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The Effect of Different Concentrations of Bee Venom on Kidney Tissue of Albino Male Rats *Rattus*

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Abstract

In this study, 60 males of albino rats (Rattus rattus) were tested to study the effect of BV on kidney tissue at two doses of 10 and 40 µg/kg and two times after 10 and 15 days. The sample included 40 rats divided into two groups; 20 rats for each group. These groups were injected intraperitoneally according to the mentioned periods and were considered as a control group. The results exhibited renal tubule dilation and slight atrophy of glomerular tuft in the kidneys of rats treated with 10 µg/kg for 10 days compared to kidneys in the control treatment. Furthermore, 10 µg/kg treatment for 15 days has caused renal tubule dilation and degeneration with cytoplasmic vacuolation. Our findings also showed a slight atrophy of the glomerular tuft as well as a reduction in the number of glomeruli in this subcapsular area. The results also indicate regeneration of kidney tubules in rats given 40 µg/kg for 10 days. Meanwhile, sections of the kidney for rats that were given 40 µg/kg for 15 days exhibited a slight atrophy of glomerular tuft hypertrophied tubular epithelial cells.

Keywords: *Bee venom, Kidney Tissues, Rattus Rattus*

1. Introduction

Bee venom is a complex mixture containing simple organic molecules, proteins, peptides, and other bioactive elements (De Lima and Brochetto-Braga, 2003)[14]. Antibacterial, anti-inflammatory, and analgesic effects, as well as the augmentation of immunological response and radiation protection, are all documented physiological activations of BV. (Kim, 1992)[25]. It is an effective and complex combination of different peptides that include: Melittin, apamin, adolapamin, protease inhibitors, peptide 401 and mast cell degranulating peptide (Saeed and Eltahir, 2017)[39]. Bellik (2015)[6] showed the structure of BV and described adoption as an essential polypeptide that consists of 103 amino acids and comprises approximately 1% of BV dry weight, and this polypeptide has analgesic and anti-inflammatory properties, also can inhibit prostaglandin synthesis by the inhibition of cyclooxygenase activity and have the ability for the inhibition of the Adolapin, a lipoxygenase from human platelets, has been showed to possess an analgesic effect. MAST Cell Degranulating Peptides (MCD), was the peptide found in bee venom consisting of 22 amino acids. This peptide is described as bicyclic

because it contained 2 desulphated bridges between cys[3,15] and cys[5,19] and constitutes about 2-3% of the dry weight of bee venom. The MCD peptide sequence was as the following: lle-Lys-Cys-Asn-Cys-Lys-Arg-Hwas-Val-IIe-Lys-Pro-Hwas-IIe-Cys-Arg-Lys-IIe-Cys-Gly-Lys-Asn, the activities of MCD peptide has been combined with allergies, its release histamine at very low concentration (Son *et al*.,2007)[40]. Banks *et al*. (1986)[5] showed the activity of MCD peptide as a potent anti-inflammatory in various animal models (at modest dosages of 0.1 mg/kg).

BV The venom comprises 88 percent water, as well as glucose, fructose, and phospholipids. were related to those found in bee's blood (Bogdanov, 2012)[7] and Histamine, dopamine, norepinephrine, and serotonin are active amines, with additional components that, to some extent, have a broad range of pharmacological qualities (Orsolic,2012)[30], and histamine, dopamine, and norepinephrine are non-peptides, as well as protease inhibitors, include hyaluronidase, lysophospholipase, -D-glucosidase and phospholipase A2 (PLA2), (Hider,1988)[19]. Recent research has found that BV raises mitochondrial membrane potential and increases reactive oxygen species (ROS) and cytoplasmic Ca 2+, which increases levels of caspase-3, PARP, FAS p53, p21, and Bax, while lowering Bcl-2 levels. The ability of BV to boost Caspases 8 and 9 are activated by increasing caspase-3 activity is responsible for its effects on DNA fragmentation (Hong *et al*., 2005[10]; Tu *et al*., 2008[42]; Ip *et al*., 2012[21]). PLA2 and melittin work together to break down the membranes of vulnerable cells, boosting their cytotoxic effect. (Damianoglou *et al*., 2010[13]; Lee *et al*., 2011[27]). Hepatocytes are activated to apoptosis by TGF-1 which is protected by an appropriate melittin dose, which inhibits the activation of the Bcl-2 protein family, as well as caspases. andpoly (adenosine diphosphate-ribose) polymerase (PARP) cleavage. Melittin has been demonstrated to have a variety of effects on diverse cell types, including antiviral, antibacterial, and anti-inflammatory activities. Melittin can also cause cell cycle detention, cell growth suppression, and death in a variety of tumor cells. (Chartsiam *et al*, 2018[11]). Phospholipid packing is significantly disrupted when multiple Peptides of melittin accumulate in the cell membrane, resulting in cell lysis (Raghuraman, 2007[36]). However, several studies have found that melittin does not damage leukocyte cell membranes at doses below 2 μM (Putz *et al*., 2006[35]).

