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Research Article

Anti-nephrotoxicity effect of bee and wasp venoms on rheumatoid arthritis-induced male albino rats *(Rattus rattus)*

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Abstract
Due to its beneficial benefits on diseases like rheumatoid arthritis, Hymenoptera venom acupuncture therapy is an alternative therapy for patients with various chronic diseases all over the world. The present study aimed to examine the modulatory effects of wasp venom (WV) and bee venom (BV) in the histological changes of the kidney caused by rheumatoid arthritis in male albino rats *(Rattus rattus)*. The dose of 40 µg/Kg body weight of lyophilized bee and wasp venoms was administered intraperitoneally (i.p.) every day for 4 weeks. The rats were randomly divided into six groups: placebo administered DW; positive control only Complete Freund's Adjuvant (CFA) injected into the right hind paw; treatment groups treated with WV or BV injected with 100µl of CFA in the right hind paw with WV or BV (i.p.) which subdivided into subgroups: 2 subgroups treated with venom (WV or BV) i.p. for 4 weeks along with CFA, and another two subgroups treated after five days from CFA injection for 4 weeks. The rats were sacrificed and the kidneys were taken out and processed for histological study. In the positive control group, many histopathological alterations were observed, such as degenerative changes in both glomeruli and renal tubule, congestion, and inflammatory cells in the kidney. Sections of the kidney from the BV-treated group showed normal glomerulus and tubules in most sections with reducing degenerative alterations and congestion. In conclusion, the BV was more effective in reducing nephrotoxicity induced by CFA than WV, which had less efficiency in reducing degenerative changes.

Keywords: Bee venom, Nephrotoxicity, Rats, Rheumatoid arthritis, Wasp venom

INTRODUCTION

The venom from red wasps *Vespa orientalis*, a member of the Vespidae family, is made up of bioactive molecules like histamine, catecholamines, serotonin, tyramine, and acetylcholine as well as proteins with high molecular weight (hyaluronidase, phospholipases, and others). It also contains peptides with low molecular weight (chemotactic peptides, wasp kinins, and mastoparans (Han *et al*., 2008; Dongol *et al*., 2014). Mastoparan is the preeminent wasp venom (WV) peptide that releases histamine from mast cells and provokes G proteins in a state eminently similar to agonist-bound receptors (Hirata *et al*., 2003). Wasp venom contains various constituents acting on the circulatory, immune, and nervous systems and possesses many allergens, enzymes, bioactive peptides, amino acids, biogenic amines, and volatile matters. In particular, some peptides show potent antimicrobial, anti-inflammatory, antitumor, and anticoagulant activity (Luo *et al*., 2022; Wu *et al*., 2022).

Bee venom is apitoxin, utilized in Eastern traditional medicine for treating various diseases, including rheumatoid arthritis, bursitis, and, tendonitis (Kim *et al*., 2004; An *et al*., 2015). The complex chemical composition of Bee venom (BV) being rich in peptides, enzymes, and amines highlights the diverse range of bioactive compounds and their therapeutic applications, which extend beyond the well-known pain-relieving and anti-inflammatory impacts (Abo-Zaid *et al*., 2023; Sadek *et al*., 2024). Melittin, apamin, mast cell degranulating peptide (MCD), histamine, norepinephrine, and

phospholipase A2 are some of the active peptides and non-peptide components found in bee venom, as well as many enzymes (El-kott and Mohanny, 2015; El-Bassion *et al*., 2016).

Bee venom acupuncture therapy (BVT) is an alternative therapy used worldwide by patients with different chronic diseases due to its therapeutic effects on conditions such as rheumatoid arthritis (RA). The effectiveness of bee venom as a treatment for RA, an autoimmune disorder that leads to inflammation in the joints due to it contains melittin and adolapin for inflammation and pain relief (Sharaf *et al*., 2022; Altaf and Iqbal, 2023). Bee venom treatment was effective in the alleviation of symptoms of the experimental rat adjuvant arthritis by means of clinical observation and serum inflammatory markers as it caused significant improvements in the clinical signs of arthritis and inflammatory markers such as in IL-1β, TNF-α, IL-6, and TOL in the 20.0 µg/kg BV-administered group (Tekeoğlu *et al*., 2020).

