Biochemistry Letters, 21 (1) 2025, pages 119-133

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Biochemical Evaluation of the Protective Role of Egyptian Cobra (Naja nubiae) Venom against Melamin Induced Kidney Toxicity in Albino Rats

Al-Shimaa M. Abas[1], Akaber T. Keshta[1] , Samah A. mohammed[1], Shimaa H.Watad[1].

[1]Biochemistry Department, Faculty of Science, Zagazig University, Egypt.

ARTICLE INFO

Received : 13/7/2025
Accepted : 10/8/2025
Accepted to Online publish: 10/8/2025

Keywords:
Nephrotoxicity ,Cobra venom, Melamine,IL-2,IL-10, INF-γ.

ABSTRACT

Background: Melamine is a common pollutant that threatens human and animal health. The elevated concentrations of these compounds cause adverse effects in humans and animals. **AIM:** This paper aims to evaluate the protective effect of Egyptian cobra snake venom against melamine-induced Nephrotoxicity in rats. **Methods**: The current study was conducted on six groups of adult albino rats as follow. group I (negative control):rats were I.P. injected with 2ml distilled water for all experiment period, group II(SV10µg/kg) :Rats were I.P. injected with 10 µg/kg snake venom, group III (SV20µg/kg):Rats were I.P. injected with 20 µg/kg Snake venom , group IV(melamine):Rats received 700mg/kg melamine ,group V(melamine+SV10µg/kg): Treated with 10 µg/kg snake venom, group VI(melamine+SV20µg/kg): Treated with 20 µg/kg snake venom. **Results:** Treatment with snake venom enhanced Nephrotoxicity induced by melamine by ameliorating kidney function and increasing apoptotic marker Caspase-3 ,and decrease level of cystatin C protein , also decrease inflammatory level marker IL-2, and expression level IL-10, INF-γ. **Conclusion**: Treatment with snake venom improved nephrotoxicity induced by melamine in albino rats.

I.

I.Introduction:

Nephrotoxicity describes the detrimental impact certain chemical agents can have on kidney function, as noted in previous studies. The kidneys filter out toxins and excrete waste products via urine. Substances that are nephrotoxic may cause acute kidney injury (AKI) either directly, by metabolizing into harmful compounds within the renal tubules, or indirectly, through hepatic metabolism that produces toxic intermediates. The principal mechanisms underlying kidney damage include oxidative stress, inflammation, apoptosis, and necrosis. Furthermore, nephrotoxic agents, drugs, and poor renal perfusion can contribute to acute renal failure (ARF), thereby exacerbating nephrotoxicity.

Melamine has attracted global attention

*** Corresponding author: Al-Shimaa Mahmoud Abas, Biochemistry Department, Faculty of Science, Zagazig University, Egypt. Email: dr_shmma@yahoo.com. Mobile:01009890366. orcid.org/0000-0002-3736-6554.**

due to its documented toxicity in food products, especially dairy. A notable example occurred when melamine-contaminated infant formula caused nephrotoxic effects in children. This incident resulted in urinary tract stones in infants and young children, affecting approximately 294,000 individuals—over 50,000 were hospitalized and six deaths occurred due to acute kidney failure. Similar renal failure and fatalities have been reported in pets consuming melamine-contaminated food. Additionally, melamine exposure has been linked to sperm abnormalities and DNA damage. Consequently, multiple strategies have been investigated to reduce or counteract melamine-induced toxicity.

Biologically active proteins and peptides make up the complex mixture that is snake venom. with distinct enzymatic and non-enzymatic properties that affect various physiological systems. Due to their pharmacological potential, snake venoms are increasingly studied for therapeutic applications. Venoms from the Naja genus, particularly . Naja nubiae, the Egyptian spitting cobra, is well-known for its anti-inflammatory, antipyretic, and analgesic properties. Some components in cobra venom modulate coagulation, acting as either pro-coagulant or anti-coagulant agents.

This study investigates the potential of cobra venom to alleviate nephrotoxic effects caused by melamine in albino rats, focusing on its influence on biochemical markers, inflammatory mediators, and histopathological changes in kidney tissues.

II.Material and Methods:

Chemicals:

Melamine was sourced from Sebra Chemical Co., located in the 10th of Ramadan City, Egypt. Venom from Naja nubiae cobras was gathered from Aswan, Egypt's Nubian region in March 2022. . Following the Mirtschin extraction technique, venom was obtained by encouraging the snakes to bite through a parafilm-covered collection container. The raw venom was lyophilized using a LABCONCO freeze-drying machine (USA), diluted 1:10 with sterile distilled water, and then stored at -20°C.

Animals:

Male albino rats (180–200 g) were acquired from Zagazig University's Experimental Animal Care Center. Prior to the experiment, Rats were kept in regular lab settings (25°C, 12-hour light/dark cycle) in the Faculty of Science animal house. All experimental procedures adhered to ethical protocols approved by the university's animal care committee (Protocol No.: ZU-IACUC/2/F/38/2022).

Induction of Nephrotoxicity:

Nephrotoxicity was induced by giving 700 mg/kg body weight of melamine orally every other day for 28 days. Warm distilled water was used to dissolve the melamine.

Lethal dosage of cobra venom median (LD_{50}) was determined in accordance with the method outlined by Meier and Theakston.

Experimental design:

48 rats were randomly assigned to six groups (n = 8 per group) following an acclimation period:
- Group I (Negative Control): Received 2 ml of distilled water daily via intraperitoneal (IP) injection.
- Group II (SV 10 µg/kg): Administered 10 µg/kg of cobra venom IP for six consecutive days.
- Group III (SV 20 µg/kg): Given 20 µg/kg of cobra venom IP for six days.
- Group IV (Melamine Only): Orally administered 700 mg/kg melamine daily for 28 days.
- Group V (Melamine + SV 10 µg/kg):

Treated with melamine as in Group IV, followed by 10 µg/kg of cobra venom IP for six days.
- Group VI (Melamine + SV 20 µg/kg): Treated with melamine for 28 days and subsequently injected with 20 µg/kg cobra venom for six days.

Blood collection:
Using light ether anesthesia, blood samples were drawn from the retro-orbital venous plexus at the conclusion of the experiment and after a 12-hour fast. Serum was isolated and kept at -20°C for biochemical analysis after samples were centrifuged for 20 minutes at 4000 rpm.