2. Materials And Methods

2.1 Experimental Animals:

The animals were acquired from the University of Al Qadisiya, animal house of Veterinary Medicine College. The animals were aired and kept at a temperature of 25 degrees Celsius and a humidity of 50 percent. The rats were given sterile food pellets to eat and were given tap water to drink. They were given a two-week acclimatization period prior to the start of the investigation. The rats weighed 200-250 grams and ranged in age from 8 to 12 weeks. The total animals were 60 males of albino rats (Rattus Rattus) 40 rats of which were divided into two groups 20 rats for each group. These two groups were treated with BV at two doses 10 and 40 µg/kg and two times 10 and 15 days. These groups were injected as intraperitoneal injection according to mentioned periods, in addition to 20 rats considered as control were injected with D.W. All animals in the study were anesthetized, slaughtered, and dissected for histological alterations in the kidneys.

2.2. Bee Venom:

BV was purchased from China's CN Lab Company. There was 17% melittin in it.100 mg of BV were dissolved in 100 mL of sterile distilled water and kept at 4°C until used. The solution was diluted to prepare the dosage required,10 and 40 g/kg according to the manufacturer's instructions (Abdu and Alahmari, 2013)[1] with few changes.

2.3. Histological Study:

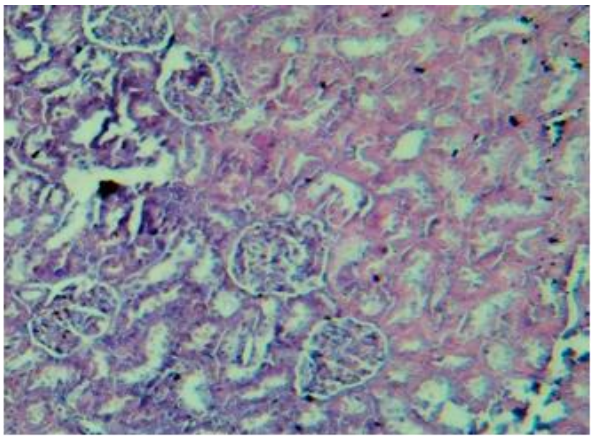
Typical histological processing is carried out for the kidney, to investigate histological alterations that may occur in the experimental group vs the negative control group. Small pieces of tissues kept in 10% formalin, dehydrated in different concentrations of alcohol, 5 micron-thick slices were obtained via embedding in paraffin blocks., and stained by Haematoxylin-Eosin (H&E) staining technique (Bancroft and Steven,1982)[4] For scanning electron microscopy (SEM), some specimens were fixed in Trump fixative {2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M Sorenson buffer (Sodium phosphate buffer at pH = 7.5)}. The fixed specimens were then washed in 0.1 M phosphate

buffer (pH=7.5) and subsequently dehydrated in different grades of alcohol. The dehydrated specimens were dried at a critical-drying point with liquid CO_2 and mounted on stubs with double conductive carbon tape and coated with 25 nm gold. The photographs were taken with a JSM-840EM (JOEL) at 12 kv Singh *et al*. (2022).

3. Result And Discussion

Hematoxylin and eosin-stained slices were examined histologically of untreated albino rat male's kidney (control) showed normal all architecture of tissue figure (1), while figure (2) showed a presence of renal tubules dilation and a slight atrophy of glomerular tuft in kidneys of animals treated with 10 µg/kg for 10 days as in compared with kidneys in the control treatment. A renal tubule dilation and degeneration with cytoplasmic vacuolation were shown in rats given a dose of 10 g/kg for 15 days figure (3).

Figure (4) exhibited slight atrophy of the glomerular tuft as well as a reduction in the number of glomeruli in this subcapsular area, and regeneration of kidney tubules, in rats who were given 40 µg/kg for 10 days. Sections of the kidney of rats who were given 40 µg/kg for 15 days, exhibited slight atrophy of glomerular tuft hypertrophied tubular epithelial cells as shown in Figure (5).