Although Hymenopteran venom is used widely in the Mediterranean area as a folk medicine for the treatment of many disorders, its effects on histological structure are still debatable; this study attempted to find out how it affected the kidney's histological structure in rheumatoid arthritis-induced rats and examine the modulatory effects of WV and BV.

MATERIALS AND METHODS

Animals

Male albino rats *(Rattus rattus)* weighing 200± 50 g, and 8–12 weeks of age were taken for the experimental study. The rats were procured from the Lab of Animals in the College of Science, University of Babylon, Iraq. Then, the rats were kept separately in lab cages and provided food and water *ad libitum*. After the adaptation period, the rats were used for the experimental studies. Rats had been kept in an environment with a controlled temperature of 25 °C, light cycle, and humidity level of 50±. Six groups of three rats each were randomly selected from the animals; the control positive received injections of Complete Freund's Adjuvant (CFA) alone, while the placebo group received injections of distilled Water (D.W.) only; treatment groups treated with WV or BV injected with 0.1 ml of Complete Freund's adjuvant in the right hind paw with 40 µg/Kg body weight of WV or BV. Both BV and WV groups are subdivided into subgroups: 2 subgroups treated with venom (WV or BV) intraperitoneally for four weeks at the time of CFA injection (along with the CFA injection group) and another 2 subgroups treated with venom (WV or BV) after five days from CFA injection for 4 weeks.

Rheumatoid arthritis Induction

Subcutaneous injection (100 ml) of Complete Freund's adjuvant (Santa Gruz Biotechnology) to the surface plantar of the right hind paw was used to induce subchronic disease (rheumatoid arthritis) (Kale and Namdeo, 2014). Each ml contained heat-killed and dried *Mycobacterium tuberculosis* (1 gm), mannide-monooleate (0.15 ml), and paraffin oil (0.85 ml).

Bee venom

The BV was provided by a Chinese company (CN Lab), and the mellitin content was 17%. BV was dissolved in sterile distilled water as stock BV (Abu-Zinadah *et al*., 2014) and kept at 4°C in darkness until utilized for the required dose (40 g/kg) at 4°C.

Wasp venom

As mentioned in Jalaei *et al*. (2014), the venom sacs were prepared, put in a clean mortar, immersed in liquid nitrogen, and then cursed using a pestle and liquid nitrogen. Following the liquid nitrogen's evaporation, 20 milliliters of 0.1 M phosphate buffer with a pH of 7.4 were added to the powder. The mixture was subsequently centrifuged at 800 xg for fifteen minutes at 4°C. The supernatant (WV) was moved to a clean tube for additional testing and kept at 20°C.

Anesthesia

Ketamine and xylazine hydrochloride were used to anesthetize rats, with 100 mg/kg injected intramuscularly (I.M) (Bhatia *et al*., 2021).

Tissue processing

The rats were sacrificed after the final day of venom injection for the histopathological study of renal tissues and both kidneys from different groups were excised., A renal tissue sample was fixed in 10% formalin, dehydrated by ethanol alcohol, cleared in the xylene, embedded in the paraffin blocks, and sectioned at 5mm thickness by eosin and hematoxylin stain (H&E) according to (Bancroft and Stevens, 1982). Histopathological changes were examined under a light microscope (Optica), and sections were photographed using a digital camera (KoPa Cam, China).

Ethical approval

The Ethics Committee of the Scientific Research Committee (ECSR) in the Biology Department at the College of Science, University of Babylon-Iraq, authorized the study procedures (NO. Z220501).

RESULTS AND DISCUSSION

The present study investigated the effects of combining BV or WV with CFA to treat nephrotoxicity brought on by arthritis. The pharmacological benefits of anti-arthritic drugs have been widely tested using the CFA as an experimental model of induced arthritis. A chronic inflammation disorder known as rheumatoid arthritis

(RA) causes synovium proliferation and cellular infiltration, progressively destroying the joints (Smolen and Aletaha, 2009). Drug treatment for RA over the last two decades has focused on using various disease-modifying anti-rheumatic drugs that are widely used today, such as methotrexate (Smolen et al., 2023), but remission or at the very least, minimal disease activity is the ultimate therapeutic aim in the treatment of RA, which may not always be attained with methotrexate monotherapy. According to a previous study, most dissatisfied arthritis sufferers are likely to consider using complementary and alternative therapy (Lee et al., 2005; Yang et al., 2017). In the sections of renal tissue in the placebo group, no histological changes in either glomeruli or renal tubules were observed (Fig.1.).

