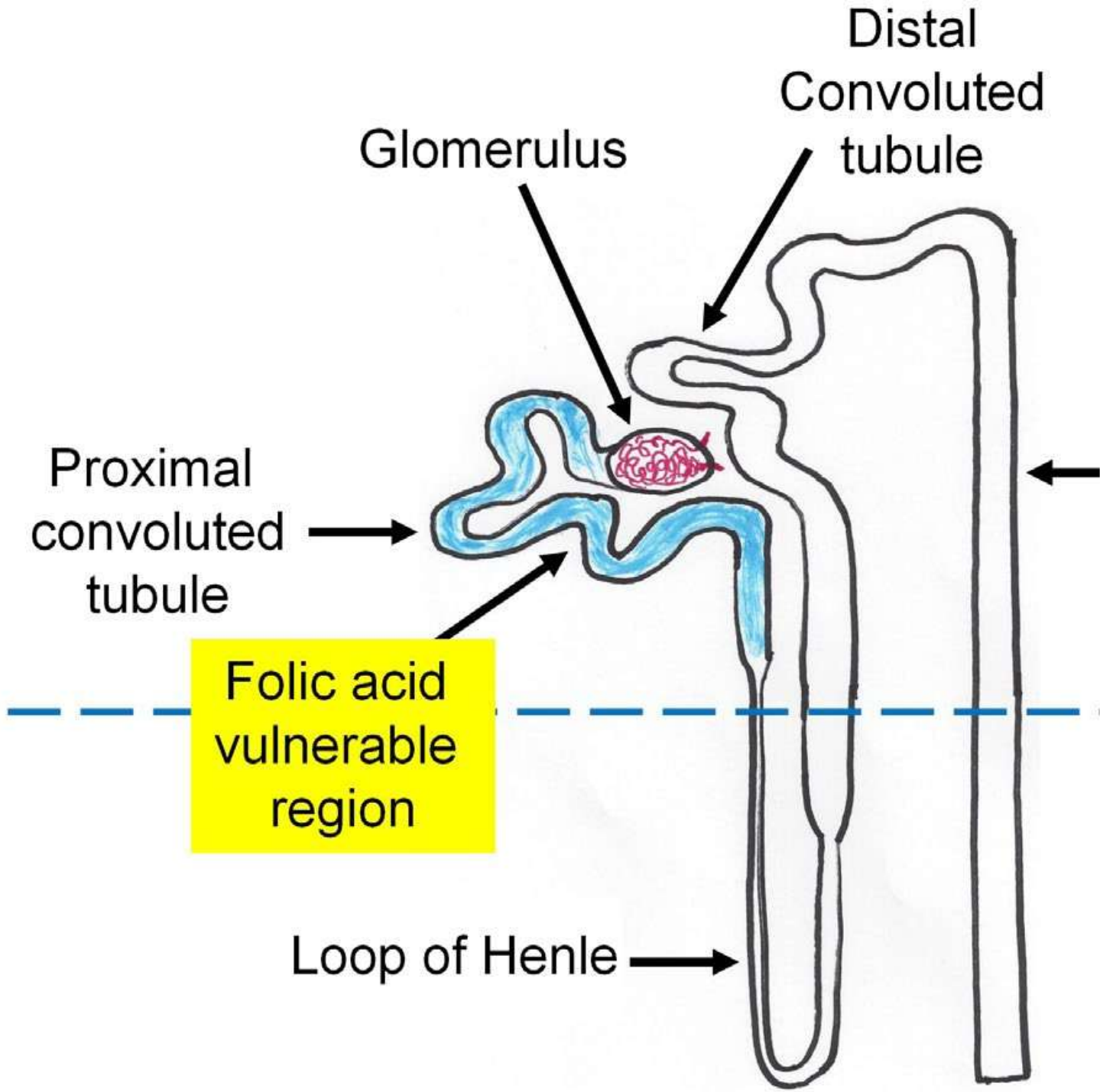


Figure 1: Chemical structure of (a) folic acid and its metabolically active derivates: (b) dihydrofolate, (c) tetrahydrofolate, (d) 5-methyltetrahydrofolate

Folic acid-induced AKI

- While a low dose of FA is nutritionally beneficial, a high dose of FA is very toxic to the kidneys
- This animal model is simple to create and is reproducible
- FA is a vitamin and is not environmentally toxic
- Administered as a simple injection, which does not require surgery and is noninvasive and animal friendly.
- FA model mainly injures the kidney and has no deleterious effects on other organs
- Mimics key features of human AKI
- As a small molecular weight compound, FA or folate is freely filtered by the glomerulus
- Renal reabsorption of folate is achieved by a high-affinity folate receptor (folate receptor 1) that is abundant on the luminal side of proximal tubular epithelial cells

- **Proximal convoluted tubule in the nephron is the most vulnerable region to folic acid (FA)-induced damage due to the presence of folate receptors.**

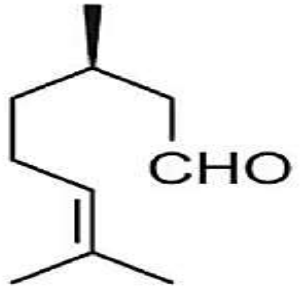


Mechanism of FA-induced AKI

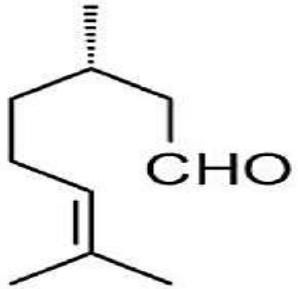
- **A single injection of FA at a dosage of 250 mg/kg body weight intraperitoneally can cause Renal folate toxicity studied after 48 hours.**

1. **Crystal formation:** Folic acid precipitates in the renal tubules forming crystals that obstruct urine flow and cause direct mechanical damage to tubular epithelial cells.
2. **Inflammation:** Crystal deposition triggers an inflammatory response activating the NF-κB signaling pathway can produce pro-inflammatory cytokines such as IL-1β and IL-6, which contributes to the development of AKI (Crystal-induced renal inflammation).
3. **Apoptosis** is a typical hallmark of AKI caused by FA, oxidative stress due to ROS causes BAX activation and the release of cytochrome-c from the mitochondria; inducing caspase-9 activation initiates the process (intrinsic pathway). Subsequently, caspase-3 is cleaved and activated, leading to the initiation of apoptosis.