Figure(1): Cross section for kidney of control animal shows normal all architectureof tissue (H&E,40X).

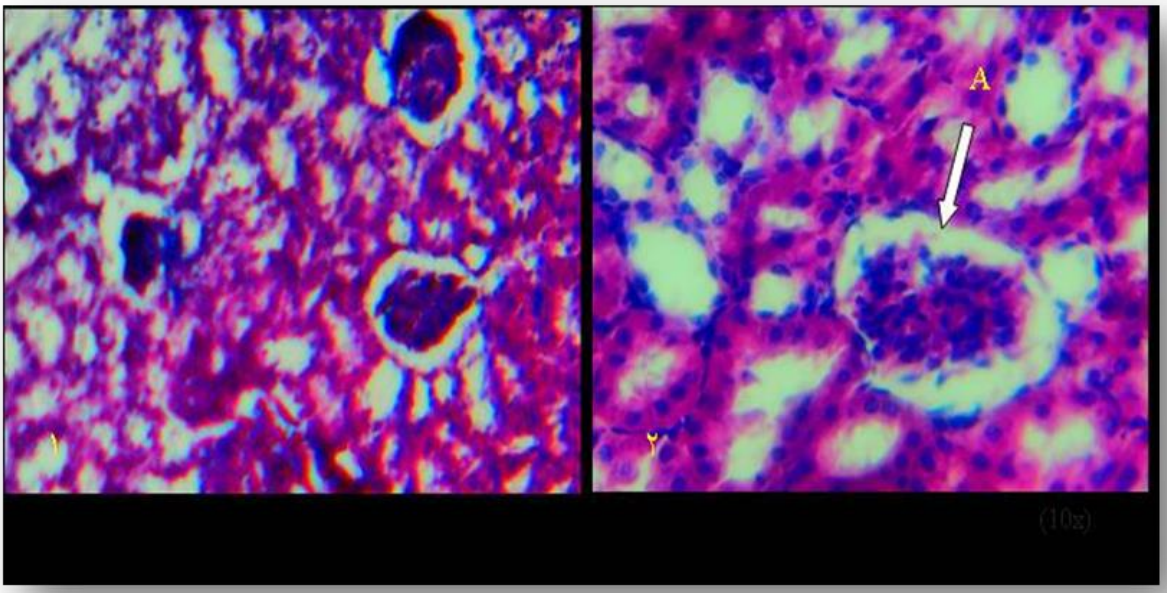
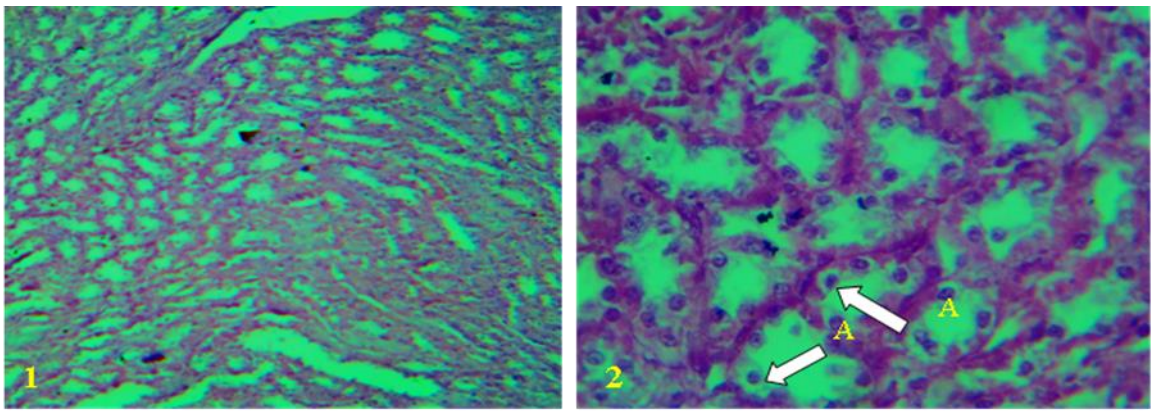


Figure (2): cross section in kidney of animals treated with 10µg/kg of BV for 10 days (1) Exhibited a renal tubules dilation (10x). (2) a slight atrophy of glomerular tuft(A) (H&E,40X).



Figure(3): cross section in kidney of animal treated with 10 µg/kg for 15 dayes (1) Exhibited renal tubules dilation (10x).(2) Degeneration with cytoplasmic vacuolation (A) (H&E,40X).