Tissue Preparation:
In order to create 10% (w/v) homogenates, kidney tissues were homogenized in ice-cold phosphate-buffered saline (pH 7.4). One portion of each sample was prepared for histological examination, while the other portion was reserved for molecular studies

Biochemical Analysis:
Kidney Function Test Urea and uric acid levels in the serum were determined using the enzymatic Berthelot method with kits from Diamond Diagnostics (Germany). Creatinine levels were quantified using a buffered kinetic Jaffé method without deproteinization, via Spinreact kits (Spain).

ELISA Assays:
Levels of IL-2, caspase-3, and cystatin C in kidney tissue homogenates were quantified using ELISA kits from MBS Biosciences.

Gene Expression Analysis (IL-10 and INF-γ):
Until the RNA was extracted, kidney tissues were kept at -80°C. The QIAamp RNeasy Mini Kit (Qiagen, Germany) was used to isolate total RNA. Metabion (Germany) provided the primer sequences. Using TransScript Green SYBR One-Step qRT-PCR SuperMix, real-time quantitative PCR was carried out. The 2^−ΔΔCt technique was used to examine relative gene expression.

Histopathological Examination:
Fixed kidney tissues were cut into sections (4–5 µm), embedded in paraffin wax, and stained with hematoxylin and eosin. A light microscope set to ×400 magnification was used for histological evaluations.

Results

Toxicity Assessment:
The median lethal dose (LD_{50}) for Naja nubiae venom was estimated to be 0.2 mg/kg body weight.

Effect on Kidney Function Markers:
Data in Table 1 and Figures 1 and 2 reveal a significant elevation in serum urea (47%, $P<0.01$), creatinine (43.7%, $P<0.001$), and uric acid (18%, $P<0.001$) in the melamine-treated group relative to controls. Cobra venom treatment significantly ameliorated these changes, particularly at the 10 µg/kg dose, demonstrating a restorative effect on renal function.

Inflammatory and Apoptotic Markers in Renal Tissue:
As shown in **Table 2 and Figure 3**, IL-2 and cystatin C levels in kidney tissues were markedly elevated in the melamine group—by 246% and 328.9%, respectively ($P<0.01$). In contrast, caspase-3 expression was reduced by 64% compared to control levels. Cobra venom administration significantly normalized all three markers, suggesting reduced inflammation and improved apoptotic regulation.

IL-10 and INF-γ Gene Expression:
Table 3 and Figure 4 demonstrate a 403% and 500% increase in IL-10 and INF-γ gene expression, respectively, in group melamine ($P<0.001$). These levels were notably reduced in venom-treated

groups, with the 10 µg/kg dose showing a stronger anti-inflammatory effect than the 20 µg/kg dose.

Histopathological Observations: Photomicrographs **(Figure 5)** showed normal renal architecture in control and venom-only groups, including intact glomeruli and tubules. Severe renal injury was evident in the melamine group, including glomerular atrophy, tubular degeneration, cytoplasmic vacuolization, and inflammatory infiltration. Treatment with cobra venom, especially at 10 µg/kg, led to partial to significant restoration of renal structure, indicating histological recovery.

.Discussion.

Nephrotoxicity refers to kidney impairment caused by various insults, including chemical agents, medications, or systemic conditions such as hypertension, obesity, liver dysfunction, septicemia, and diabetes mellitus [31]. It is distinct from renal pathology of non-chemical origin, though both can present with overlapping clinical features.

Previous research has established that melamine can cause multi-organ damage in humans and animals, with significant implications for the central nervous system, cardiovascular health, reproductive function, liver, and especially the kidneys [32]. In the present study, melamine exposure was associated with marked renal dysfunction, evidenced by elevated biochemical markers, inflammatory cytokines, and observable histological damage. These findings align with prior reports documenting the nephrotoxic potential of melamine and its capacity to induce urolithiasis, nephrolithiasis, and hydronephrosis [32].

Snake venoms are rich sources of biologically active molecules with potential pharmacological applications [33]. For instance, phospholipase A2 (PLA2) enzymes from *Cerastes* and *Macrovipera lebetina* species have demonstrated anticancer activity [34]. PLA2s also degrade phospholipid membranes and have been reported to exert antimicrobial effects [35]. Beyond antimicrobial and antitumor effects, components of snake venom possess analgesic and anti-inflammatory properties that have been studied for decades [36]. However, their therapeutic utility is limited by challenges in targeted delivery.

In the current investigation, melamine administration significantly elevated serum levels of urea, creatinine, and uric acid—classic indicators of impaired kidney filtration. These findings support earlier research suggesting that melamine disrupts podocyte function and glomerular integrity, leading to increased proteinuria and elevated waste product accumulation [16,37–41]. Treatment with cobra venom reversed many of these effects. Improved renal function in treated groups, especially those receiving the 10 µg/kg dose, supports previous observations that venom components can restore glomerular filtration and reduce protein leakage [42,43].

Our results also showed a significant increase in IL-2 levels following melamine exposure. IL-2 is a key pro-inflammatory cytokine involved in T-cell proliferation and immune activation via NF-κB pathway activation [57,59,60]. Elevated IL-2 levels indicate ongoing renal inflammation. Notably, groups treated with cobra venom showed normalization of IL-2 levels, suggesting that venom components may attenuate the inflammatory cascade—a finding consistent with prior work [42,43].

In terms of apoptosis, melamine significantly reduced caspase-3 expression. This result is consistent with studies showing that melamine-induced

ROS production, mitochondrial dysfunction, and reduced caspase activity lead to impaired apoptotic regulation in kidney tissue [46,49]. However, treatment with *Naja nubiae* venom restored caspase-3 levels, pointing to improved mitochondrial stability and regulated cell death pathways in the kidneys [51].

Another important biomarker, cystatin C, was significantly increased in the melamine group. This reflects glomerular filtration rate (GFR) impairment and early-stage kidney injury [52–55]. Cobra venom treatment normalized cystatin C levels, in agreement with studies reporting venom-induced enhancement in renal clearance and GFR [56].

Elevated gene expression of IL-10 and INF-γ in melamine-treated rats further confirmed the presence of active inflammation and cytokine dysregulation. These cytokines are known to promote IκB kinase (IKK) activation and NF-κB-mediated signaling, contributing to kidney injury [57,58,61–65]. The reduction of these markers in venom-treated groups indicates that cobra venom may exert a regulatory effect on pro-inflammatory signaling pathways, likely through inhibition of IKKs, as reported in previous literature [42].

Histological findings supported all biochemical and molecular data. Rats exposed to melamine exhibited severe renal tissue degeneration, including tubular necrosis, glomerular damage, and inflammatory cell infiltration. These changes are in line with earlier findings on melamine-induced kidney pathology [44,45]. Conversely, histological recovery was observed in venom-treated groups, particularly those given 10 µg/kg, showing regeneration of tubular structure and restoration of glomerular integrity—similar to observations by other authors investigating the therapeutic effects of *Naja nubiae* venom [43].

