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Effects of Bee Venom on Cadmium Toxicity in Albino Rats

Mariam Ismail Ali[1]*, Rania Helmi Abdou[1], Mary Ayad Sargious[2], Omnia Mohamed Khattab[2], Kawthar Abdelwahed Elhady[1]

[1]Deptartment of Forensic Medicine and Toxicology, Faculty of Veterinary , Medicine, Suez Canal University, Ismailia, Egypt; [2]Genome Research Unit, Animal Health Research Institute, Dokki, Giza, Egypt.

Abstract | The present study aimed to confirm the mitigation effects of bee venom at a dose of 2mg/kg against cadmium chloride hepatotoxicity, nephrotoxicity, and neurotoxicity in rats. The study found that cadmium treatment in rats increased inflammatory markers (TNF-α 264.3% and IL-1β 170.7%), and oxidative markers(MDA 140.5%), Higher liver enzymes, histopathological lesions, and genes expression screened for hepatotoxic effects. Bee venom effectively reduced inflammation (TNF-α 49% and IL-1β 41.4%) and oxidation (MDA 36.8%), reduced liver enzymes, and enhanced histopathologic lesions with score 1, as confirmed by the difference in regulation of inflammatory genes (TNF-α 37% increase, IL-1β 15% reduction, and NF-kB19% reduction) and massive increase of pro-apoptotic Bax in the liver. Not only the potential beneficial role of bee venom on cadmium hepatotoxic effects but also nephrotoxic and neurotoxic effects and reducing hepatotoxicity caused by cadmium exposure via regulating, Bax, and NF-kB signaling pathways in rats by considering its possible antioxidant, anti-inflammatory, and anti-apoptotic effects. The results showed the nephrotoxic effect of cadmium: a significant increase in serum creatinine, urea, and histopathological lesions in the kidney of cd-treated rats. Bee venom showed nephroprotective effects, decreasing serum creatinine and urea by 16.9% and73.4% respectively and also improving histopathologic lesions in the kidney. Also, the neuroprotective effect of bee venom appeared in the improvement of the histopathological lesions in the brain.

Keywords: Cadmium, Bee venom, Oxidative stress, Inflammation, Apoptosis

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***Correspondence** | Mariam Ismail Ali, Deptartment of Forensic Medicine. and Toxicology, Faculty of Veterinary, Medicine, Suez Canal University, Ismailia, Egypt; **Email:** mariam_ismail@vet.suez.edu.eg
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INTRODUCTION

Cd2+ is a major industrial and environmental pollutant considered hazardous to both human and animal health. Moreover, Cd2+ is now placed seventh on the Agency for Toxic Substances and Disease Registry's list of the top twenty hazardous substances. Exposure to cadmium mostly affects the liver, kidneys, lungs, bones, and testes. In addition, Parkinson's disease, Alzheimer and other neurodegenerative illnesses are brought due to exposure to cadmium.

Cd2+can disrupt cellular functions and cause tissue damage through multiple toxicological pathways. First, Oxidative Stress Caused by Cadmium affects transcription factors, including NF-κB and AP-1, leading to increased NOX gene

expression. These transcription factors are active during inflammatory responses, and their activation can result in increased mRNA levels of NOX subunits, which improves enzyme complex production and assembly. Oxidative stress impairs the cellular antioxidant defense system. Key antioxidant enzymes that include superoxide dismutase (SOD) (Qu F *et al*., 2024).

Cd2+second mechanism is disruption of cellular signaling pathways. It could activate p38 MAPK pathway, leading to the synthesis and release of cytokines including TNF-α and IL-6. These inflammatory molecules play a crucial role in how cells deal with toxicity (Mijit *et al*., 2020). Furthermore, p38 MAPK is associated with apoptotic pathways, and its activation can either promote or prevent apoptosis, with the specific effect varying depending on cell type and stress environment.

In a comprehensive investigation of rats, cadmium exposure resulted in a considerable increase in lipid peroxidation (LPO), indicating oxidative damage within neural tissues. Cadmium exposure was also linked to higher serum urea and blood urea nitrogen levels, which are signs of renal impairment (poli *et al*., 2022).