Citronellol



(*R*)-**1**

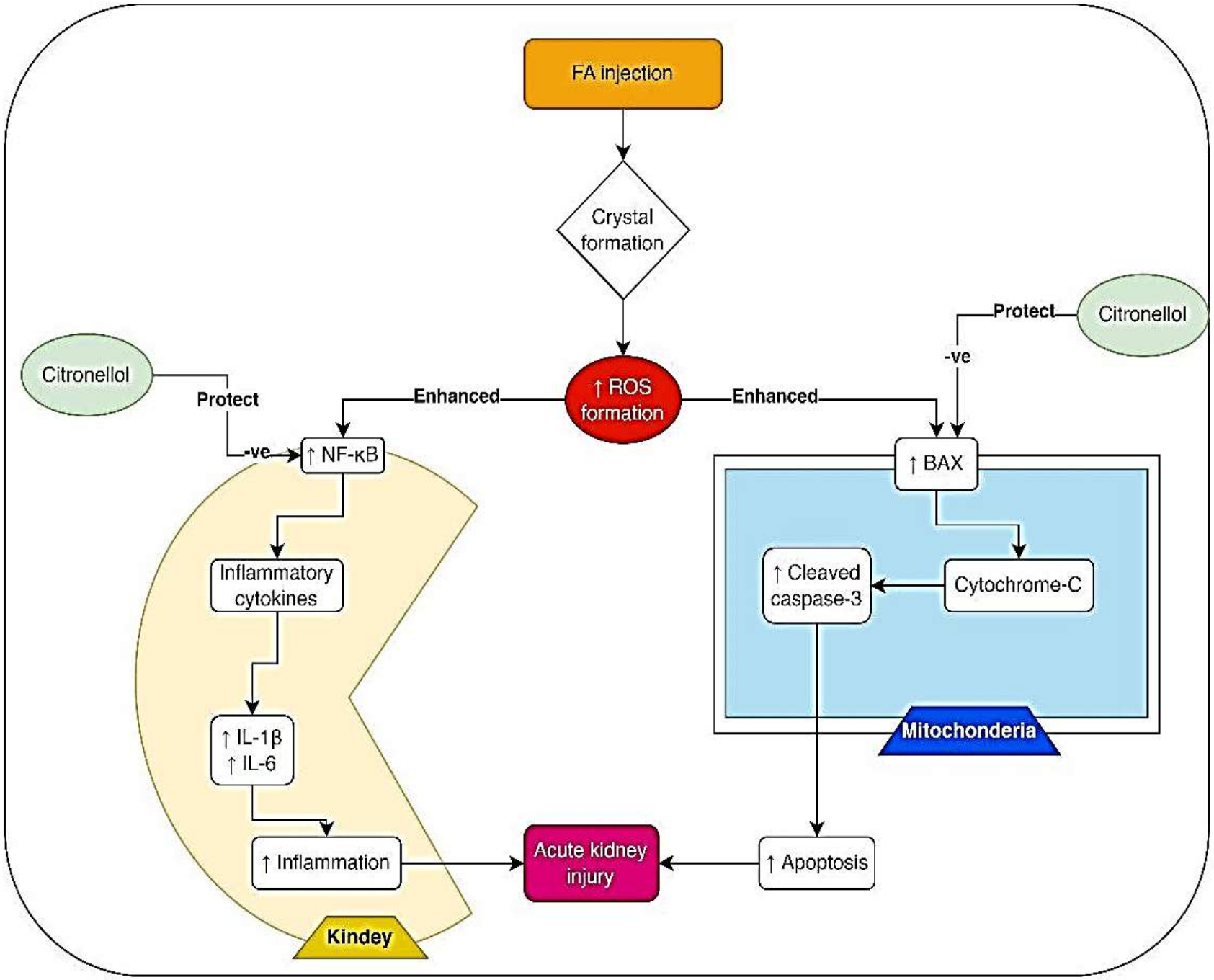


(*S*)-**1**

Citronellol (CT) with the molecular formula C10H18O (3,7-Dimethyl-6-octen-1-ol) is a naturally occurring monoterpene alcohol found in the essential oils of aromatic plant species such as *Cymbopogon citratus, Cymbopogon winterianus, and Lippia alba*, used in cooking and traditional medicine

Demonstrates several therapeutic benefits, including anti-inflammatory, anti-diabetic, cardioprotective, antioxidant, and anti-apoptotic properties.

Figure 2. Proposed schematic diagram showing the effect of citronellol in FA– induce AKI.

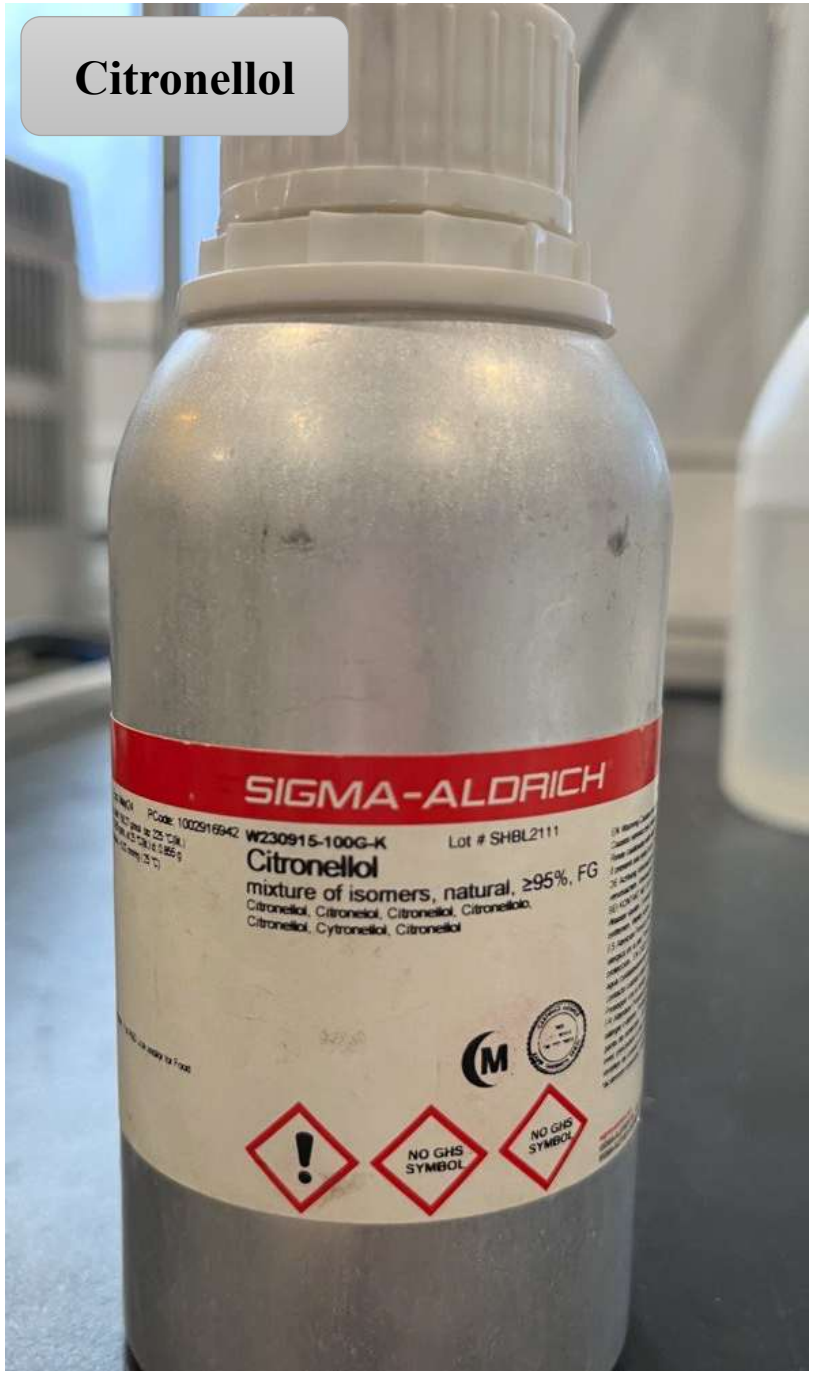


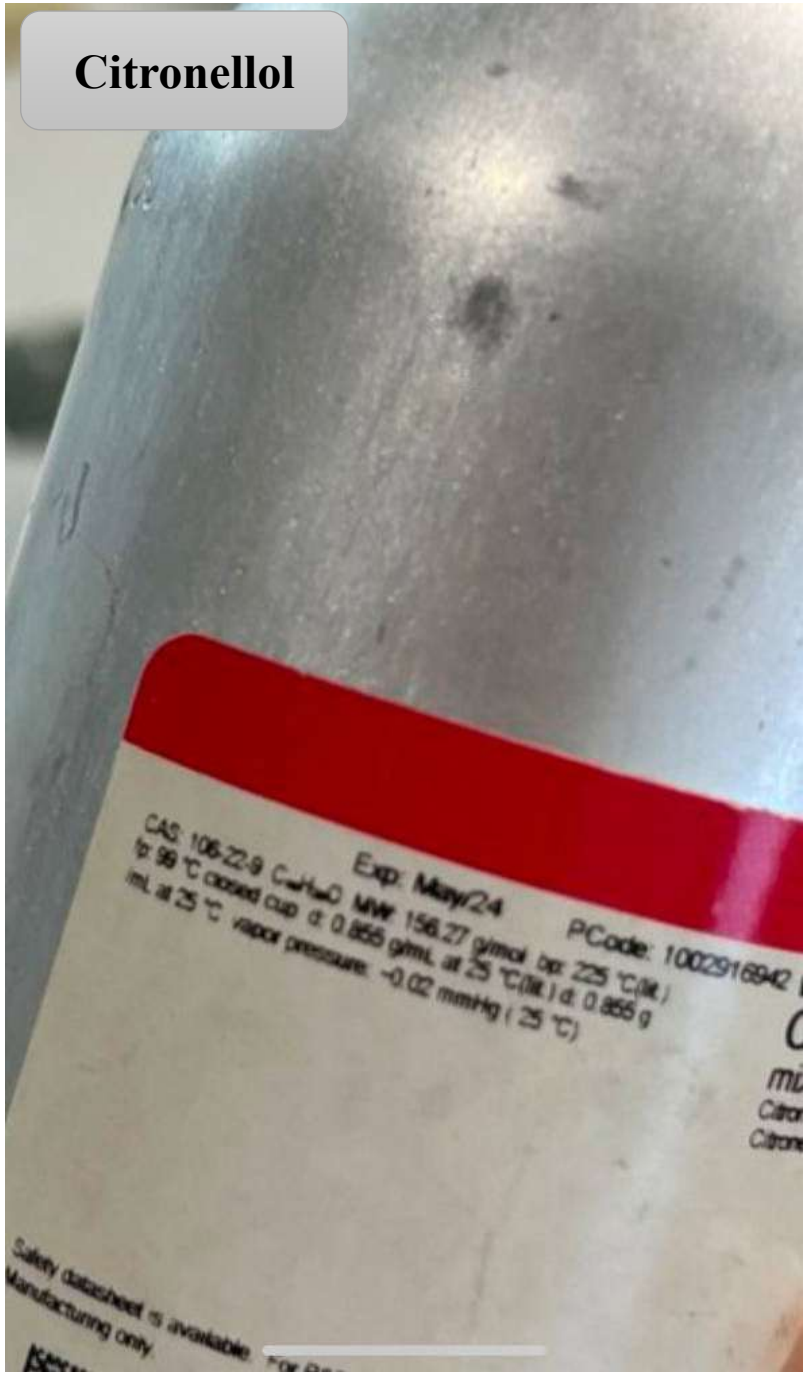
Aims of the Study:

1. To evaluate Citronellol's renal protective effect in FA-induced AKI in mice model.
2. To evaluate Citronellol's anti-inflammatory effect in the FA-induced AKI in mice model.
3. To evaluate Citronellol's anti-apoptosis effect in the FA-induced AKI in mice model.
4. To suggest a mechanism of action of citronellol renal effects.