Final thoughts:

The study's conclusions indicate that *Naja nubiae* (Egyptian cobra) venom, particularly at a dose of 10 µg/kg, offers significant protection against melamine-induced nephrotoxicity in albino rats. Treatment with this dose effectively mitigated renal dysfunction, suppressed inflammatory responses, and improved apoptotic and anti-apoptotic biomarkers. Additionally, histological improvements in renal tissue further supported the therapeutic potential of the venom. Based on the evidence, the 10 µg/kg dose appears to be safer and more efficacious than the 20 µg/kg dose, making it a promising candidate for further investigation as a potential nephroprotective agent.

References

[1] Al-Naimi M. S., Rasheed H. A., Hussien N. R., Al-Kuraishy H. M. and Al-Gareeb A. I. (2019). "Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury." Journal of advanced pharmaceutical technology & research 10(3): 95.

[2] Popovi´c, D.; Koci´c, G.; Kati´c, V.; Jovi´c, Z.; Zarubica, A.; Veli˘ckovi´c, L.J.; Nikoli´c, V.; Jovi´c, A.; Kundali´c, B.; Raki´c, V. Protectiveeffects of anthocyanins from bilberry extract in rats exposed to nephrotoxic effects of carbon tetrachloride. Chem. Biol. Interact. 2019, 304, 61–72. [CrossRef] [PubMed]

[3] Uddin, M.J.; Dorotea, D.; Pak, E.S.; Ha, H. Fyn kinase: A potential therapeutic target in acute kidney injury. Biomol. Ther. 2020, 28, 213. [CrossRef] [PubMed]

[4] Safhi, M.M. Nephroprotective effect of Zingerone against CCl4-induced renal toxicity in Swiss albino mice: Molecular mechanism.

Oxidative Med. Cell. Longev. 2018, 2018, 2474831. [CrossRef]

[5] R.L.M. Dobson, S. Motlagh, M. Quijano, R.T. Cambron, T.R. Baker, A.M. Pullen, B.T. Regg, A.S. Bigalow-Kern, T. Vennard, A. Fix, R. Reimschuessel, G. Overmann, Y. Shan, G.P. Daston, Identification and Characterization of Toxicity of Contaminants in Pet Food Leading to an Outbreak of Renal Toxicity in Cats and Dogs, Toxicol. Sci. 106 (2008) 251-262. https://doi.org/10.1093/toxsci/kfn160.

[6] C.-Y. Chien, C.-F. Wu, C.-C. Liu, B.-H. Chen, S.-P. Huang, Y.-H. Chou, A.-W. Chang, H.-H. Lee, C.-H. Pan, W.-J. Wu, J.-T. Shen, M.-Y. Chang, C.-H. Huang, J. Shiea, T.-J. Hsieh, M.-T. Wu, High melamine migration in daily-use melamine-made tableware, J. Hazard. Mater. 188 (2011) 350-356. https://doi.org/10.1016/j.jhazmat.2011.01.128.

[7] V. Bhalla, P.C. Grimm, G.M. Chertow, A.C. Pao, Melamine nephrotoxicity: an emerging epidemic in an era of globalization, Kidney Int. 75 (2009) 774-779. https://doi.org/10.1038/ki.2009.16.

[8] Z. Wang, H. Luo, W. Tu, H. Yang, W.H. Wong, W.T. Wong, K.F. Yung, N. Zhou, J. Zhang, X. Li, W. Guo, D. Mu, F. Li, M. Mao, Y.L. Lau, Melamine-tainted milk product-associated urinarystones in children, Pediatr. Int. 53 (2011) 489-496. https://doi.org/10.1111/j.1442-200X.2010.03284.x

[9] B. Puschner, R.H. Poppenga, L.J. Lowenstine, M.S. Filigenzi, P.A. Pesavento, Assessment of melamine and cyanuric acid toxicity in cats, J. Vet. Diagn. Invest. 19 (2007) 616-24. https://doi.org/10.1177/104063870701900602.

[10] J.Y. Yhee, C.A. Brown, C.H. Yu, J.H. Kim, R. Poppenga, J.H. Sur, Retrospective study of melamine/cyanuric acid-induced renal failure in dogs in Korea between 2003 and 2004, Vet. Pathol .46 (2009) 348-54. https://doi.org/10.1354/vp.46-2-348.

[11] C.Y. Chu, K.O. Chu, J.Y.W. Chan, X.Z. Liu, C.S. Ho, C.K. Wong, C.M. Lau, T.L. Ting, T.F. Fok, K.P. Fung, C.C. Wang, Distribution of melamine in rat foetuses and neonates, Toxicol. Lett. 199 (2010) 398-402. https://doi.org/10.1016/j.toxlet.2010.10.004.

[12] Q.-x. Zhang, G.-y. Yang, J.-t. Li, W.-x. Li, B. Zhang, W. Zhu, Melamine induces sperm DNAdamage and abnormality, but not genetic toxicity, Regul. Toxicol. Pharmacol. 60(1) (2011) 144-150. https://doi.org/10.1016/j.yrtph.2011.03.004.

[13] A.F. Hamouda, A.A.E. Amin, S.S. Ibrahim, M.A. Mahmoud, Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats, World Vet. J. 9 (2019) 146-157. https://dx.doi.org/10.36380/scil.2019.wvj19.

[14] Y.M. Abd-Elhakim, W.A.M. Mohamed, K.M. El.Bohi, H.A. Ali, F.A. Mahmoud, T.M. Saber, Prevention of melamine-induced hepatorenal impairment by an ethanolic extract of Moringa oleifera: Changes in KIM-1, TIMP-1, oxidative stress, apoptosis, and inflammation-related genes, Gene 764 (2021) 145083. https://doi.org/10.1016/j.gene.2020.145083.

[15] Mohamed AT, Garcia SA, Stockand JD. Snake Venoms in Drug Discovery: Valuable Therapeutic Tools for Life Saving. Toxins (Basel). 2019; 11(10): 564.

[16] Theakston RDG, Warrel DA, Griffith E. "Report of a WHO

workshop on the standardization and control of antivenoms,". ; 2003.

[17] Abdel-Daim M, Altawil H, Abdelrahman M, Elnaggar MS, El-Khayat ZA. Analgesic, Antipyretic and AntiInflammatory Activities of the Egyptian Spitting Cobra, Naja Nubiae Venom. Journal of Forensic Toxicology and Pharmacology. 2015; 4: 1.