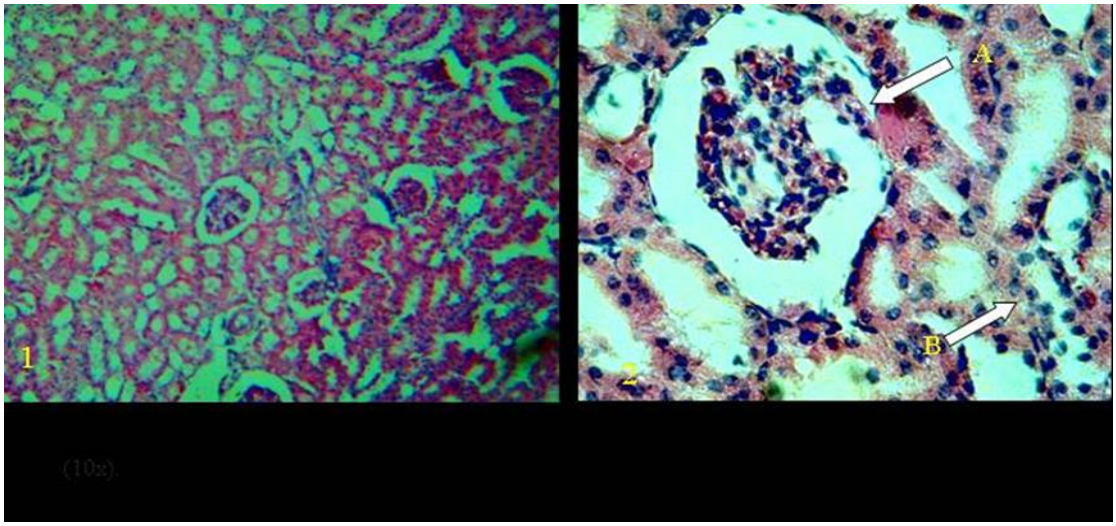
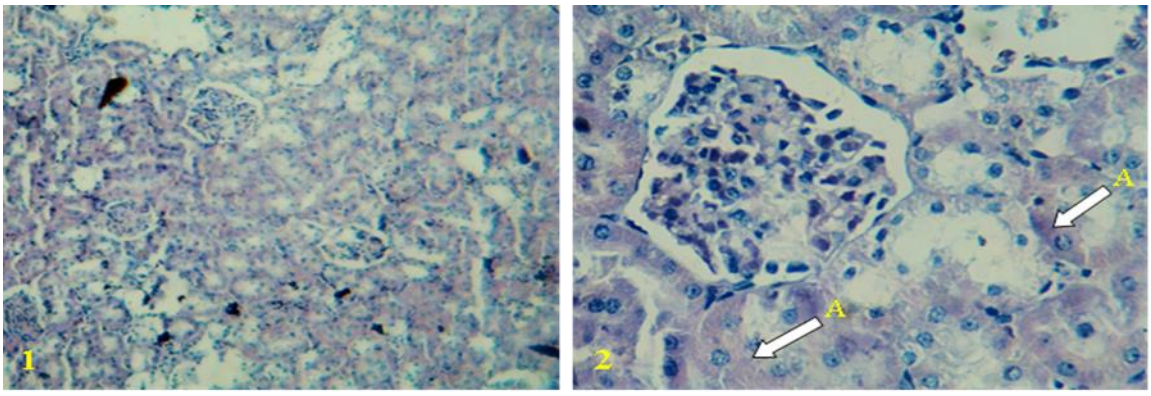


Figure. (4): cross section in kidney of animals treated with 40µg/kg for 10 days (1) Exhibited slight atrophy of glomerular tuft (10x). (2) The number of glomeruli in this subcapsular area is reduced (A), Regeneration of kidney tubules(B) (H&E,40X).



Figure(5): cross section in kidney of animal treated with 40 µg/kg for 15 dayes (1) Exhibited slight atrophy of glomerular tuft (10x).(2) Hypertrophied tubular epithelial cells. (H&E,40X).