In the present study, it was found that kidneys from the CFA-treated group showed marked histopathological

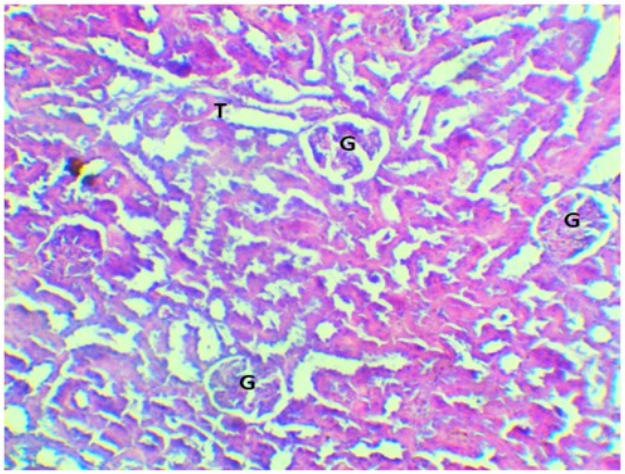

Fig. 1. *Section of the kidney from the placebo group shows both glomeruli (G) and tubules (T) with normal histological structures H&E (100X).*

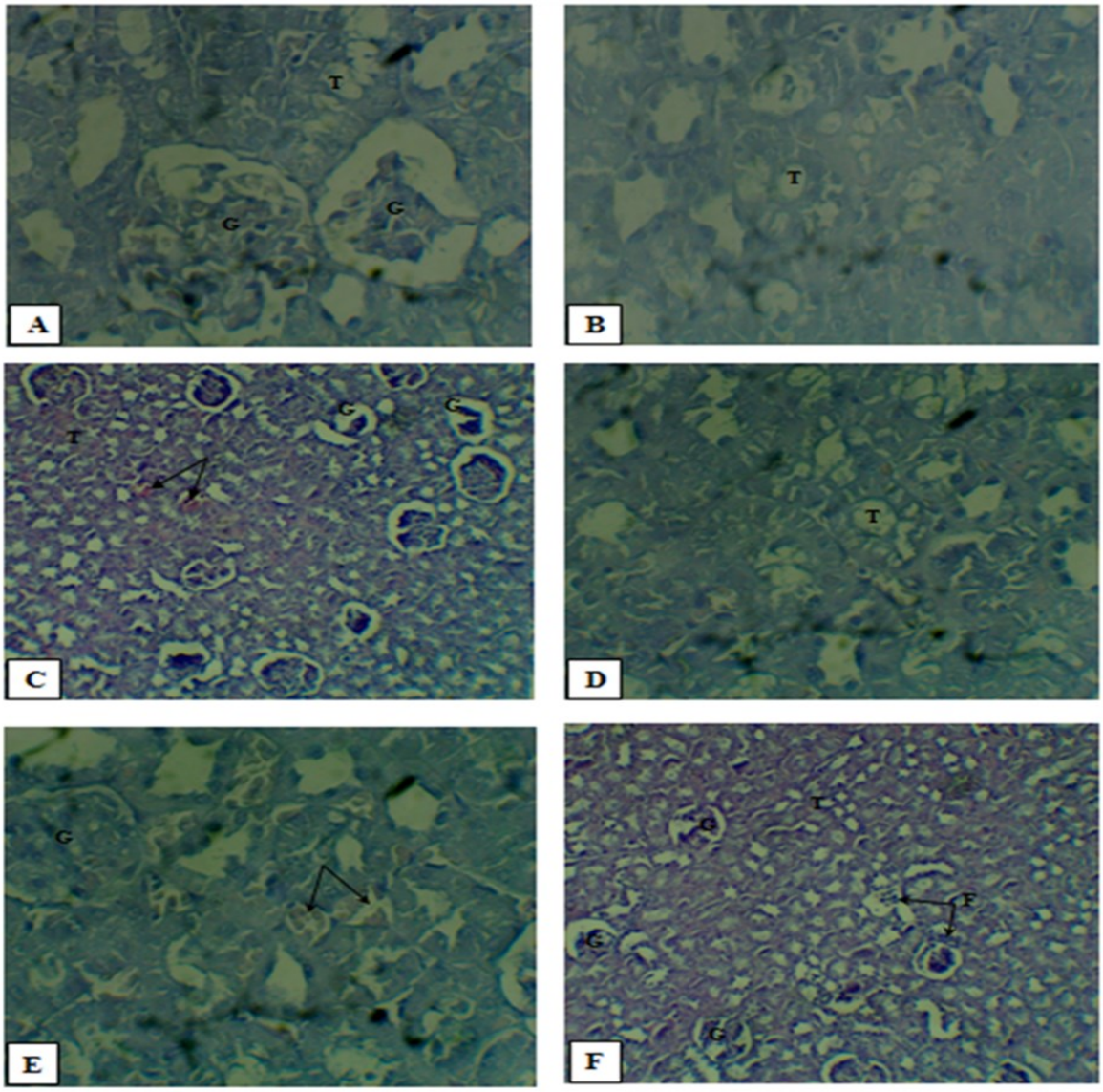

Fig. 2. *Sections of the kidney from the CFA-treated group show some histopathological changes as degenerative changes of both glomeruli (G) (A, C&D) and renal tubules (B&D). The presence of congestion (⟶) (C&E) and inflammatory cells (F) were evident. A, B (400X); C, D, E, F, H&E (100X).*