[18] Kim H, Keum DJ, Kwak JW, Chung HS, Bae H. Bee Venom Phospholipase A2 Protects against AcetaminophenInduced Acute Liver Injury by Modulating Regulatory T Cells and IL-10 in Mice. PLoS ONE. 2014; 9(12): e114726

[19] Carlile SR, Shiels J, Kerrigan L, Delaney R, Megaw J, Gilmore BF, et al. Sea snake cathelicidin (Hc-cath) exerts a protective effect in mouse models of lung inflammation and infection. Scientific Reports. 2019; 9: 6071.

[20]Early,R.,J,Yu,H.,Mu,X.P.,Xu,H., Guo,L.,Kong,Q.,Zhou,J.,He,B.,Yang, X.,Huang,H.,Hu,E.,Jiang, Y.,2013. Repeat oral dose toxicity studies of melamine in rats and monkeys .Arch. Toxical 87,517-527.

[**21] Meier, J., and Theakston, R.D.G. (1986):** Approximate LD50 determination of snake venom using eight to ten experimental animals. Toxican, 24 (4), 395-401.

[22]Waynforth,H.,1980. Rats as laboratory animals. Surgery, Experimental. Academic Press,NewYork,NY,USA.

[23] Fawcett JK, Soctt JE. A rapid and precise method for the determination of urea. Journal of Clinical Pathology. 1960; 13: 156-159.

[24] Schirmeister J. Determination of creatinine in serum. Deutsche Medizinische Wochenschrift. 1964; 89: 1940.

[25]Hollander, G. A.; Zuklys, S.; Morel, C.; Mizoguchi, E.; Mobisson, K.; Simpson, S.; Terhorst, C.; Wishart, W.; Golan, D. E.; Bhan, A. K.; Burakoff, S. J. (1998). Monoallelic expression of the interleukin-2 locus. Science 279: 2118-2121,

[26]Pirttila, T. J.; Manninen, A.; Jutila, L.; Nissinen, J.; Kalviainen, R.; Vapalahti, M.; Immonen, A.; Paljarvi, L.; Karkola, K.; Alafuzoff, I.; Mervaala, E.; Pitkanen, A.: Cystatin C expression is associated with granule cell dispersion in epilepsy. Ann. Neurol. 58: 211-223, 2005.

[27] Yuan, J.S.; Reed, A.; Chen, F. and Stewart, C.N. (2006): Statistical analysis of real-time PCR data. BMC Bioinformatics, 7:85.

[28] Bancroft JD, Stevens A (2016). Theory and Practice of histological techniques. Churchill Livingstone, London. Edition: 7th. P: 120:131.

[31] Jia Y., et al. (2022). "Chronic kidney damage pathology score for systematic assessment of the non-neoplastic kidney tissue and prediction of post-operative renal function outcomes." Human Pathology 124: 76-84.

[32] Parsutkar Y. (2022). "Overview of Melamine: Source, Contamination, Toxicology and Health Aspects." Pract Clin Invest 4(1): 17-25.

[33] Suvilesh K., et al. (2017). "Snake venom proteinases as toxins and tools." Proteases in Physiology and Pathology: 485-515.

[34] Vyas V. K., et al. (2013). "Therapeutic potential of snake venom in cancer therapy: current perspectives." Asian Pacific journal of tropical biomedicine 3(2): 156-162.

[**35] Bocian A. and Hus K. K. (2020).** "Antibacterial properties of snake venom components." Chemical Papers 74: 407-419.

[36] Mohamed Abd El-Aziz T., et al. (2019). "Snake venoms in drug discovery: Valuable therapeutic tools for life saving." Toxins 11(10): 564.

[37] I.-C. Lee, S.-H. Kim, H.-S. Baek, S.-S. Kang, J.-C. Kim, Synergistic effect of melamine incombination with cyanuric acid on urinary tract toxicity in rats, J. Biomed. Res. 14 (2013) 145-153

[38] T. Yasui, T. Kobayashi, A. Okada, S. Hamamoto, M. Hirose, K. Mizuno, Y. Kubota, Y. Umemoto, N. Kawai, K. Tozawa, B. Gao, K. Kohri, Long-term follow-up of nephrotoxicity in ratsadministered both melamine and cyanuric acid, BMC Res. Notes 7 (2014) 87.https://doi.org/10.1186/1756-0500-7-87.

[39] S. Peerakietkhajorn, N. Huipao, S. Hiranyachattada, Effects of Melamine and Cyanuric Acidon Renal Function and Structure in Rats, Sains Malays. 48 (2019) 1721-1728.

[40] D. Kerjaschki, "Caught flat-footed: podocyte damage and the molecular bases of focal glomerulosclerosis," Journal of Clinical Investigation, vol. 108, no. 11, pp. 1583–1587, 2001.

[41] K. Zandi-Nejad, A. A. Eddy, R. J. Glassock, and B. M. Brenner, "Why is proteinuria an ominous biomarker of progressive kidney disease?" Kidney International, Supplement, vol. 66, no. 92, pp. S76–S89, 2004.

[42] M.N. Al-Seeni, H.A. El Rabey, S.M. Al-Solamy, The protective role of bee honey against thetoxic effect of melamine in the male rat kidney, Toxicol. Ind. Health 31(2015) 485-93.https://doi.org/10.1177/0748233714551765.

[43]Ayman.s.mohamed,hanan farag Elsayed,Nagat saleh Hussein and Abdeljalil Mohamed Alshawoush 2021: Naja nubiae venom ameliorate hepatorenal toxicity induced byGentamicin in rats.GSC Adavanced research and Reviews.09(03),030_038.

[44] Yang, W.; Liang, C.; Zhang, X.; Tian, X.; Ren, C.; Chen, S.; Wang, J.; Zhang, J. Melamine Induced Changes in Histopathology of the Main Organs and Transcriptional Levels of MAPK Signaling Genes in Kidneys of Female Mice. Environ. Toxicol. 2022, 37, 585–592. [CrossRef].

[45] A. Melekoğlu, H. Ekici, A. Esra, S. Karahan, Evaluation of melamine and cyanuric acidcy totoxicity: an in vitro study on L929 fibroblasts and CHO cell line, Ankara Üniversitesity Veteriner Fakültesi Dergisi 67 (2020) 399-406.
https://doi.org/10.33988/auvfd.664059.