Bee venom is made up of a combination of peptides, proteins, and low-molecular-weight components with proteins and peptides accounting for the majority of the venom. Two of the most important enzymes are phospholipase A2 and hyaluronidase. Melittin, apamin, phospholipids, some histamine and myoglobin, epinephrine, norepinephrine, aminobutyric acid, alpha-amino acids, glucose, fructose, complex ethers, phosphorus, and magnesia are all present in bee poison (Grisotto *et al*.,2006[17]; Arruda *et al*., 2007[3]; Oliveira *et al*., 2007[29]; Bogdanov *et al*., 2016[7]). Melittin is the most important component, accounting for nearly half of the dry weight (Park *et al*., 2011[33]) Biologically active amines, nonpeptide components, and enzymes, including hyaluronidase acid, phosphomonoesterase, and D glucosidase, are all found in BV (Han *et al*., 2013[13]).

The anti-inflammatory chemical melatonin, which is a hundred times more powerful than cortisone, is responsible for the beneficial effects of bee stings. Unfortunately, certain elements in bee venom can cause an allergic reaction response, which can be fatal in a hypersensitive person. Hemolysis, renal damage, hepatotoxicity, and myocardial infarction have all been linked to several stings (Galera *et al*., 2009[16]).

The toxicity of bee stings has been studied using animal models, Bee stings generate hemoconcentration, which could explain the venom's considerable edema. Different cytokines, such as interleukin (IL)-1, IL-6, tumor necrosis factor-, and others, increase in response to bee stings. A mouse model employing the subcutaneous method showed rapid elevations in blood alanine

aminotransferase and aspartate aminotransferase transaminases, creatinine, urea nitrogen, uric acid, sodium and chloride electrolytes, and creatine kinase, indicating injury to the liver, kidneys, and skeletal muscle. (Daher *et al*., 2003[12]; Bresolin, *et al*., 2002[10]). A huge injection of bee venom can induce adult respiratory distress syndrome (ARDS), pancreatitis, liver injury, heart damage, skin necrosis, shock, hypertension, hemorrhage, thrombocytopenia, hemolysis, and rhabdomyolysis are just a few of the disorders that might arise (Gabriel *et al*., 2004[15]).

Acute kidney injury (AKI) is defined as a period of compromised renal function followed by renal failure, resulting in the buildup of electrolyte imbalance and nitrogen products. A direct toxic effect on renal function has been described in cases of bee venom inoculation. On the other hand, the exact process by means of which kidney injury develops is unknown. Complications include shock, rhabdomyolysis, hemolysis, and direct tubular nephrotoxicity are thought to always have a role in this feature (Kim *et al*., 2019[23]; Wehbe *et al*., 2019[43]). The most prevalent Acute tubular necrosis, which can be induced by both ischemia and venom component nephrotoxicity, is a biopsy finding in these cases. Reis et al (1998)[38] found no alterations. AKI caused by bee venom toxins was studied in an experimental setting, found poisons in the glomeruli, interstitium, and renal arteries. Some toxins found in bee venom have been seen in clinical and experimental research to cause direct damage to renal tubules.

Some bee venom toxins have been shown in clinical and experimental studies to cause direct damage to renal tubules (Reis *et al*., 1997[37]; Bersolin *et al*., 2002[10]). Because of the substantial reabsorption of poisonous chemicals in this region, the renal tubules' proximal portion has been discovered to be more vulnerable to the venomous effects of bees, which is connected to high metabolic activity, energy consumption, and enzymatic susceptibility (Reis *et al*., 1998[38]). Bee stings have caused systemic allergic reactions in up to 3.4 percent of children and 7.5 percent of adults. These allergic symptoms might be modest, limited to the skin, or moderate to severe, with life-threatening anaphylaxis as a possibility. As a consequence, before employing a bee venom treatment, an allergy test should be done (Kim *et al*., 2013[26]; Sturm *et al*., 2018[41]).

Apitoxin induces low-molecular-weight chemicals that can cause hazardous or allergic reactions. compounds. In the short term, these drugs may cause acute discomfort, local inflammation, itching, and irritation, which will diminish in a few hours (Bellik, 2015[6]; Al-Ameri and Alhasan, 2020[2]). Melittin is the most common cause of allergic reactions. According to Moreno and Giralt, (2015)[38] when mastocytes interact with lg E, a cascade of mediators like enzymes, leukotrienes, platelet activation factors, histamines, peptides, and other chemical compounds are released. The liver cell damage was caused by a reversible prothrombotic condition generated by a bee sting (Park *et al*., 2010[31]).