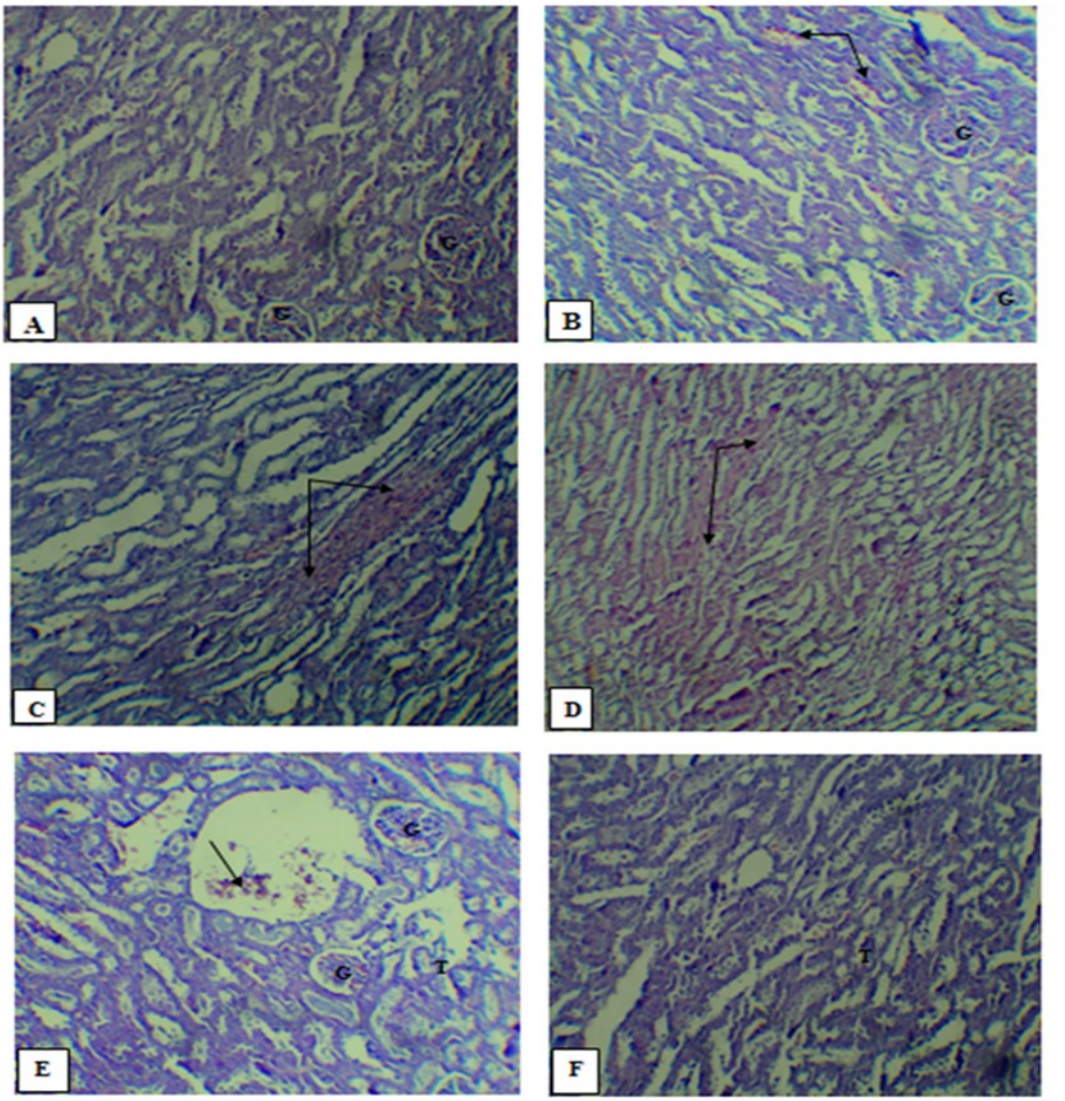

Fig. 3. *Sections of the kidney from the WV-treated group along with CFA injection show the normal histological structure of glomerulus (G) and tubules (T) in some sections (A&B) whereas the presence of congestion (⟶) (C, D&E) and the presence of degenerative changes of renal tubules (T) was evident in most sections (E&F) were evident. (100X), H&E.*

changes such as shrunken glomeruli and extensive damage and degenerative changes to both glomeruli and renal tubules structures, the presence of congestion and inflammatory cells (inflammation) was evident. The treatment with BV or WV demonstrated regenerative changes and reduced congestion was still seen in some places.

Sections of kidney from both subgroups of the WV-treated group (40 µg/Kg) along with CFA injection and WV-treated group after 5 days from CFA injection, although few sections showed normal histological structure (Fig.3A&B) several changes deviating from normal structure were observed in most sections, including shrunken glomeruli, degenerative changes of renal tissue and the presence of the congestion in most sections was also seen (Fig.3B, C, D, F). Furthermore, there was a disarrangement of renal tubules and congestion (Fig.4).

Chemically, wasp venom includes a variety of proteins, peptides, volatile compounds, and bioactive substances such as decoralin, phospholipase A2, antigen 5, and mastoparan. The bioactive components possess anti-inflammatory, anti-microbial, and anti-cancer activities. The insufficient amount of WV and an absence of advanced techniques for synthesizing bioactive components in WV continue to be barriers to wasp venom's effective use. Wasp venom has the potential to be an innovative natural source for the creation of novel medicines and new drug development agents (El-Wahed *et al.,* 2021). In addition to serotonin, histamine, dopamine, noradrenaline, and adrenaline, the venom of bees and wasps contains complex combinations of physiologically active proteins and peptides, such as phospholipases, hyaluronidase, phosphatase, and -