[46] A. Albihn, J. Lovén, J. Ohlsson, L.M. Osorio, M. Henriksson, c-Myc-dependent etoposideinduced apoptosis involves activation of Bax and caspases, and PKCdelta signaling, J. Cell. Biochem. 98 (2006) 1597-1614. https://doi.org/10.1002/jcb.20816.

[47] A.J. Yiu, C.-L. Ibeh, S.K. Roy, B.C. Bandyopadhyay, Melamine induces Ca2+-sensingreceptor activation and elicits apoptosis in proximal tubular cells, Am. J. Physiol. Cell Physiol. 313 (2017) C27-C41. https://doi.org/10.1152/ajpcell.00225.2016.

[48] D.R. McIlwain, T. Berger, T.W. Mak, (2015): Caspase functions in cell death and disease [published correction appears in Cold Spring Harb Perspect Biol. Apr;7(4). pii: a026716. doi: 10.1101/cshperspect.a026716], Cold Spring Harb.Perspect.bilo.5(4)(2013),a008656 (published2013Apr1.doi:10.1101/cshperspect.a008656).

[49] A. Albihn, J. Lovén, J. Ohlsson, L.M. Osorio, M. Henriksson, c-Myc-dependent etoposide induced apoptosis involves activation of Bax and caspases, and PKCdelta signaling, J. Cell. Biochem. 98 (2006) 1597-1614. https://doi.org/10.1002/jcb.20816.

[50]Togashi Y, Imura N, Miyamoto Y (2013) Urinary cystatin C as arenal biomarker and its immunohistochemical localizationin

anti-GBM glomerulonephritis rats. Exp Toxicol Pathol65:1137–1143.

[51]Ghys L, Paepe D, Smets P, Lefebvre H, Delanghe J, Daminet S(2014) Cystatin C: a new renal marker and its potential usein small animal medicine. J Vet Intern Med 28:1152–1164.

[52] G. van Pottelbergh, L. van Heden, C. Mathe¨ı, and J. Degryse, "Methods to evaluate renal function in elderly patients: a systematic literature review," Age and Ageing, vol. 39, no. 5, Article ID afq091, pp. 542–548, 2010.

[53] M. Lipcsey, M. Furebring, S. Rubertsson, and A. Larsson, "Significant differences when using creatinine, modification ofdiet in renal disease, or cystatin C for estimating glomerularfiltration rate in ICU patients," Upsala Journal of MedicalSciences, vol. 116, no. 1, pp. 39–46, 2011.

[54] D. Fliser and E. Ritz, "Serum cystatin C concentration as amarker of renal dysfunction in the elderly," The AmericanJournal of Kidney Diseases, vol. 37, no. 1, pp. 79–83, 2001.

[55] B. A. Perkins, R. G. Nelson, B. E. P. Ostrander et al., "Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study," Journal of the American Society of Nephrology, vol. 16, no. 5, pp. 1404–1412, 2005.

[56] Fatima LD, Fatah C. Pathophysiological and Pharmacological Effects of Snake Venom Components:Molecular Targets. Journal of Clinical Toxicology. 2014; 4: 2.

[57] Khorami, S. H. H. et al. Serum levels of interleukin-4, interleukin-10 and interferon-γ in patients with chronic hepatitis B infection. Hepat. Mon. 18(4), e60377. https://doi.org/10.5812/hepatmon.60377 (2018).

[58] Bare Y, Marhendra APW, Sasase T, Fatchiyah F. Differential Expression of IL-10 Gene and Protein in Target Tissues of Rattus Norvegicus Strain Wistar Model Type 2 Diabetes Mellitus (T2DM). Acta Inform Med. 2018 Jun;26(2):87-92. doi: 10.5455/aim.2018.26.87-92. PMID: 30061777; PMCID: PMC6029922.

[59] Danko S. Batusic, Alexander von Bargen, Sabine Blaschke, Jozsef Dudas & Giuliano Ramadori (2011): Different physiology of interferon-α/-γ in models of liver regeneration in the rat. Histochem Cell Biol 136, 131–144 (2011). https://doi.org/10.1007/s00418-011-0838-7.

[60] Kassab, R.B.; Theyab, A.; Al-Ghamdy, A.O.; Algahtani, M.; Mufti, A.H.; Alsharif, K.F.; Abdella, E.M.; Habotta, O.A.; Omran, M.M.; Lokman, M.S.; et al. (2022): Protocatechuic Acid Abrogates Oxidative Insults, Inflammation, and Apoptosis in Liver and Kidney Associated with Monosodium Glutamate Intoxication in Rats. Environ. Sci. Pollut. Res. , 29, 12208–12221.

[61] Abd-Elhakim Y. M., et al. (2021). "Prevention of melamine-induced hepatorenal impairment by an ethanolic extract of Moringa oleifera: Changes in KIM-1, TIMP-1, oxidative stress, apoptosis, and inflammation-related genes." Gene 764: 145083.

[62] M. Gałażyn-Sidorczuk, M.M. Brzóska, M. Jurczuk, J. Moniuszko-Jakoniuk, Oxidative damage to proteins and DNA in rats exposed to cadmium and/or ethanol, Chem. Biol. Interact. 180 (2009) 31-38. https://doi.org/10.1016/j.cbi.2009.01.014.

[63]Neubauer K, Lindhorst A, Tron K, et al. Decrease of PECAM-1-gene expression induced by

proinflammatory cytokines IFN-gamma and IFN-alpha is reversed by TGF-beta in sinusoidal endothelial cells andhepatic mononuclear phagocytes. BMC Physiol 2008;8:9.

[64] Ahmed, Z.S.O.; Galal, M.K.; Drweesh, E.A.; Abou-El-Sherbini, K.S.; Elzahany, E.A.M.; Elnagar, M.M.; Yasin, N.A.E. Protective Effect of Starch-Stabilized Selenium Nanoparticles against Melamine-Induced Hepato-Renal Toxicity in Male Albino Rats. Int. J. Biol. Macromol. 2021, 191, 792–802. [Google Scholar] [CrossRef] [PubMed]

[65] Y.M. Abd-Elhakim, W.A.M. Mohamed, K.M. El.Bohi, H.A. Ali, F.A. Mahmoud, T.M. Saber, Prevention of melamine-induced hepatorenal impairment by an ethanolic extract of Moringaoleifera: Changes in KIM-1, TIMP-1, oxidative stress, apoptosis, and inflammation-related genes, Gene 764 (2021) 145083. https://doi.org/10.1016/j.gene.2020.145083.