Materials and Methods


Folic acid
powder


Citronellol
SIGMA-ALDRICH
W230915-100G-K
Lot # SHBL2111
Citronellol
mixture of isomers, natural, ≥95%, FG
NO GHS SYMBOL
NO GHS SYMBOL


Citronellol
Exp: May/24
PCode: 1002916942

Experimental Design of the Study Protocol:

- **Forty Albino mice weighing 25-30 g were included in the study. Four groups of ten mice each (n=10) were allocated as follows:**

Group Number	Group name	Description
Group I	Control group	Mice received corn oil orally for 4 consecutive days. On day 4 mice received an IP injection of 0.1 ml of 0.3M $NaHCO_3$ and then sacrificed after 48 hours.
Group II	Folic acid -model group	Mice received a single IP injection of folic acid (250 mg/kg) dissolved in 0.3M $NaHCO_3$ on day 4 and then sacrificed after 48 hours.
Group III	Treatment Group: Citronellol 50mg/kg	Mice administered Citronellol (50mg/kg/day) orally for 4 consecutive days. On day 4, mice received a single dose of IP injection of Folic acid (250 mg/kg) and then sacrificed after 48 hours.
Group IV	Treatment Group: Citronellol 100mg/kg	Mice administered Citronellol (100mg/kg/day) orally for 4 consecutive days. On day 4, mice received citronellol orally and an IP injection of Folic acid (250mg/kg) and then sacrificed after 48 hours.

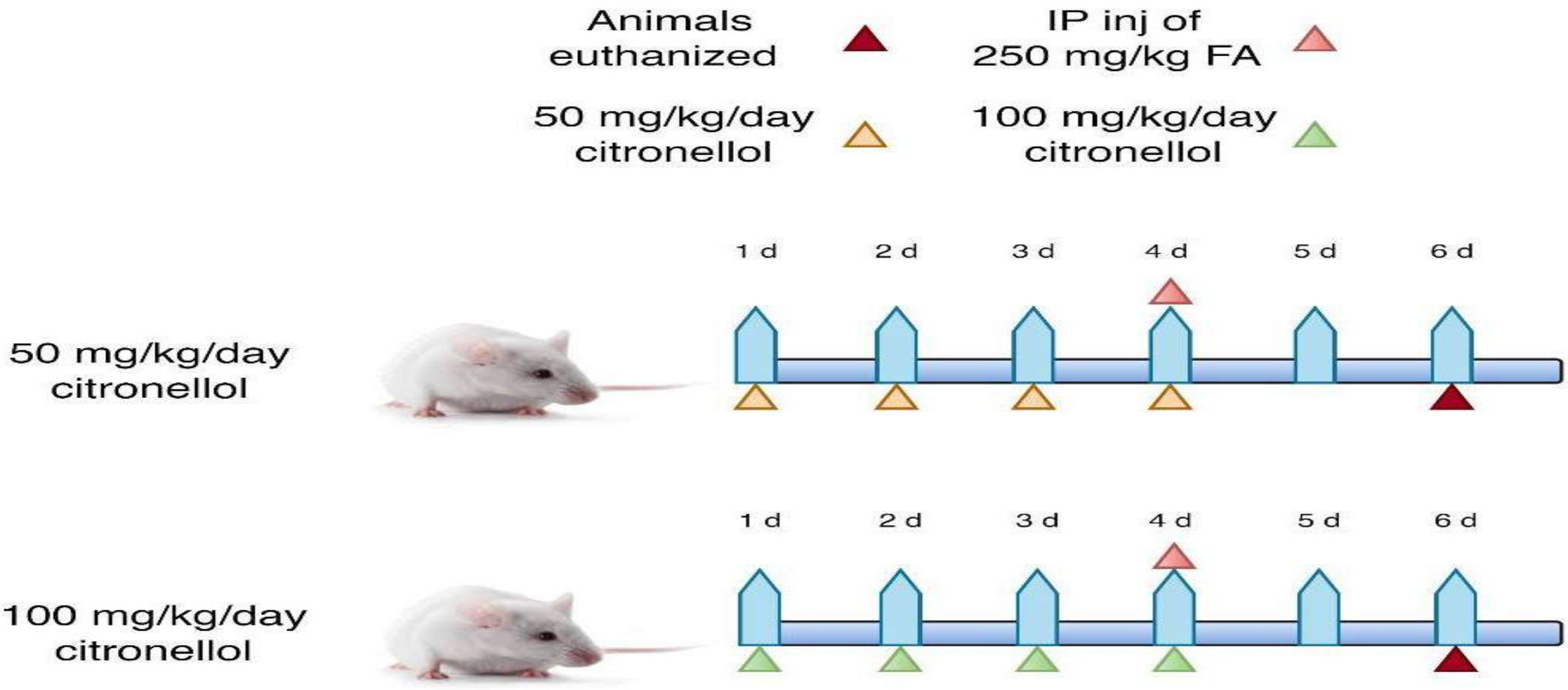


Figure 3. Schematic presentation of the experiment protocol

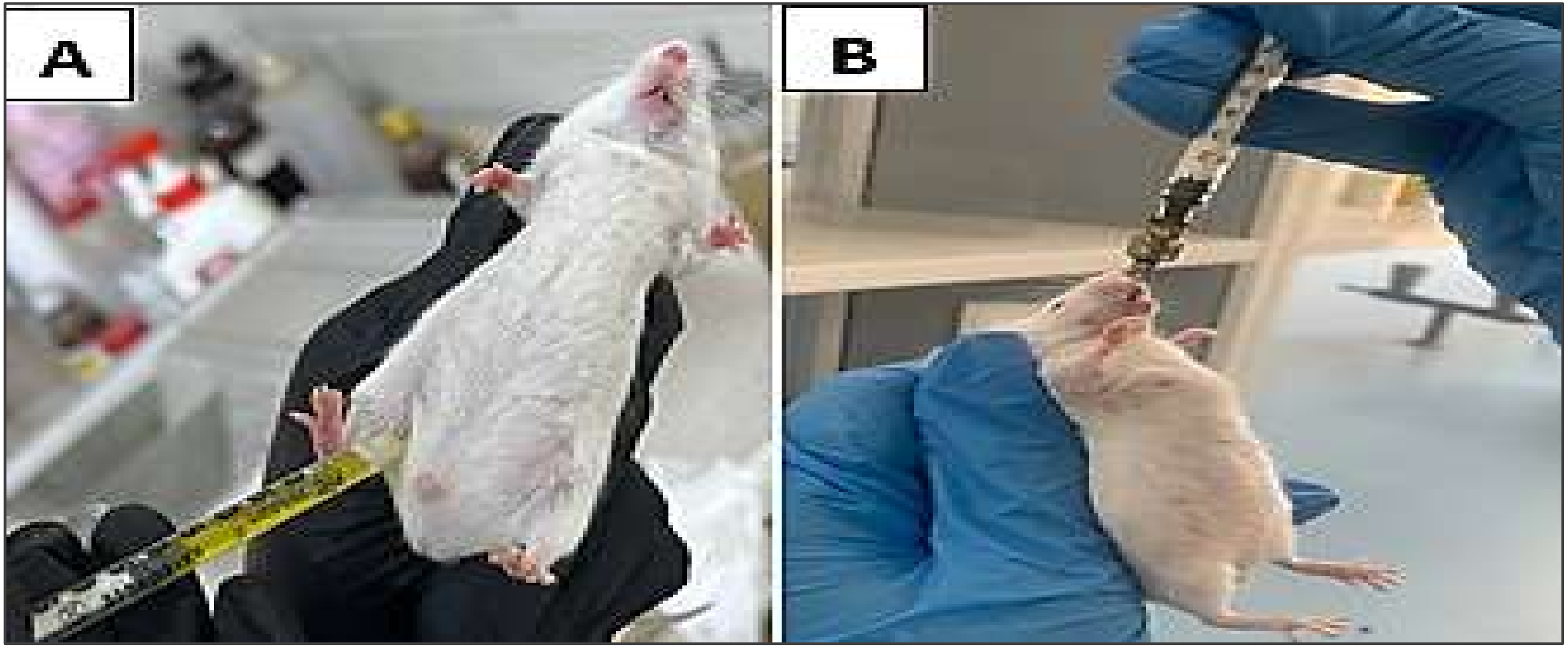


Figure 4. Images were taken during the experiment. A) Folic acid administered by IP injection. B) Citronellol administered by oral gavage.

On day 6, immediately before euthanizing, retro-orbital sampling was used to gather the blood. Serum obtained for urea and creatinine levels measurment.

At the end of the experiment (on day 6), after euthanization by diethyl ether; and then sacrificed by cervical dislocation, the right kidneys of ten mice from each group were removed and rinsed with cold phosphate buffer saline (PBS, pH 7.4) then separated into two pieces for ELISA and PCR analysis.