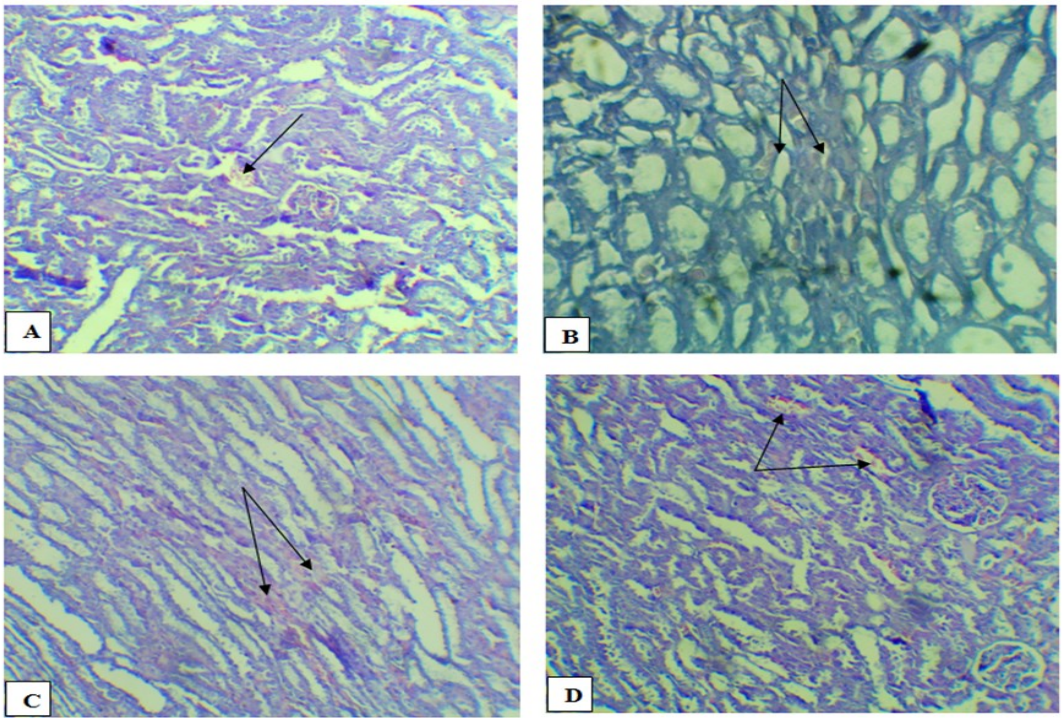

Fig. 4. *Sections of the kidney from the WV-treated group after 5 days from the CFA injection group show degeneration in both glomeruli and renal tubules and congestion (⟶) in some sections, H&E (100X).*

glucosidase. Melittin, apamin, and mast cell degranulating peptides are exclusive to bees, while mastoparan and bradykinin are found solely in wasps (Pak, 2016).

In both subgroups of arthritic rats treated with BV (40 µg/Kg) along with CFA (Fig. 5 A) and five days after injection of CFA (Fig.5 B), BV could retain some of the histopathological changes in renal tissues caused by CFA and most sections showed overt improvement of renal histological structure as compared to arthritic rats without treatment (CFA treated rats). Moreover, some sections still had degenerative alterations in glomeruli and renal tubules and congestion (Fig.5 C, D, E, F).

The BV is used worldwide to treat various ailments and diseases because it contains therapeutic characteristics (Abaci and Orhan, 2022; Dumitru et al., 2022). The BV therapy utilizes live bee stings (or injected venom) to treat different illnesses, including rheumatoid arthritis, multiple sclerosis, heart and blood system problems, skin diseases, and other diseases. The primary active ingredient works against bacteria, viruses, and inflammation. In terms of BV, 2.8 mg/Kg for body weight was the median lethal dose (LD50). Consequently, if carefully applied, bee stings are safe for treating human diseases (Eze et al., 2016).

Thus, the present study demonstrates that BV treatment improved histological changes, probably owing to BV's active components. These changes can result from interactions between the different venom components., mainly melittin, and enzymes like phospholipases (Carpena et al., 2020). The results of this study were in line with other studies and demonstrated that, in comparison to Verapamil's negative effects, BV treatment (150 mg/kg/BW) led to improvements in the parenchymatous structures, glomerulus, and tubules of the kidney. Additionally, neither the proximal nor the distal convoluted tubules' ultrastructures were altered in the BV-treated group. When verapamil was combined with the BV group, the ultrastructure of the proximal and distal convoluted tubules improved. With its anti-fibrotic properties associated with regulating inflammatory responses and deactivating many cytokines and growth factors, BV may offer therapeutic potential for managing the symptoms of renal fibrosis. This is because it interacts with many growth factor-mediated pro-fibrotic genes (Seleem, 2016). Furthermore, it is widely known that BV protects against renal tubular damage (Kim et al., 2020).