[66]. Abd-Elhakim YM, Mohamed WA, Bohi KME, Ali HA, Mahmoud FA, Saber TM. Prevention of melamine-induced hepatorenal impairment by anethanolic extract of Moringa oleifera: changes in KIM-1, TIMP-1, oxidative stress, apoptosis, and inflammation-related genes. Gene2021; 764:145083

[67]. Chang L, Yue Z, She R, Sun Y, Zhu J. The toxic effect of a mixture of melamine and cyanuric acid on the gastrointestinal tract and liver in mice. Research in Veterinary Science. 2015;102:234–237

Table 1: Comparison of renal function biomarkers (urea, creatinine, uric acid) across experimental groups following melamine and/or cobra venom administration.

Groups	Negative control	SV (10/µg/dl)	SV (20/µg/dl)	Melamine	Melamine+ SV (10/µg/dl)	Melamine+ SV (20/µg/dl)	P value
Urea (mg/dl)	**51.5±0.64**[b]	**50.75±1.37**[b] -1.45%	**50.5±0.64**[b] -1.9 %	**75.75±8.6**** 47%	**52±4.02**[b] 0.97%	**61.3±2.02** **19%**	**0.002**
Creatinine (mg/dl)	**0.55±0.008**[c]	**0.53±0.04**[c] -3.6%	**0.58±0.02**[b] 5.45%	**0.79±0.02***** 43.6%	**0.51±0.03**[c] -7.3%	**0.63±0.02**[a] 14.5%	**0.000**
Uric acid (mg/dl)	**1.44±0.014**[c]	**1.41±0.052**[b] -2%	**1.55±0.023**[b] 7.6%	**1.70±0.022***** 18%	**1.56±0.016***[b] 8.3%	**1.59±0.037** **10%**	**0.000**

Regarding the control group, * P<0.05, ** P<0.01, and *** P<0.001 were observed. aP<0.05, bP<0.01, and cP<0.001 in comparison to the positive control group. The mean difference is significant at P<0.05. The proportion of change is expressed as a percentage of the control group.

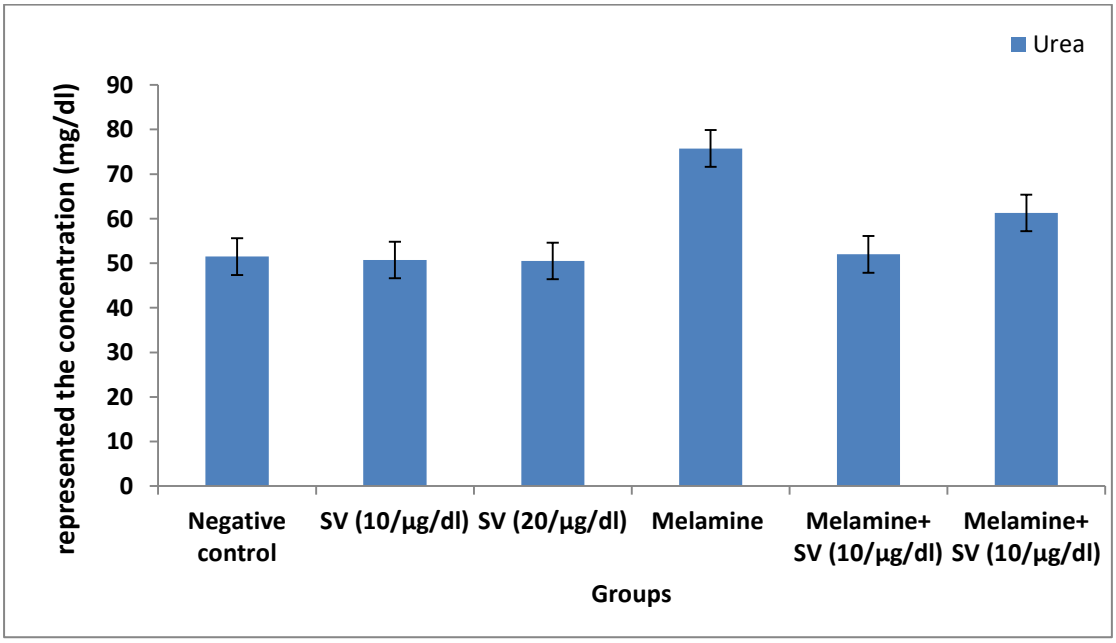

Figure 1: Average serum urea levels across all treatment and control groups.

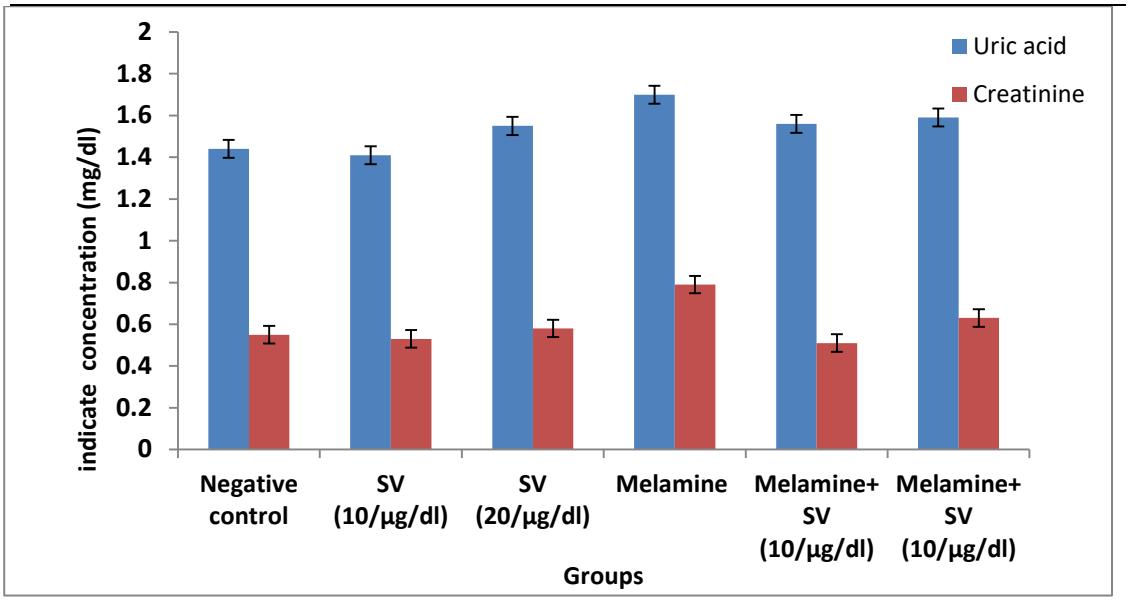

Figure 2: Comparative analysis of uric acid and creatinine concentrations among different experimental groups.