Park et al. (2012)[31] demonstrated that melittin (a significant component of BV) reduced the liver's apoptosis and inflammatory response, which protected mice from D-galactosamine/LPS-induced liver failure. In several models of induced hepatic damage, investigations have BV has been demonstrated to reduce blood aminotransferase enzymes, and pro-inflammatory cytokine production is inhibited (Kim et al., 2014[22]). Furthermore, Park et al. (2010)[31] found that component of BV is PLA2 (phospholipase A2)., which regulates Tregs (CD4 + CD25 + Foxp3 + T cells) and IL-10 (interleukin-10) in mice with acetaminophen-induced hepatotoxicity. In acetaminophen-injected mice, Kim et al. (2003)[24] found that PLA2 increased the synthesis of anti-inflammatory cytokines, which may protect against hepatic impairment.

4. Conclusion

Our findings demonstrated a catastrophic side effect of injecting different doses of bee venom into the kidneys and livers of rats. This might be linked to the allergic effect of the bee venom component on tissue. As a result, in some cases, we recommend care while using bee venom as a therapy. Finally, histological examinations of hematoxylin and eosin-stained liver and kidney sections indicated hepatic and renal problems when bee venom was delivered to the rats' group in various amounts. Multiple mechanisms, including intravascular hemolysis, rhabdomyolysis, hypotension, and venom

component direct toxicity to renal tubules, can cause acute kidney damage (AKI). Arterial hypotension is a key factor in this kind of acute kidney damage, which leads to an ischemic renal lesion.

Acknowledgments
None

Ethical Clearance
The study was approved the University of Al Qadisiya, Veterinary Medicine College.

Conflict of interest:
The authors declare no conflict of interest.

References:
Abdu, F., & Alahmari, A. (2013). Anti-inflammatory effect of Melittin on Mice Jejunum. *Global Advanced Research Journal of Environmental Science and Toxicology*, 2(3), 68-76.

Al-Ameri, D. T., & Alhasan, A. S. (2020). Study of the genetic diversity of Iraqi honeybee (Apis mellifera L.) populations utilizing the mt-DNA COI region. *Int. J. Agricult. Stat. Sci*, 16, 1775-1778. https://connectjournals.com/03899.2020.16.1775

Arruda, V. M., Alves Jr, V. V., Moraes, M., Chaud Netto, J., & Suárez, Y. R. (2007). Análise morfológica da glândula de veneno de Apis mellifera L. (Hymenoptera: Apidae) em populações de Mato Grosso do Sul. *Neotropical Entomology*, 36, 203-209. https://doi.org/10.1590/S1519-566X2007000200006

Bancroft, D.J. and Stevens, A. (1982) Theory and Practice of HistologicalTechniques.2nd Edition. Chrchill Livingstone. *Medical Division of Longman Group Limitted*.

Banks, B. E., & Shipolini, R. A. (1986). Chemistry and pharmacology of honey-bee venom. *Venoms of the Hymenoptera: Biochemical, pharmacological and behavioural aspects*, 329-416.

Bellik, Y. (2015). Bee venom: its potential use in alternative medicine. *Anti-infective agents*, *13*(1), 3-16.

Bogdanov, S. (2016) Bee venom: composition, health, medicine: a review. Muehlethurnen: Bee Product Science;. [cited 2017 Jan 31]. Available from: http://www.bee-hexagon.net/files/file/fileE/Health/VenomBookReview.pdf

Bogdanov, S. (2015). Bee venom: composition, health, medicine: a review. *Peptides*, *1*, 1-20.

Bresolin, N. L., Carvalho, F. C., Goes, J. C., Fernandes, V., & Barotto, A. M. (2002). Acute renal failure following massive attack by Africanized bee stings. *Pediatric nephrology*, *17*, 625-627. https://doi.org/10.1007/s00467-002-0888-0

Tipgomut, C., Wongprommoon, A., Takeo, E., Ittiudomrak, T., Puthong, S., & Chanchao, C. (2018). Melittin induced G1 cell cycle arrest and apoptosis in Chago-K1 human bronchogenic carcinoma cells and inhibited the differentiation of THP-1 cells into tumour-associated macrophages. *Asian Pacific journal of cancer prevention: APJCP*, *19*(12), 3427. https://doi.org/10.31557%2FAPJCP.2018.19.12.3427

Daher, E. D. F., Silva Junior, G. B. D., Bezerra, G. P., Pontes, L. B., Martins, A. M. C., & Guimarães, J. A. (2003). Acute renal failure after massive honeybee stings. *Revista do Instituto de Medicina Tropical de São Paulo*, *45*, 45-50. https://doi.org/10.1590/S0036-46652003000100010

Damianoglou, A., Rodger, A., Pridmore, C., R Dafforn, T., A Mosely, J., M Sanderson, J., & R Hicks, M. (2010). The synergistic action of melittin and phospholipase A2 with lipid membranes: development of linear dichroism for membrane-insertion kinetics. *Protein and peptide letters*, *17*(11), 1351-1362.