Conclusion

The treatment of rats with arthritis by BV or WV revealed an improvement in nephrotoxicity severity caused by CFA-induced arthritis at the end of the study in comparison with arthritic non-treated rats (control positive group), but the BV was more effective in reducing nephrotoxicity induced by CFA due to BV and WV possesses a wide variety of bioactive compounds. Also, this study demonstrated the curative effects of BV against arthritis nephrotoxicity, which could inhibit the progression and development of nephrotoxicity in the rat model of arthritis. Therefore, additional research on the impact of BV and WV venoms on ultra-structural

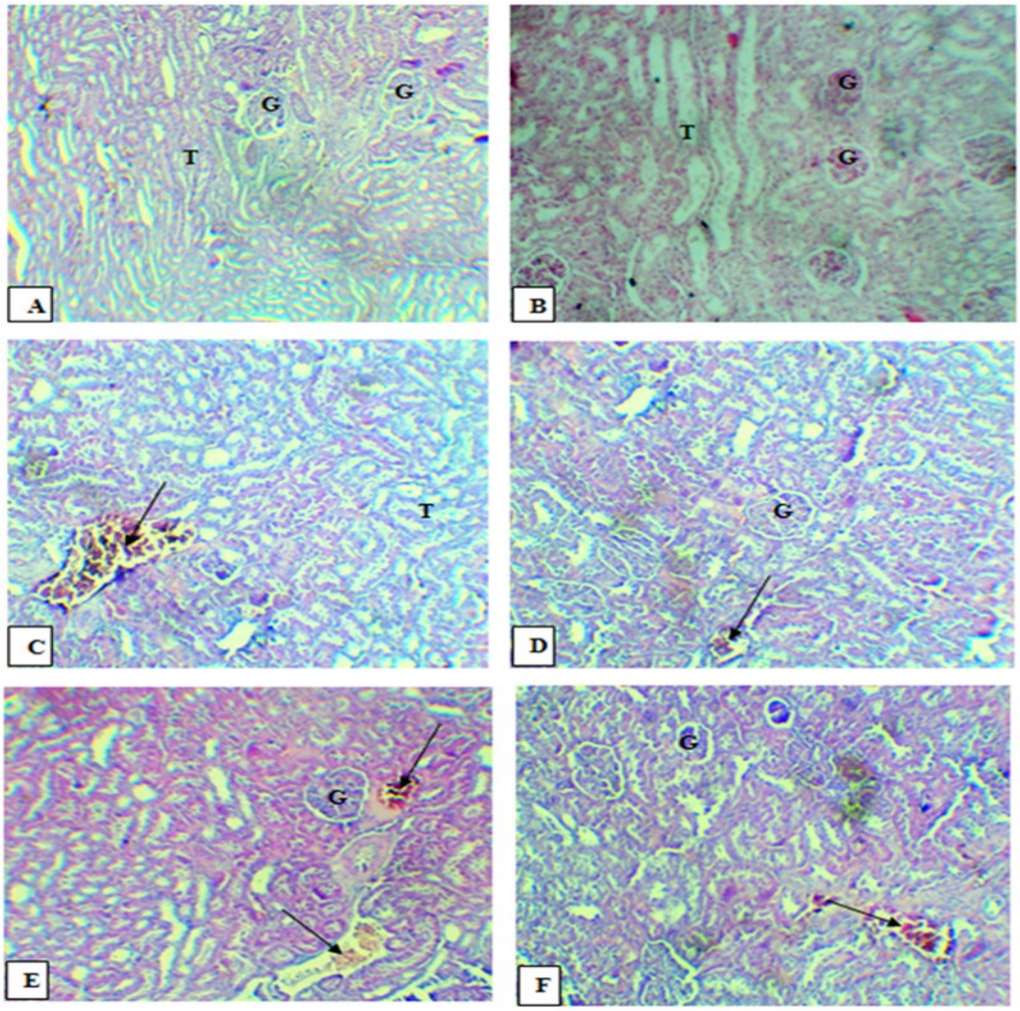

Fig. 5. *Sections of the kidney from the BV-treated group show normal glomerulus (G) and tubules (T) in most sections (A&B) although the presence of degenerative alterations and congestion (⟶) in some sections (C, D, E, F). H&E (100X).*

alterations in the kidney and measurements of kidney function biomarkers.

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Conflict of interest

The authors declare that they have no conflict of interest.

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