50 mg of tissue utilized in the ELISA was divided into small pieces and put in a micro-centrifuge tube with 0.45 ml of cold PBS for measuring cleaved caspase 3 and Bax

50–100 mg of kidney tissue was added to 1 ml of TRIzol solution before being frozen, to estimate the gene expression level of IL-1β, KIM-1, NF-κB, and IL-6 relative to the housekeeping gene GAPDH as a reference gene in renal tissue samples

Each animal's left kidney was carefully removed, cleaned with PBS, then preserved in 10% formaldehyde per standard protocol for histopathological slides and stained with H&E

Samples

Kidney Tissue

Retro-orbital blood

Right kidney

Left kidney

To obtain serum for urea and creatinine measurements

Upper piece in PBS for ELISA Determination of cleaved caspase 3 and Bax

Lower piece in Trizol for Rt-qPCR analysis of IL-1β, KIM-1, NF-κB, and IL-6

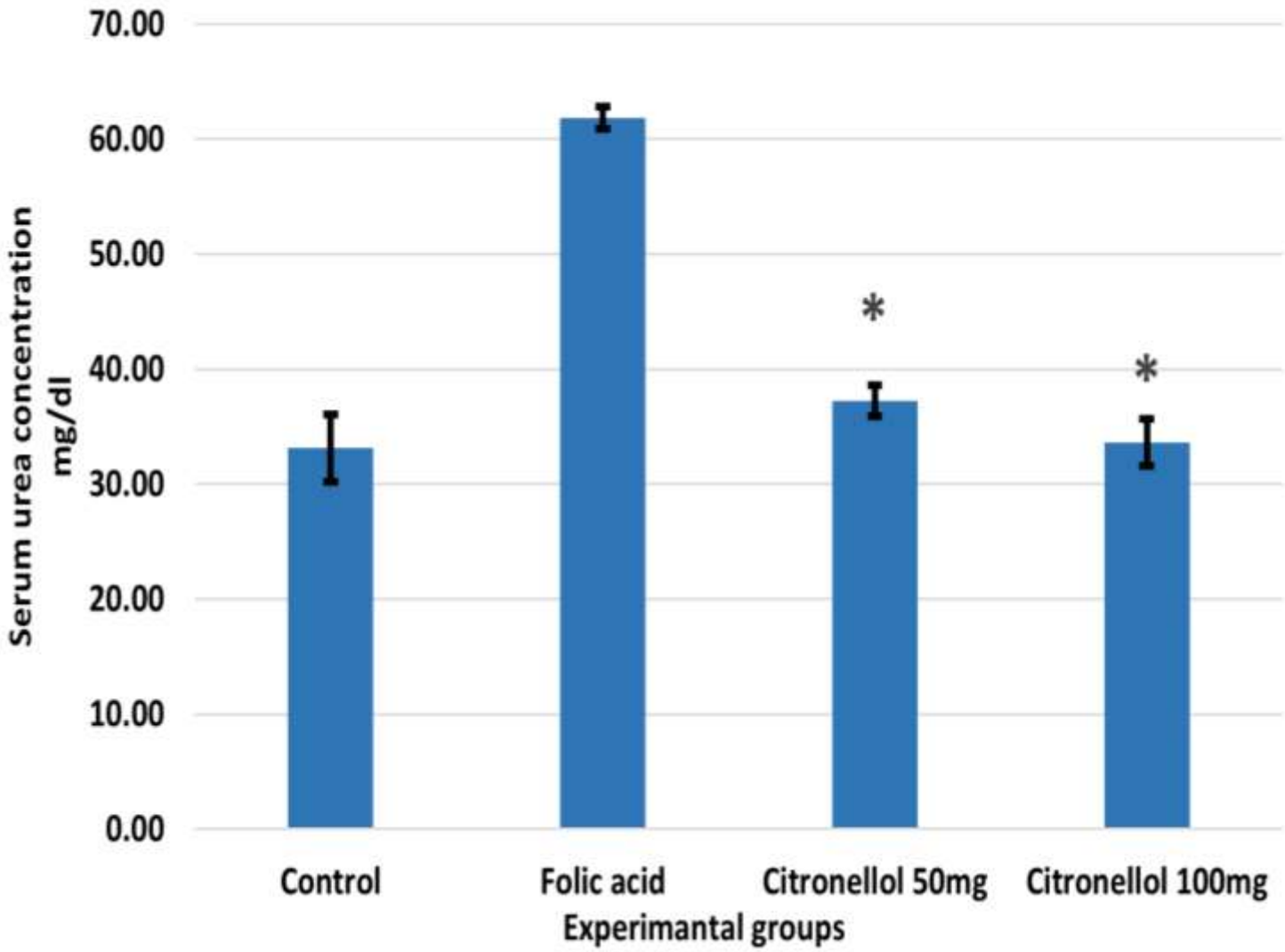
Histopathological analysis

Results and Discussion

1.Effects of Citronellol on renal function:

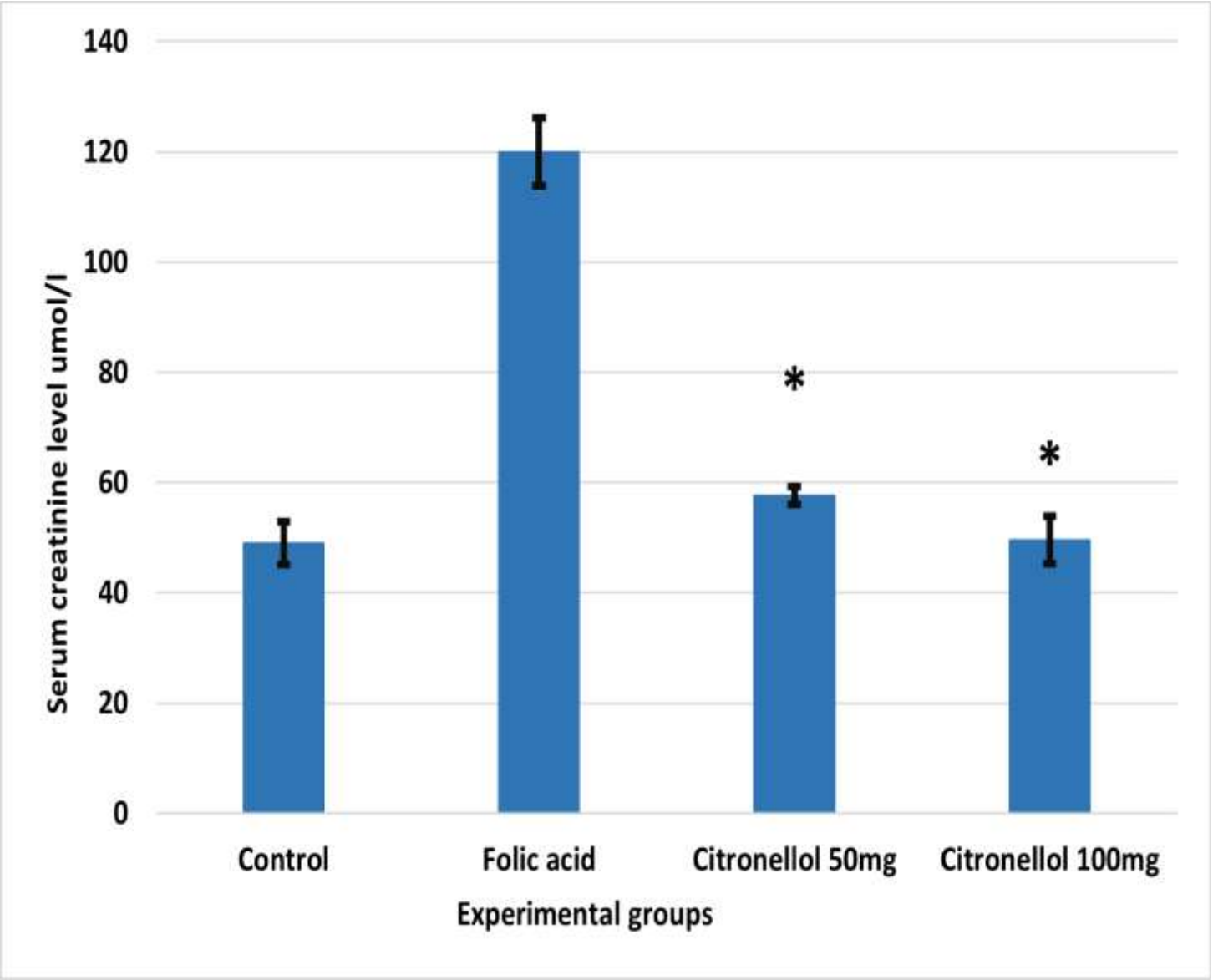
1.1 Effect of Citronellol on serum urea level in folic acid-induced AKI in mice

- **Figure 1: Effect of Citronellol on serum urea level in Folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