De Lima, P. R., & Brochetto-Braga, M. R. (2003). Hymenoptera venom review focusing on Apis mellifera. *Journal of Venomous Animals and Toxins including Tropical Diseases*, *9*, 149-162. https://doi.org/10.1590/S1678-91992003000200002

Gabriel D.P., Rodrigues A.G. Jr, Barsante R.C., dos Santos Silva V., Caramori J.T., Martim L.C., Barretti P.,and Balbi A.L., (2004). Severe acute renal failure after massive attack of Africanized bees. *Nephrology Dialysis Transplantation*. 19:2680

Galera, C., Soohun, N., Zankar, N., Caimmi, S., Gallen, C., & Demoly, P. (2009). 9 Severe Anaphylaxis to Bee Venom Immunotherapy: Efficacy of Pretreatment and Concurrent Treatment With Omalizumab. *Journal of investigational allergology & clinical immunology*, *19*(3), 225.

Grisotto, L. S., Mendes, G. E., Castro, I., Baptista, M. A., Alves, V. A., Yu, L., & Burdmann, E. A. (2006). Mechanisms of bee venom-induced acute renal failure. *Toxicon*, *48*(1), 44-54. https://doi.org/10.1016/j.toxicon.2006.04.016

Han S.M., Park K.K., Nicholls Y.M., Macfarlane N., and Duncan G. (2013) Effects of honeybee (Apis mellifera) venom on keratinocyte migration in vitro. *Pharma Magazine*,9:220–226.

Hider, R. C. (1988). Honeybee venom: a rich source of pharmacologically active peptides. *Endeavour*, *12*(2), 60-65. https://doi.org/10.1016/0160-9327(88)90082-8

Hong, S. J., Rim, G. S., Yang, H. I., Yin, C. S., Koh, H. G., Jang, M. H., ... & Chung, J. H. (2005). Bee venom induces apoptosis through caspase-3 activation in synovial fibroblasts of patients with rheumatoid arthritis. *Toxicon*, *46*(1), 39-45. https://doi.org/10.1016/j.toxicon.2005.03.015

Ip, S. W., Chu, Y. L., Yu, C. S., Chen, P. Y., Ho, H. C., Yang, J. S., ... & Chung, J. G. (2012). Bee venom induces apoptosis through intracellular Ca2+-modulated intrinsic death pathway in human bladder cancer cells. *International Journal of Urology*, *19*(1), 61-70. https://doi.org/10.1111/j.1442-2042.2011.02876.x

Kim, H., Keum, D. J., Kwak, J. W., Chung, H. S., & Bae, H. (2014). Bee venom phospholipase A2 protects against acetaminophen-induced acute liver injury by modulating regulatory T cells and IL-10 in mice. *PLoS One*, *9*(12), e114726. https://doi.org/10.1371/journal.pone.0114726

Kim, H., Park, S. Y., & Lee, G. (2019). Potential therapeutic applications of bee venom on skin disease and its mechanisms: A literature review. *Toxins*, *11*(7), 374. https://doi.org/10.3390/toxins11070374

Kim, H. W., Kwon, Y. B., Ham, T. W., Roh, D. H., Yoon, S. Y., Lee, H. J., ... & Lee, J. H. (2003). Acupoint stimulation using bee venom attenuates formalin-induced pain behavior and spinal cord fos expression in rats. *Journal of veterinary medical science*, *65*(3), 349-355. https://doi.org/10.1292/jvms.65.349

Kim M.H. (1992). The bee venom therapy and the bee sting therapy. *Korea Educational Communications*, Seoul: 67-141.