The molecular mechanism of toxicity in Cd2+ is mainly due to in vivo Apoptosis. Cadmium is characterized by extended biological half-life in humans due to the inability of the body to remove it effectively (Tzirogiannis *et al*., 2003). Persistent exposure to it is the main cause of Cd's acute and chronic toxicity (Kasuya *et al*., 2000; Genchi *et al*., 2020).

Bee venom, also known as Apitoxin (Apitox®). It has been utilized for the treatment of many diseases Multiple sclerosis (MS), Parkinson's disease (PD), rheumatoid arthritis (RA), liver fibrosis (LF), multiple sclerosis (MS), inflammatory disorders, and neurodegenerative diseases (Lee *et al*., 2005). Bee venom contains biogenic amines, polypeptides, and enzymes, primarily phospholipase A2 (PLA2), hyaluronidase, acid phosphatase, lipids like mast cell degranulation peptide, basic peptides, and non-enzymatic proteins (primarily melittin and apamin), histamine, bioactive amines, and non-peptide substances like glucose and fructose (Dennis *et al*., 2011). Bee venom reduces the quantity of free radical species (ROS) that cause oxidative damage.

Melittin is the main component of Bee Venom, accounting for 60% of its composition. It activates PAL2 and produces anti-inflammatory and anti-arthritic effects. Apamin is the smallest neurotoxin. It crosses the blood-brain barrier and blocks the calcium potassium channel. Melittin inhibit inflammatory cytokines like interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Moreover, melittin decreases signaling pathways that activate inflammatory cytokines, including nuclear factor-kappa B (NF-κB) (Wehbe *et al*., 2019).

Alanine and aspartate transaminases ALT and AST, and their changes have been used as tools to investigate differences in liver cell viability and cell membrane permeability because of cadmium toxicity. High levels of ALT and AST are the most important markers for liver damage. In addition, the significant decline in GSH levels in cadmium toxicity was previously recorded which may be explained by cadmium's strong affinity for the thiol group of GSH. Cadmium-induced oxidative stress is caused by liver inflammation. One major source of inflammation is Cd-induced inflammatory mediators like IL-1β, TNF-α, IL-6, and IL-8 due to the activation of Kupffer cells (Yamano *et al*., 2000). Cadmium exposure upregulates proinflammatory gene expression, including interleukin-1, interleukin-6, tumor necrosis factor-α, and interferon-γ, while downregulating anti-inflammatory cytokine IL-10 in rat testes (Sivaprakasam and Nachiappan, 2016). Contrarily Kim *et al*., (2010) suggest that Bee venom possesses anti-fibrogenic properties that are mediated by the suppression of pro-inflammatory cytokines and fibrogenic gene expression like IL-1beta and TNF-alpha.

Excessive cadmium-induced renal damage primarily affects the proximal tubules, interfering with the tubular cells' cellular and functional integrity, which is the main sites where the metal accumulates in the renal cells (Bernard, 2008) and also a significant increase in plasma urea and creatinine in cadmium intoxication (Ibrahim *et al*., 2018).

The study aims to investigate the role of Bee venom therapy in the protection of rats against cadmium toxicity regarding the biochemical parameters of the liver and kidney in addition to confirming the effect of bee venom as a protective tool against cadmium toxicity by confirming the downregulation of an inflammatory gene during cadmium administration and in case of Cd2+ and bee venom administration.

MATERIAL AND METHODS

Animals

Forty healthy albino rats, weighing 150 – 250g, were used in this study which were obtained from the Laboratory Animal House at the Faculty of Veterinary Medicine Suez Canal University, Ismailia, Egypt. They were maintained under standard laboratory animal housing conditions and fed a standard diet and water ad libitum. All animals, including the control and experimental groups, were housed under identical environmental conditions throughout the study. These conditions included consistent temperature, humidity, a light/dark cycle, and free access to food and water. The only difference between the groups was the treatment administered; the experimental groups received bee venom

and/or cadmium, and the control group received no treatment. All the humanity and ethical respects, in addition to the animal's euthanasia, were taken into consideration and performed.