Effects of treatment plans on kidney levels of anti-apoptotic (cystatin C), apoptotic (caspase-3), and inflammatory (IL-2) indicators in all rat groups are shown in Table 2.

Groups	Negative control	SV (10/µg/dl)	SV (20/µg/dl)	Melamine	Melamine+ SV (10/µg/dl)	Melamine+ SV (20/µg/dl)	P value
Kidney iL-2(ng/g tissue)	6.35±0.54[b]	5±0.23[b] -21%	4.75±1.23[b] -25%	22±1.50** 246%	9.9±0.46*[a] 55.9%	12.7±0.23**[a] 100%	0.000
Kidney Cystatin –c (ng/g tissue)	14.15±0.60[b]	12.65±0.43[b] -10.6%	13.3±1.15[b] -6%	60.7±3.0** 328.9%	25.5±2.24**[b] 80%	22.35±1.02**[b] 58%	0.000
Kidney Caspase-3 (ng/g tissue)	25.05±1.35[b]	26.85±1.18[c] 7%	27.85±1.58[b] 11%	8.9±1.5** -64%	17.05±0.95[b] -32%	20.9±0.69[c] -16.5%	0.000

P<0.05, **P<0.01, and *** P<0.001 in comparison to the control group, respectively. lower than the positive control group (aP<0.05, bP<0.01, cP<0.001). The percentage change is the percentage of change relative to the control group. The mean difference is significant at P<0.05

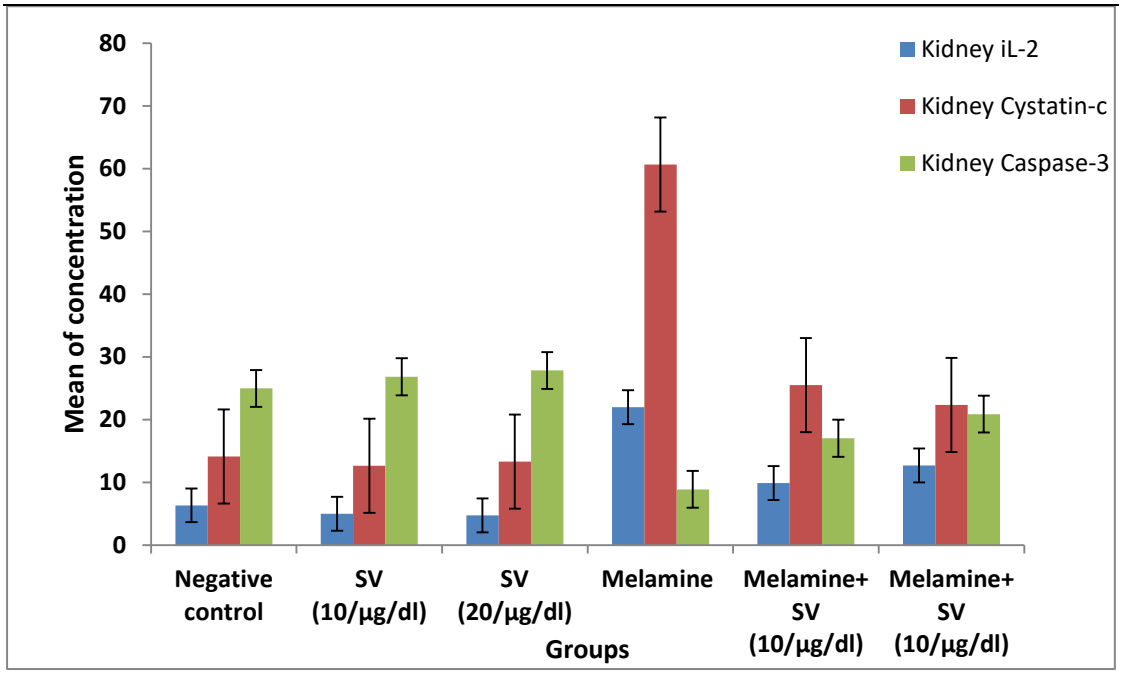

Figure 3 : Group-wise mean levels of renal biomarkers reflecting inflammation, apoptosis, and cellular repair.

Table3: Differential expression of IL-10 and INF-γ genes in renal tissues among the various experimental groups.

Groups		Negative Control	SV (10/µg/dl)	SV (20/µg/dl)	Melamine	Melamine+ SV (10/µg/dl)	Melamine+ SV (20/µg/dl)	P value
Gene expression of IL-10	**Kidney**	**1±0.00^{c}**	**1.02±0.002*^{c}** 2%	**1.11±0.002***^{c} 11%	**5.03±0.02*** 403%	**2.13±0.12*^{b}** 113%	**2.34±0.04***^{c} 134%	**0.000**
Gene expression of INF-γ	**Kidney**	**1±0.00^{c}**	**1.04±0.004**^{c}** 4%	**1.33±0.03*^{c}** 33%	**6.00±0.012*** 500%	**2.55±0.01***^{c} 155%	**2.98±0.04***^{c} 198%	**0.000**

* P<0.05, ** P<0.01, and *** P<0.001 relative to the control group respectively. compared to aP<0.05, bP<0.01, and cP<0.001 for the positive control group. The difference in mean is significant at P<0.05. Change percentage is the percentage of change compared to the control group.