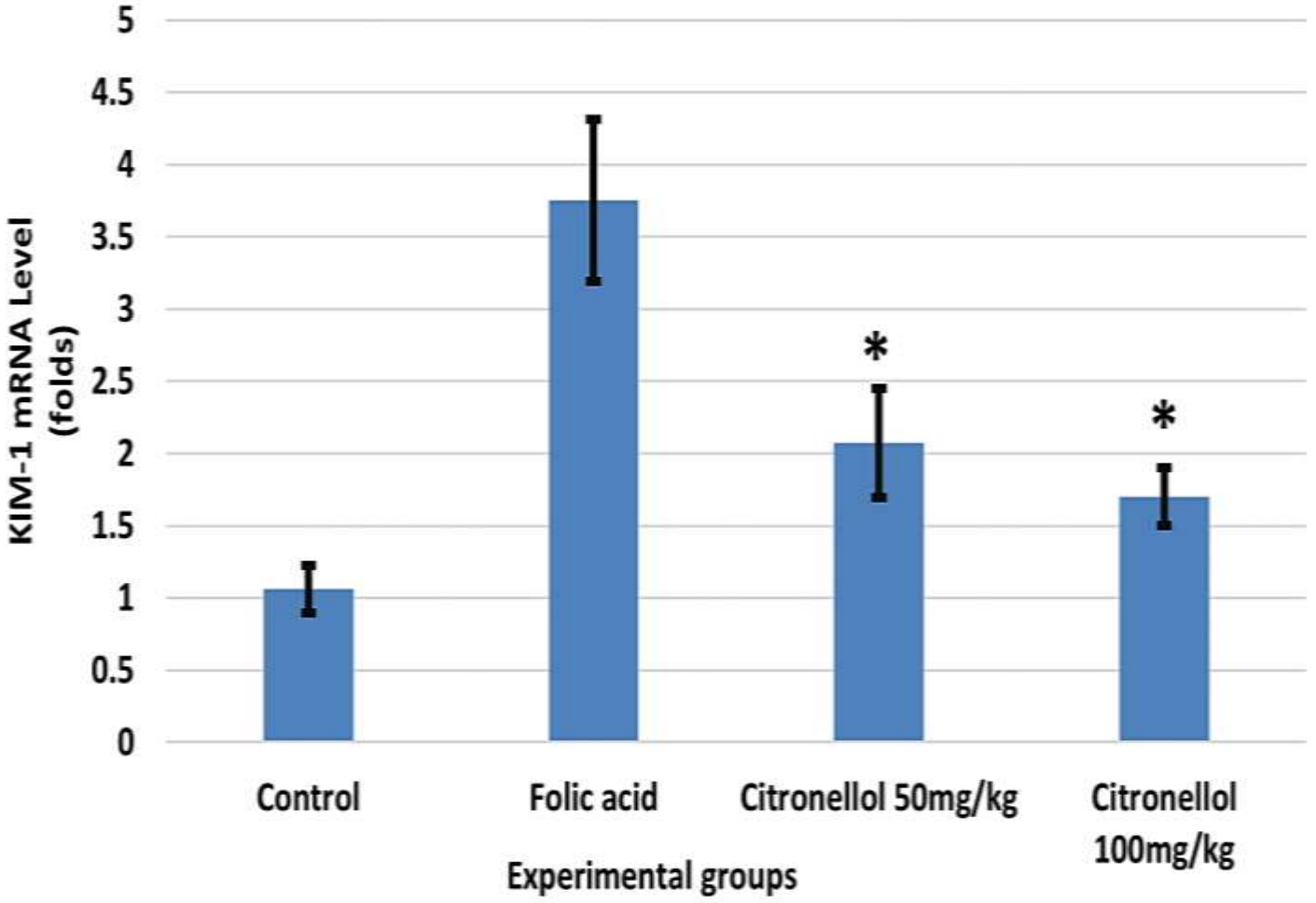
1.2 Effect of citronellol on serum creatinine level in folic acid-induced AKI in mice

- **Figure 2: Effect of Citronellol on serum creatinine level in folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



1.3. Effect of Citronellol on Kidney injury molecule-1 (KIM-1) in folic acid- induced AKI model

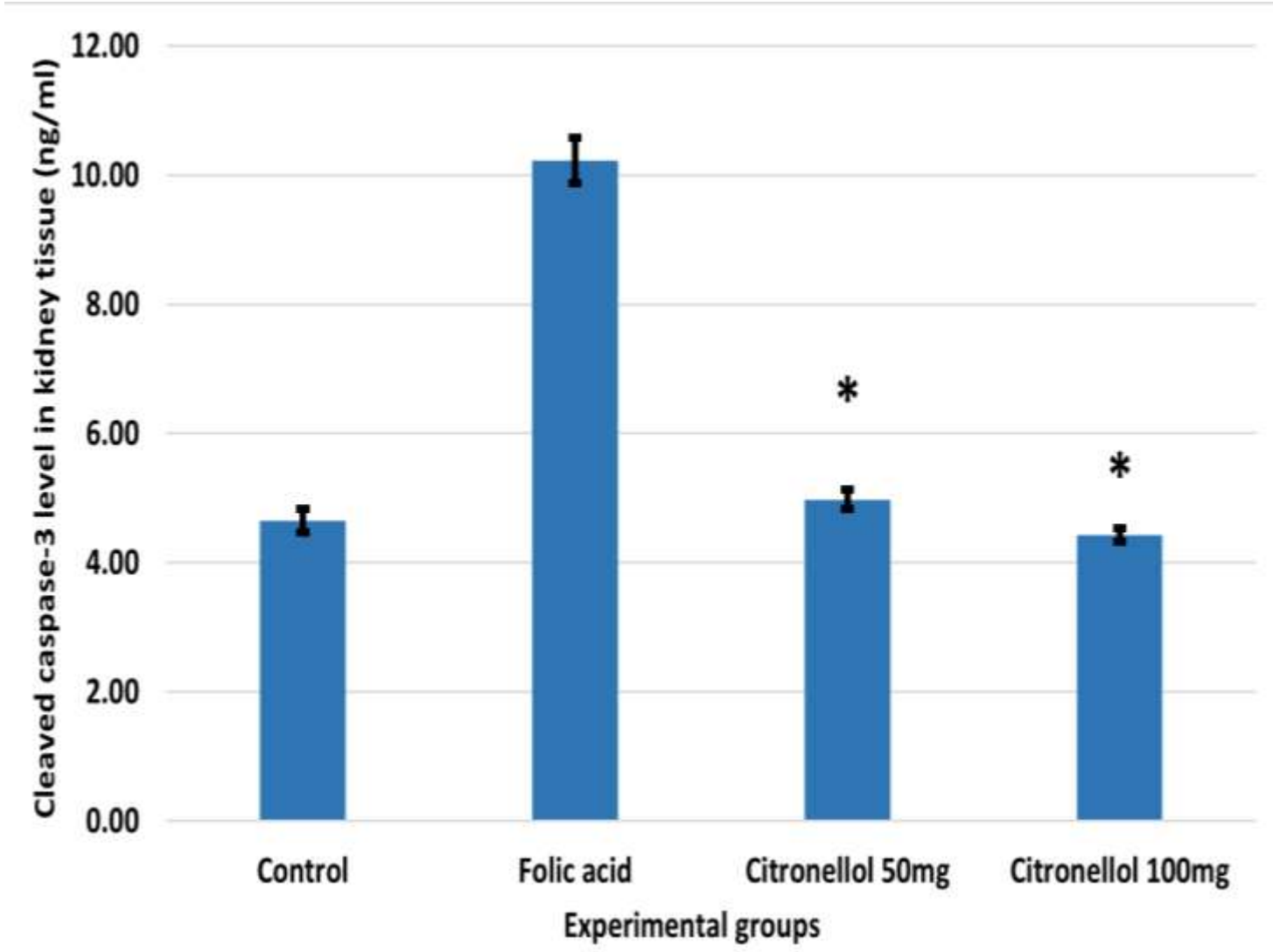
- **Figure 3: Effect of Citronellol on KIM mRNA expression in Folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