Chemicals

Bee venom *(Apis mellifera)* which was purchased in crude form from (The Department of Bee Research, Plant Protection Institute, Agriculture Research Centre in Dokki, Giza governorate, Egypt), kept desiccated at 4 ◦C and reconstructed for use with sterile saline The bee venom (BV) inintraperitoneally injected with Bee venom at a dose of 2mg/kg BW according to (Hassan *et al*., 2019). Cadmium chloride ($cdcl_2$) was purchased from LOBAL Chemie (LABORATORY REAGENTS AND FINE CHEMICALS) UN No.2570, CAS No. 35668-66-2 was dissolved in distilled water to a 5 mg/ml concentration. according to (Ojo *et al*., 2023) and Renugadevi and Prabu (2010).

Experimental Design and Treatments

After an acclimation period of 2 weeks, the rats were randomly grouped into 4 groups (n = 10): 1st group:(Control negative, (c)). 2nd group: (The bee venom (BV) intraperitoneal injected with Bee venom at a dose of 2mg/kg BW.3rd group: the Cd-treated group, was orally gavaged with cdcl2 at a dose of 5mg/kg, Cdcl2. 4th group: Cdcl2/Bee venom group(both)(B), orally dosed cdcl2 followed by Bee venom by the same doses and routes as in groups 3 and 2). The exposure was daily for six weeks.

Blood samples

The first blood sample was collected after the third week, and the second blood sample at the end of the sixth week of the experiment. The rats were weighed and then fasted 12-14 hours before sample collection, Blood was collected using the retro-orbital method. For biochemical assessments. (the blood was further centrifuged at a speed of 3,000 rpm for 10 minutes). The clear serum was collected using a clean dropper in sterilized tubes, properly labeled, and then frozen at -20°C until the biochemical analysis of serum AST, ALT "IFCC Method for Alanine Aminotransferase," (Bergmeyer *et al*., 1986). IL-1β according to Durum *et al*. (1985), TNF-α described by Beutler *et al*. (1985), IL-10 described by Fichtlscherer *et al*. (2004), GSH described by Baker *et al*. (1990), SOD described by Nishikimi *et al*. (1972) with few modifications, MDA according to Botsoglou *et al*., (1994)., serum creatinine and urea by the methods of Ilstrup *et al*. (1985) and Bartels *et al*. (1972).

Tissue Samples

At the end of the experimental period (after 6 weeks), rats from the control and dosed groups were deeply anesthetized by diethyl ether then tissues were dissected, the selected organs (brain, liver, and kidney) were dissected out and washed several times in saline (0.9% NaCl). The liver from all rats was divided into 2 groups, a formalized group for histopathological examination and the other group kept frozen for molecular examination.

The kidney from all rats was kept formalized for the histopathological examination while the brain from all rats was kept preserved in a Bouin's solution for histopathological examination.

The liver and kidney of the control and all treated animals were collected and harvested in 10% neutral buffered formalin, meanwhile, cerebral, and cerebellar tissue specimens were sliced and fixed in Bouin's solution. After proper fixation, all tissue samples were dehydrated in ascending grades of alcohol, cleared in 3 changes of xylene, and then embedded into paraffin at 60 °C. The paraffin blocks were prepared and 5-7 µm thick tissue sections were obtained and placed onto glass slides. The tissue slices were then deparaffinized and stained with hematoxylin and eosin according to the method adopted by Bancroft *et al*. (2013).

The obtained histological sections were viewed, and photomicrographs were captured and collected using an OLYMPUS BX43 research optical microscope equipped with an OLYMPUS SC100 digital camera. The magnification scale bar was reported on the collected photomicrographs.

Lesion Scoring Evaluation

To assess morphological damage to the liver after treatment with Cd and Bee Venom: pyknotic nuclei, Kupffer cell hyperplasia, Congestion of the central vein, and Mononuclear cell infiltration.