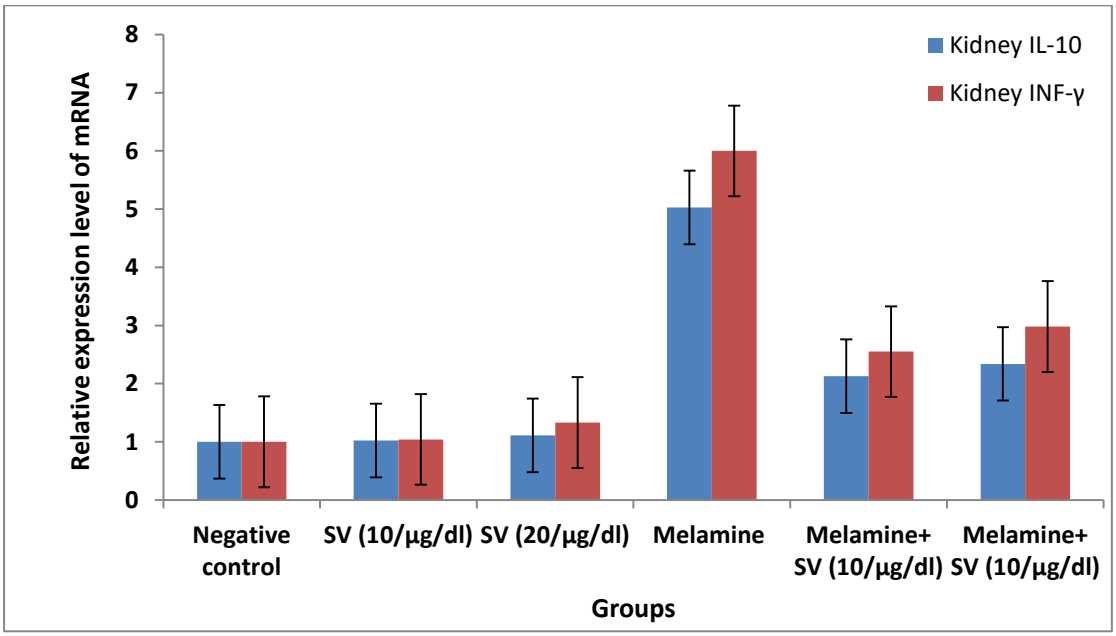

Figure 4: Relative expression level of mRNA INFγ and IL10 in renal tissues of all test groups.

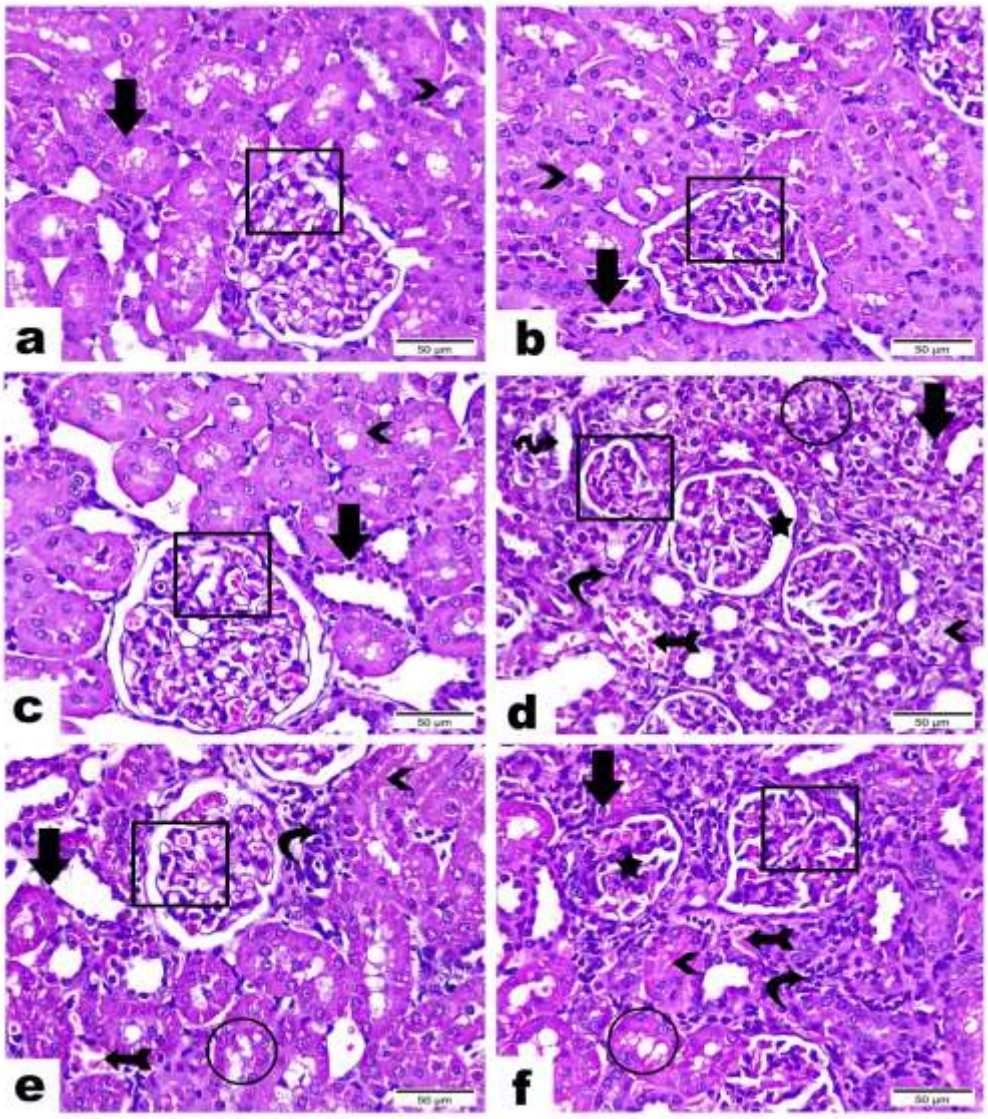

Figure 5: Photomicrographs presented Hematoxylin and Eosin Stain, Magnification Power = x400, and Scale Bar = 50µm were used to examine the histopathological differences in kidney tissue slices (renal cortex area) between the groups under investigation A control group, a 10µg/kg cobra venom group, and a 20µg/kg cobra venom group Venom exhibiting the typical histological structure of the renal cortex, forming a renal corpuscle with intact proximal (arrowheads) and distal (arrows) convoluted tubules, as well as Bowman's capsule (rectangles). (d) Section from melamine Group revealing severe renal damage including atrophy of renal corpuscle with degenerated capsule (rectangle), dilated glomerular space (star), vascular congestion (arrow with tail), interstitial edema (wave arrow), and inflammatory cells infiltration (curvy arrow). Furthermore, most renal tubules display degenerative changes with loss of its organization (circle), other renal tubules noticed with cytoplasmic vacuolization, pyknotic nuclei of its lining cells (arrow) and hyaline cast in their lumen (arrowhead). (e) Section from Treated Group with 10µg/kg CobraVenom showing a significant tissue recovery with most renal tubules and a virtually normal renal corpuscle with slight congestion (rectangle) restore its normal organization (circle) except few noticed with epithelial desquamation (arrowhead) and deep basophilic apoptotic nuclei (arrow). Moreover, few number of aggregated inflammatory cells (curvy arrow) as well as scarce interstitial hemorrhage (arrow with tail) were also seen. (f) Section from Treated Group with 20µg /kg cobraVenom demonstrating few improvements that assembled in some renal corpuscle still with atrophy (star) and others appeared intact (rectangle), vascular congestion (arrow with tail), and high number of inflammatory cells infiltration (curvy arrow). Additionally, some renal tubules restore its organization (circle), other renal tubules detected with epithelial desquamation (arrowhead), and cytoplasmic vacuolization of its lining cells (arrow).