2 Effect of Citronellol on renal tissue apoptosis

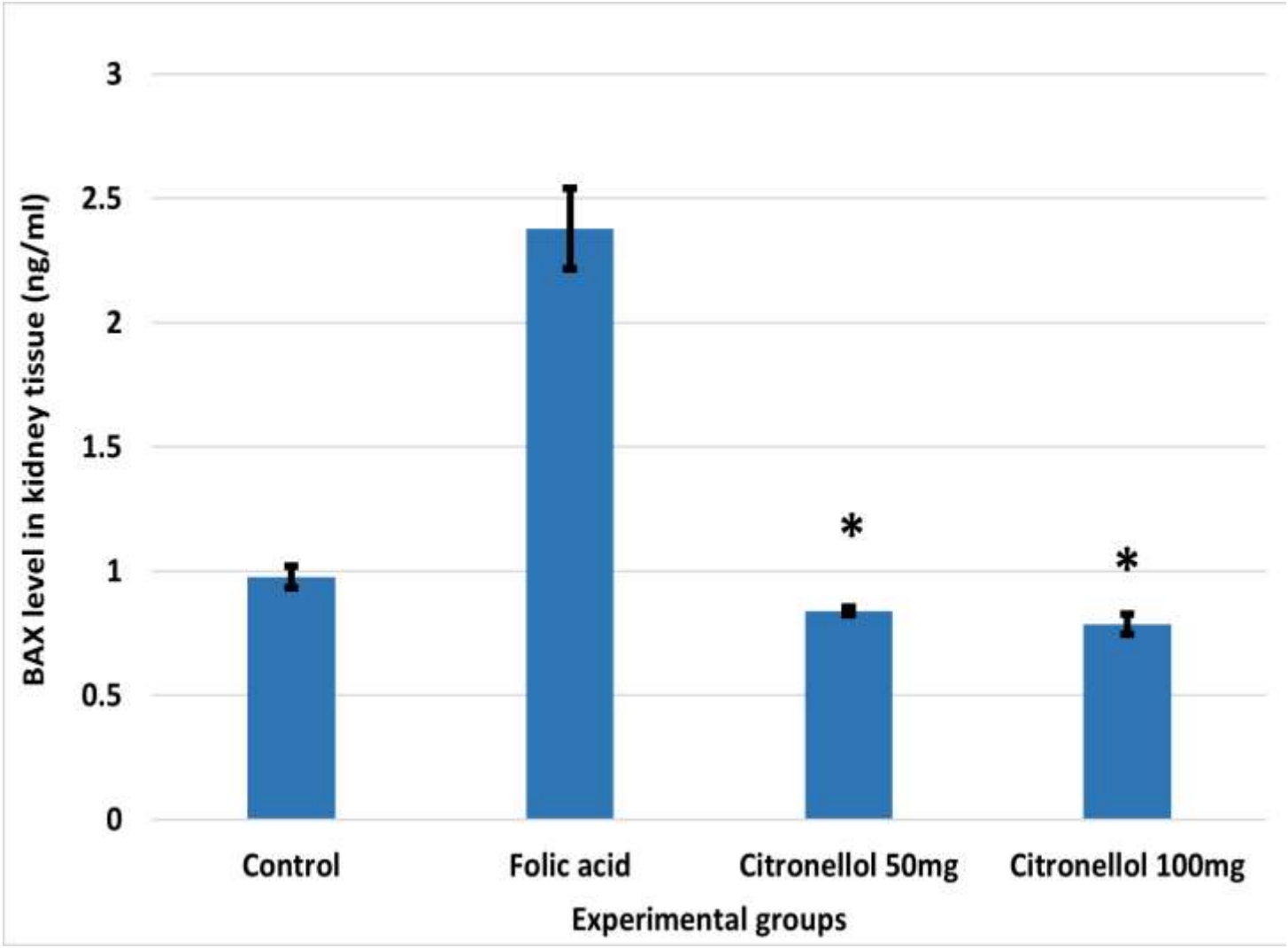
2.1 Effect of Citronellol on cleaved caspase-3 in folic acid-induced AKI in mice

- **Figure 4: Effect of Citronellol on cleaved caspase-3 level in folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



2.2 Effect of Citronellol on Bax level in folic acid-induced AKI in mice

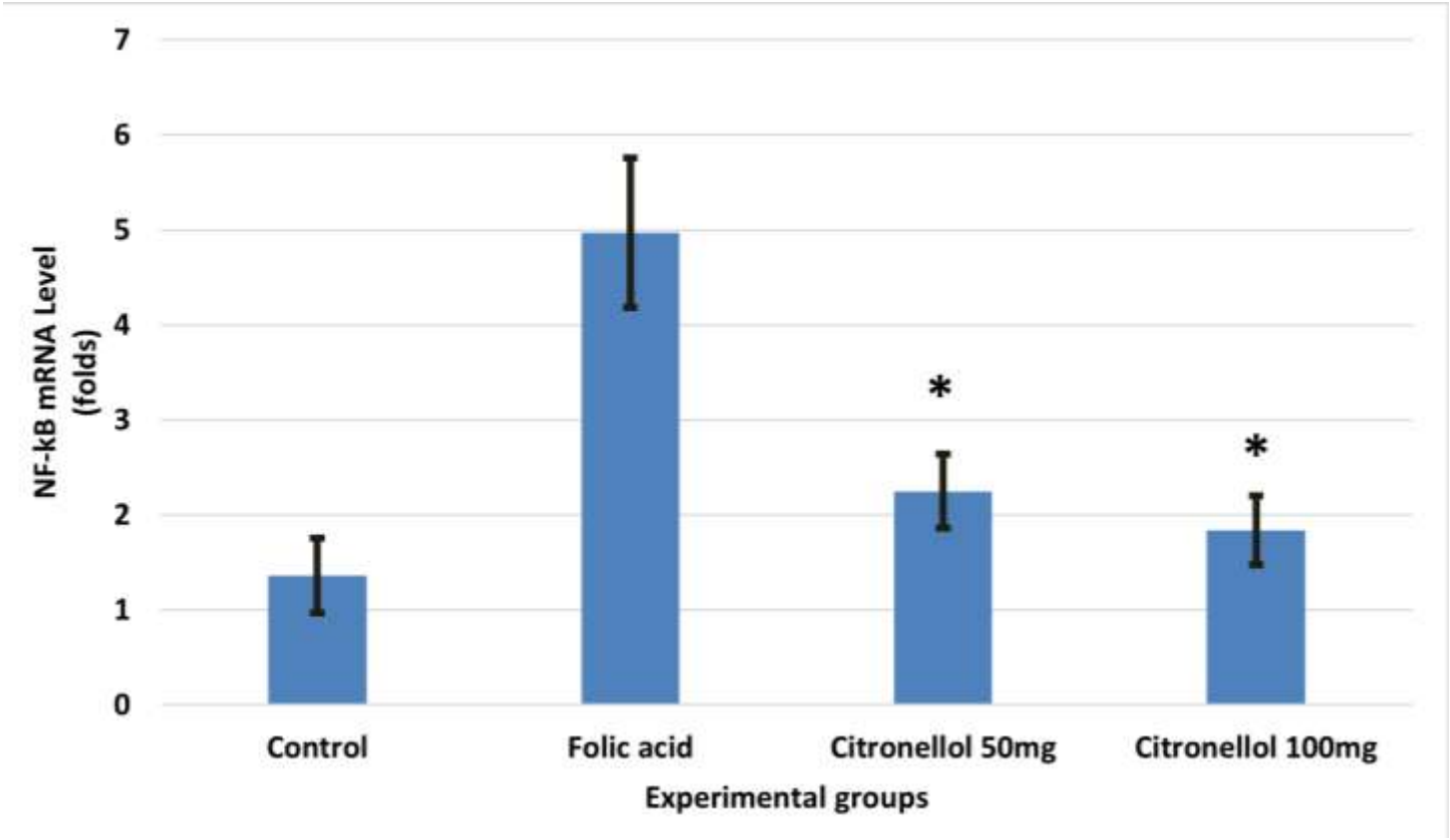
- **Figure 5: Effect of Citronellol on Bax level in folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



3. Effects of citronellol on renal tissue inflammation

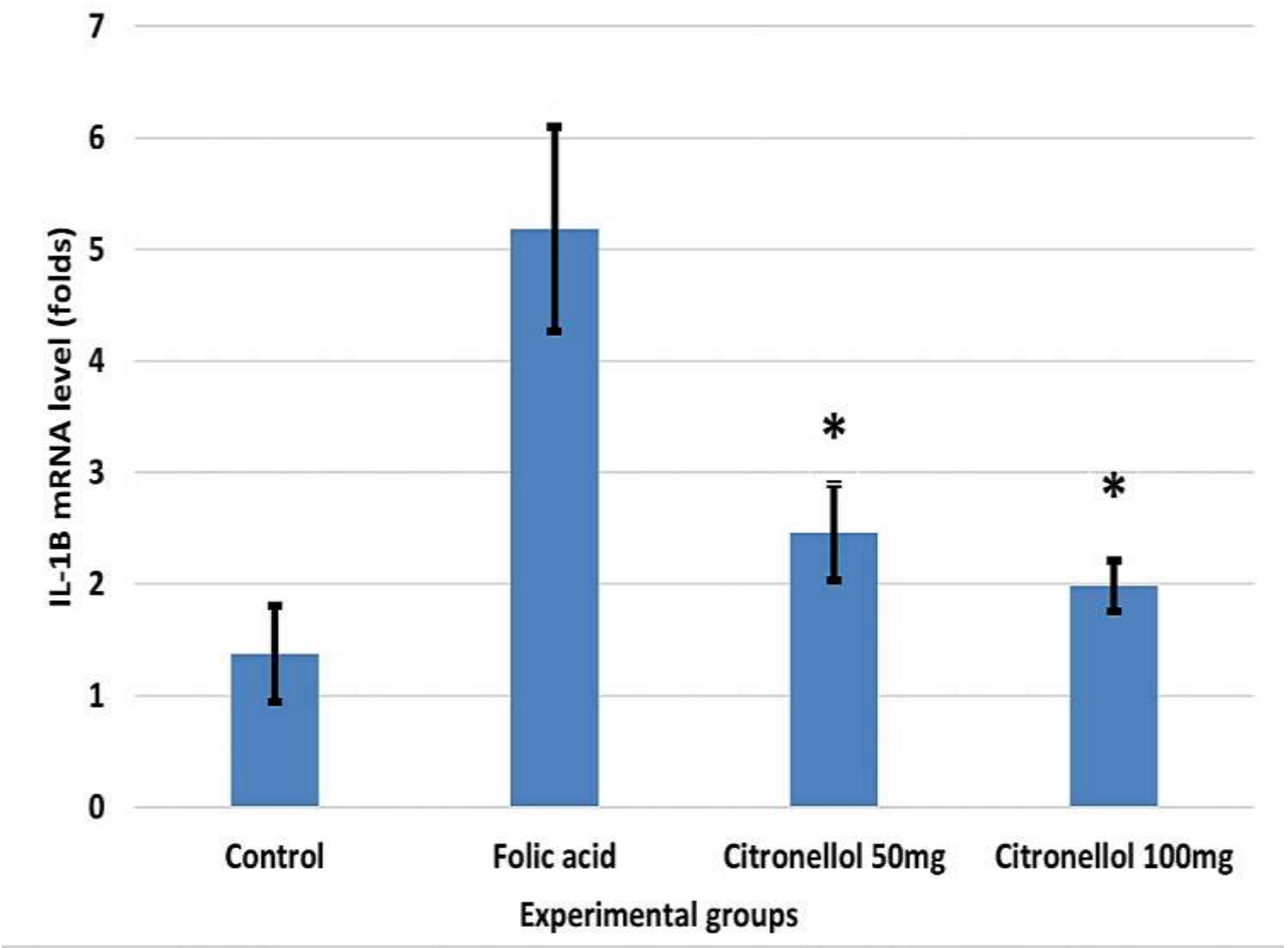
3.1 Effect of Citronellol on NF-κB in folic acid-induced AKI model