The Histopathological scoring of kidney lesions sections was carried out to assess morphological damage to the kidney in the cortex and medulla after treatment with Cadmium and Bee Venom: Congestion of the inter-tubular capillaries, glomerular and tubular degeneration as glomeruli shrinkage, necrosis of tubules, and presence of inflammatory cells.

To assess morphological damage to the brain after treatment with Cadmium and Bee Venom: Congestion of blood vessels, degenerated neurons(chromatolysis), intracellular vacuoles in Purkinje cell, and vacuolar space around pyramidal cells.

These measures were evaluated on a scale from 0 to 4, which ranged from not present (0) to mild (1), moderate (2), severe (3), and very severe (4).

Real-Time Quantitative PCR (RT-qPCR) for Analysis of Gene Expression

RNA extraction: Total cellular RNA was extracted from fresh and frozen tissues by RNeasy Mini Kit (50 RNeasy Mini Spin Columns, (Qiagen, USA.). cat.

no.74104 following the manufacturer's instruction. Reverse transcription of total RNA (1µg) into cDNA was carried out using the HiSenSc2.4. sript™ RH (–) cDNA Synthesis Kit (iNtRON Biotechnology Co., South Korea).

Real-Time Polymerase Chain Reaction (qPCR)

We used (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) as the housekeeping gene for normalization, ensuring consistent and reliable comparison of gene expression levels across samples. The PCR primers for the genes used in this method are listed in (Table 1).

Table 1: F forward primer, R reverse primer Gene Primers for quantitative real-time PCR.

Gene	Primer sequence (5′–3′)
GAPDH	F: CCCCCAATGTATCCGTTGTG
	R: TAGCCCAGGATGCCCTTTAGT
IL-1β	F: GGAAGGCAGTGTCACTCATTGTG
	R: GGTCCTCATCCTGGAAGCTCC
TNF-α	F: AGCCCTGGTATGAGCCCATGTA
	R: CCGGACTCCGTGATGTCTAAGT
NF-κB	F: AGCACCAAGACCGAAGCAA
	R: TCTCCCGTAACCGCGTAGTC
Bax	F: CCAGGACGCATCCACCAAGAAG
	R: CCCAGTTGAAGTTGCCGTCTGC

The HERA SYBR® Green qPCR master mix is a ready-to-use real-time PCR kit optimized for the real-time quantification of target DNA. intercalating dye, which binds to double-stranded DNA and releases fluorescence during each reaction cycle, allowing for the genotype of the sample to be quantified. The PCR cycling conditions included an initial denaturation at 95°C for 15 min followed by 40 cycles of denaturation at 95°C for 30s, annealing at 60°C for 60s, and extension at 72°C for 60s. After obtaining the Ct values using the 2-ΔΔCt method for the reference gene GAPDH, the expression level of the target genes was normalized to that of GAPDH. The relative fold changes in the gene expression were estimated based on the comparative 2– ΔΔCT (Ct: cycle threshold) method with the GAPDH gene as an internal control to normalize target gene expression levels. All mixed reagents in a nuclease-free Eppendorf. Reagents required per sample HERA SYBR® Green Master Mix (2x) 10µl, Forward primers 20x (200nM) 1µl (up to 1µM each), Reverse primers 20x (200nM) 1µl (up to 1µM each), Nuclease free water To 20µl DNA template (up to 250ng) Volume varies.

Statistical Analysis

Results were expressed as the mean ±standard error (Mean ±SE). Statistical analysis was done using the one-way analysis of variance (ANOVA) followed by the post-hoc Tukey's test. All statistics were carried out using SPSS. In this study, p values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Serum Biochemical Assessments, Liver Function Tests

Effects of cadmium chloride and bee venom on serum (ALT and AST) levels in rats: ALT and AST measurements tabulated in Table 2 and graphically represented by Figure 1 panel A, it was denoted that a significant increase in the level of ALT, AST ($p < 0.05$) was recorded in Cadmium chloride treated rats (group 3) after the end of the third week and the end of the experimental period compared with the negative control group (group 1). Bee Venom-treated rats (group 2) showed no significance in the level of ALT, and AST compared to the negative control group (group 1). In contrast, the administration of cadmium chloride and Bee venom group (group 4) showed a significant decrease compared to the cadmium chloride group (group 3).