Kim, C.M.H. (2013) Apitherapy—Bee Venom Therapy. In Biotherapy-History, Principles and Practice. A Practical Guide to the Diagnosis and Treatment of Disease using Living Organisms; *Springer: Heidelberg*, Germany, pp. 77–112

Lee, W. R., Park, J. H., Kim, K. H., Park, Y. Y., Han, S. M., & Park, K. K. (2011). Protective effects of melittin on transforming growth factor-β1 injury to hepatocytes via anti-apoptotic mechanism. *Toxicology and applied pharmacology*, *256*(2), 209-215. https://doi.org/10.1016/j.taap.2011.08.012

Moreno, M., & Giralt, E. (2015). Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. *Toxins*, *7*(4), 1126-1150. https://doi.org/10.3390/toxins7041126

Oliveira, E. C., Pedroso, P. M., Meirelles, A. E., Pescador, C. A., Gouvêa, A. S., & Driemeier, D. (2007). Pathological findings in dogs after multiple Africanized bee stings. *Toxicon*, *49*(8), 1214-1218. https://doi.org/10.1016/j.toxicon.2007.01.020

Oršolić, N. (2012). Bee venom in cancer therapy. *Cancer and metastasis reviews*, *31*, 173-194. https://doi.org/10.1007/s10555-011-9339-3

Park, J. H., Kim, K. H., Kim, S. J., Lee, W. R., Lee, K. G., & Park, K. K. (2010). Bee venom protects hepatocytes from tumor necrosis factor-α and actinomycin D. *Archives of Pharmacal Research*, *33*, 215-223. https://doi.org/10.1007/s12272-010-0205-6

Park, J. H., Kim, K. H., Lee, W. R., Han, S. M., & Park, K. K. (2012). Protective effect of melittin on inflammation and apoptosis in acute liver failure. *Apoptosis*, *17*, 61-69. https://doi.org/10.1007/s10495-011-0659-0

Park, J. H., Kum, Y. S., Lee, T. I., Kim, S. J., Lee, W. R., Kim, B. I., ... & Park, K. K. (2011). Melittin attenuates liver injury in thioacetamide-treated mice through modulating inflammation and fibrogenesis. *Experimental Biology and Medicine*, *236*(11), 1306-1313. https://doi.org/10.1258/ebm.2011.011127

Park, S., Chun, H. J., Keum, B., Seo, Y. S., Kim, Y. S., Jeen, Y. T., ... & Ryu, H. S. (2010). Anaphylactic shock-induced ischemic proctocolitis following bee stings: first case report. *Endoscopy*, *42*(S 02), E153-E154. https://doi.org/10.1055/s-0029-1244101

Putz, T., Ramoner, R., Gander, H., Rahm, A., Bartsch, G., & Thurnher, M. (2006). Antitumor action and immune activation through cooperation of bee venom secretory phospholipase A2 and phosphatidylinositol-(3, 4)-bisphosphate. *Cancer Immunology, Immunotherapy*, *55*, 1374-1383. https://doi.org/10.1007/s00262-006-0143-9

Raghuraman, H., & Chattopadhyay, A. (2007). Melittin: a membrane-active peptide with diverse functions. *Bioscience reports*, *27*(4-5), 189-223. https://doi.org/10.1007/s10540-006-9030-z

dos Reis, M. A., Costa, R. S., Coimbra, T. M., Dantas, M., & Gomes, U. A. (2004). Renal changes induced by envenomation with Africanized bee venom in female Wistar rats. *Kidney and Blood Pressure Research*, *20*(4), 271-277. https://doi.org/10.1159/000174157

Saeed, W., & Khalil, E. (2017). Toxic effects and safety of bee venom protein [Melittin] in mice: Search for natural vaccine adjuvants. *IJNPR*, *3*(1), 111-114.

Son, D. J., Lee, J. W., Lee, Y. H., Song, H. S., Lee, C. K., & Hong, J. T. (2007). Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacology & therapeutics*, *115*(2), 246-270. https://doi.org/10.1016/j.pharmthera.2007.04.004

Sturm, G. J., Varga, E. M., Roberts, G., Mosbech, H., Bilò, M. B., Akdis, C. A., ... & Muraro, A. (2018). EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. *Allergy*, *73*(4), 744-764. https://doi.org/10.1111/all.13262

Tu, W. C., Wu, C. C., Hsieh, H. L., Chen, C. Y., & Hsu, S. L. (2008). Honeybee venom induces calcium-dependent but caspase-independent apoptotic cell death in human melanoma A2058 cells. *Toxicon*, *52*(2), 318-329. https://doi.org/10.1016/j.toxicon.2008.06.007

Wehbe, R., Frangieh, J., Rima, M., El Obeid, D., Sabatier, J. M., & Fajloun, Z. (2019). Bee venom: Overview of main compounds and bioactivities for therapeutic interests. *Molecules*, *24*(16), 2997. https://doi.org/10.3390/molecules24162997