- **Figure 7: Effect of Citronellol on NF-κB mRNA expression in folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



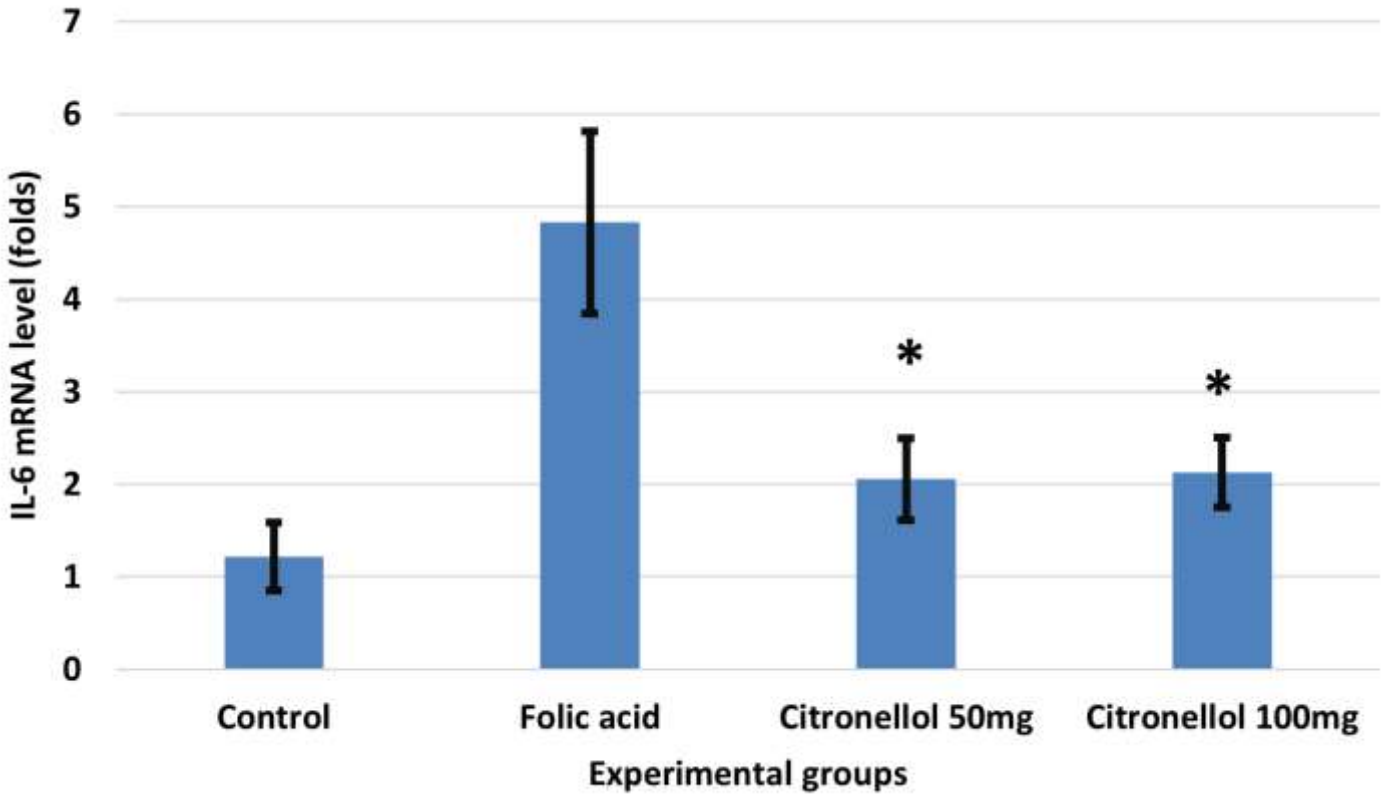
3.2 Effect of Citronellol on IL-1β expression in folic acid-induced AKI model

- **Figure 6: Effect of Citronellol on IL-1β mRNA expression in folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



3.3 Effect of Citronellol on IL-6 expression in folic acid-induced AKI model

- **Figure 8: Effect of Citronellol on IL-6 mRNA expression in folic acid-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



4. Effect of citronellol on kidney histology in FA-induced AKI in mice

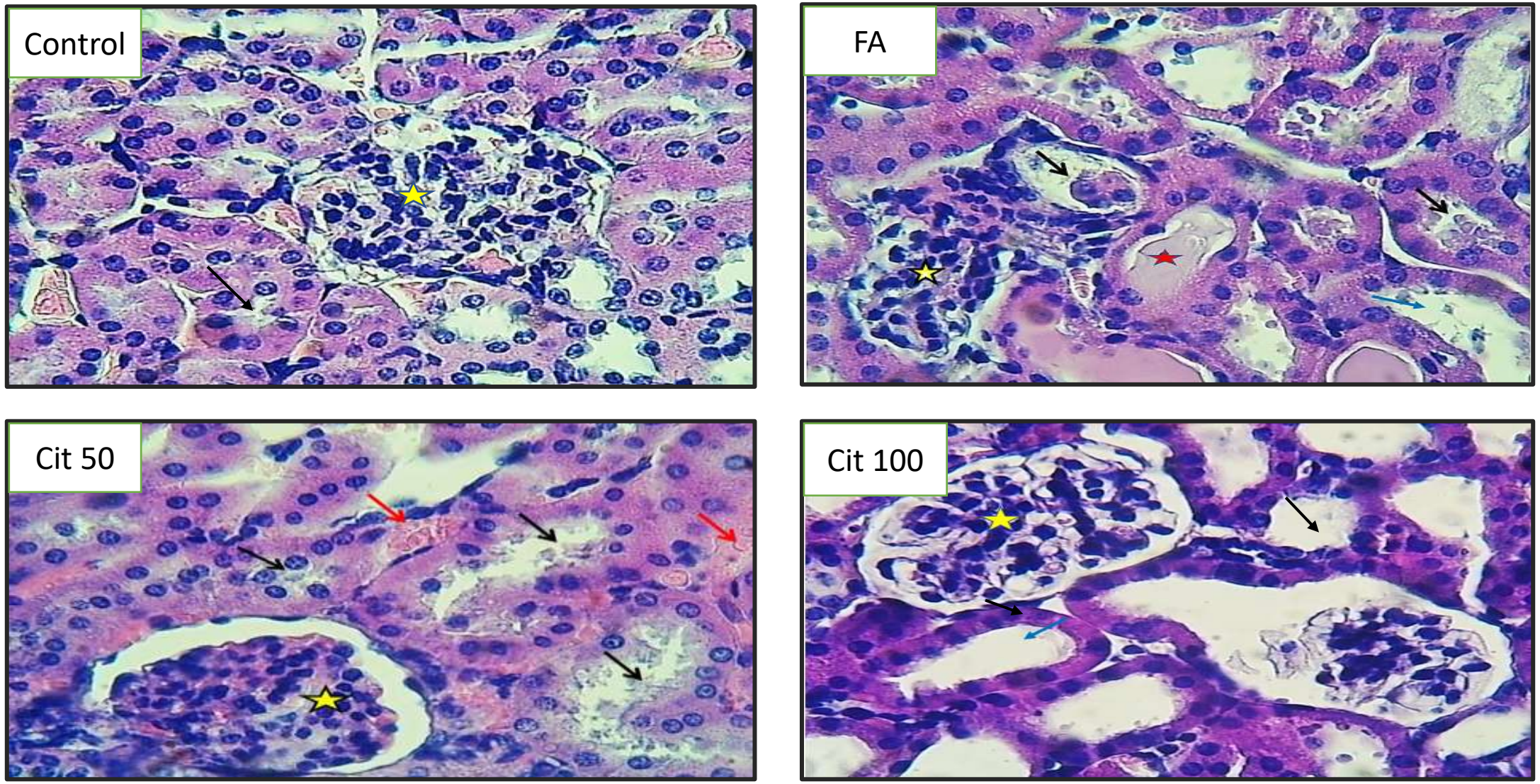
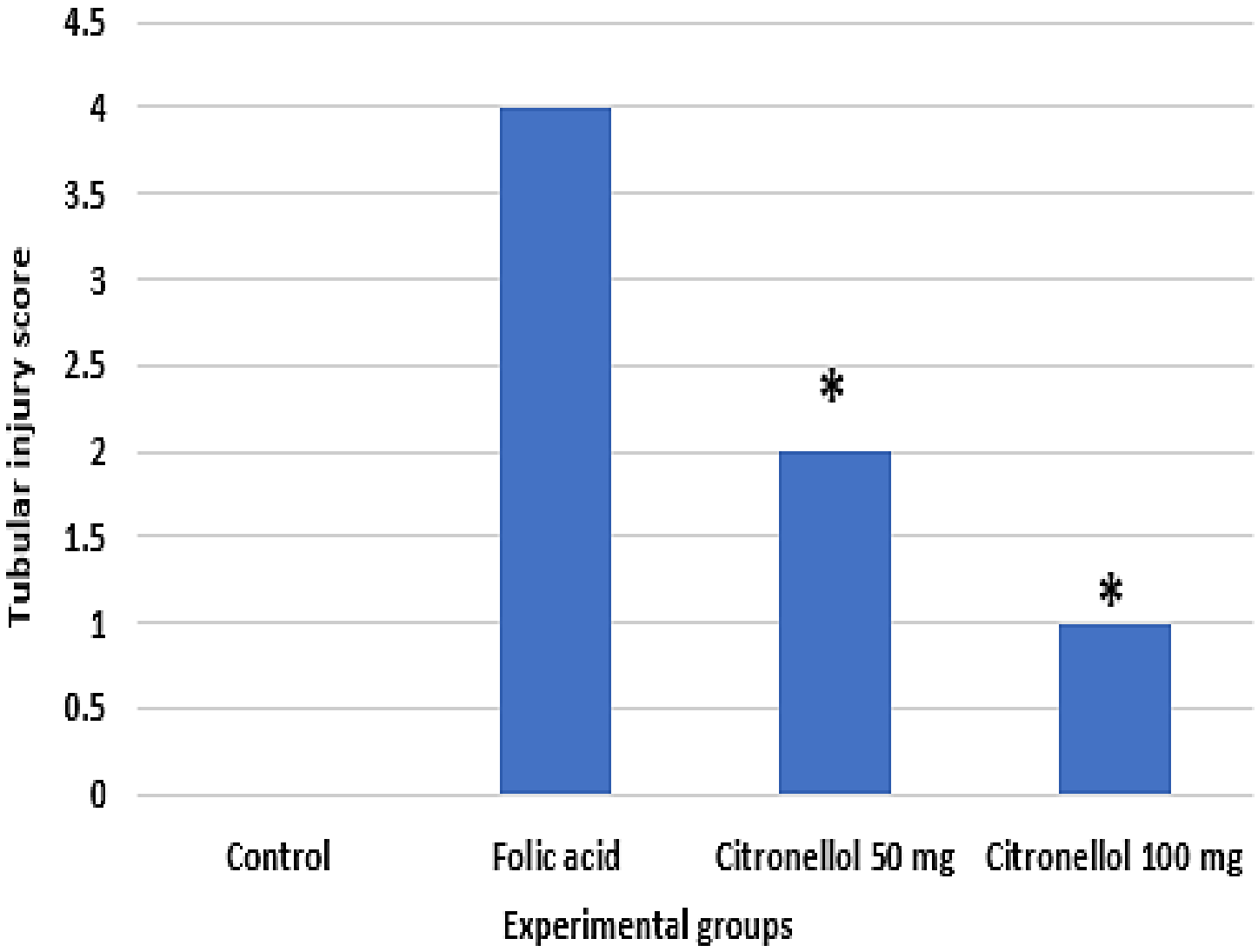
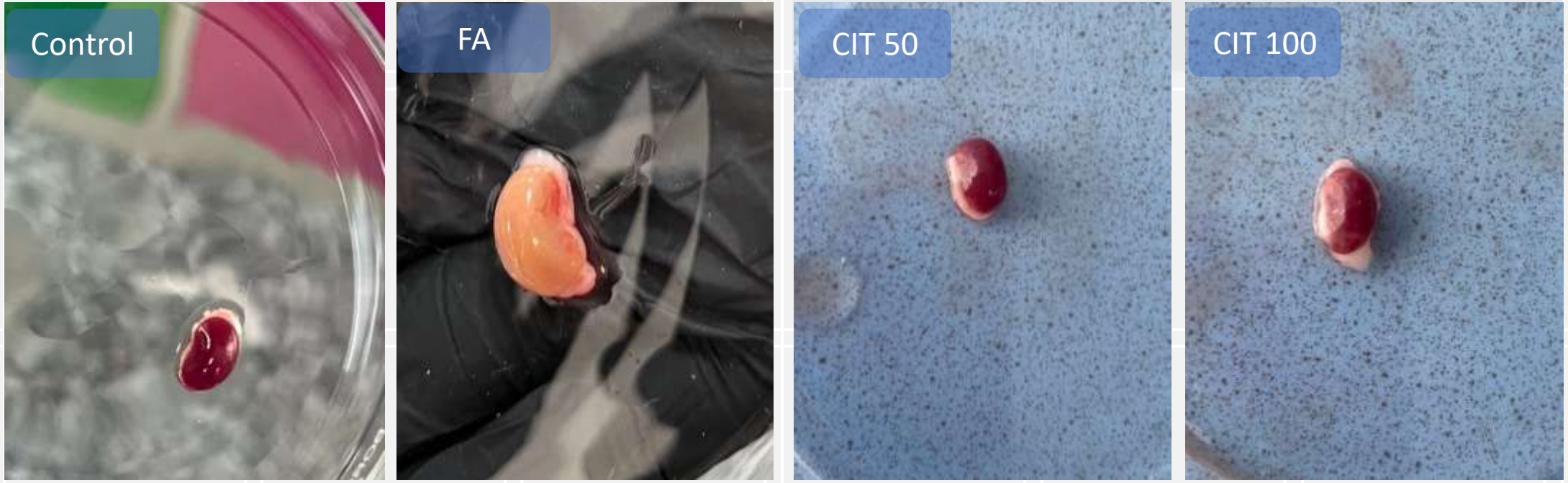


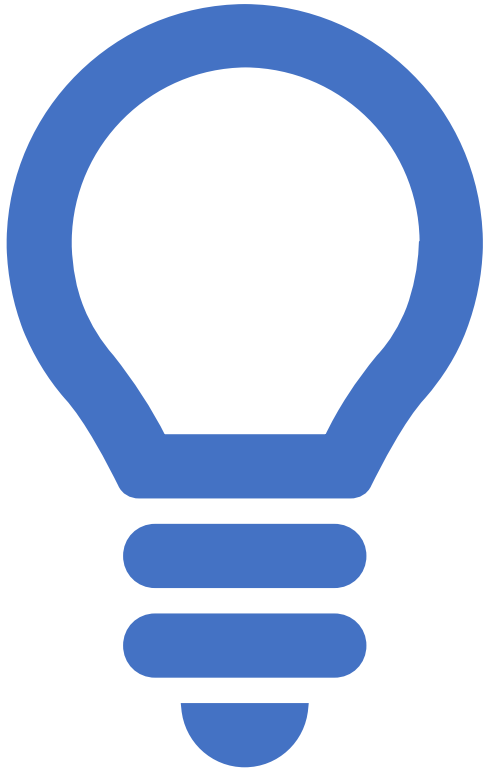
Figure 9: Effect of Citronellol on pathological kidney changes in folic acid-induced AKI in mice.

- **Figure 10: Effect of Citronellol on renal tubular injury score in FA-induced AKI in mice.**
- **Data was expressed as Mean ± SEM.**
- **(*) indicates a statistically significant difference when compared to the folic acid model group (*P*<0.05).**



Gross view of kidneys





Conclusion and Recommendations

Conclusions:

- Citronellol has a reno-protective effect against FA-induced AKI in mice model.
- Citronellol exerted an anti-inflammatory effect against FA-induced AKI in mice model.
- Citronellol exerted an anti-apoptotic effect against FA-induced AKI in mice model.
- Citronellol produces its anti-inflammatory effect by inhibiting NF-κB dependent signaling pathway.
- Citronellol produces its antiapoptotic effect by inhibiting the intrinsic pathway.

Recommendation for further studies:

- Investigation of the protective effect of citronellol in animal models of other drugs or toxins-induced AKI.
- Evaluate the effect of citronellol on the chronic kidney disease model induced by FA.
- Evaluate the protective effects of citronellol on other tissues such as the liver and heart.
- Investigation of the NLRP3 inflammasome implication in the inflammatory pathway.
- Evaluation of more doses of citronellol to precisely define its dose-response effect.

Thank you