Elevated serum hepatic marker enzyme levels, which signify cellular leakage and a loss of functional integrity of the hepatic membrane architecture, are a reliable indicator of

Table 2: Effect of Bee venom and Cadmium chloride treatment in the activity of ALT and AST (U/L) in the serum of rats at the end of the third week and the end of the experiment.

	1st group (c)		2nd group (Bee venom)		3rd group (Cd)		(Both) cd +Bee venom	
	21 days	45 days	21 days	45 days	21 days	45 days	21 days	45 days
ALT	24.68 ±0.2658	24.7±0.2309	24.33 ±0.2136	23.77 ±0.318	55.85 ±1.498	69.97 ±1.312 183.3% increase	34.35 ±0.9887	42.9 ± 0.7506 38.6% decrease
AST	91.08±0.5573	91.7±0.7371	90.15±0.7354	90.3±1.026	187.3±1.526	237.4 ±2.352 160.6% increase	138.6 ±1.127	171.8 ±1.903 27.6% decrease

Values are expressed as Mean ± (SE), (n= 10/group). using the post hoc Tukey's test, one-way ANOVA. (1st group: (control negative group),2nd group: (bee venom group),3rd group: (Cd-treated group),4th group: (cdcl2/BV group). Different small superscript letters indicate significance in the same row ($p<0.05$);NB: percentage change calculated in cadmium group compared to the control group; Percentage change/improvement of bee venom as a treatment calculated compared both group to the cadmium group.

liver injury following Cd exposure. ALT, AST, and other liver enzymes are important indicators of biliary function and liver cell damage that are measured in liver function tests. It is essential to comprehend these enzymes to diagnose liver disorders and track the effectiveness of treatment (Lala *et al* , 2024).

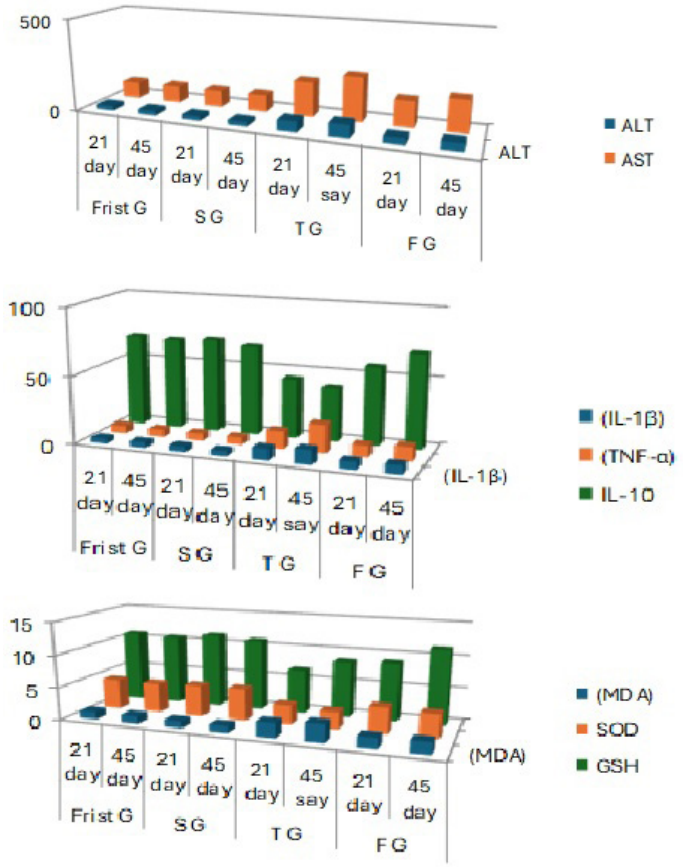

Figure 1: Effect of treatment of bee venom and cadmium toxicity on ALT, AST (U/L estimated in different groups panel A. Pro-inflammatory cytokines IL-1β, TNF-α, IL10 in(pg/mL)were detected panel B. Serum oxidative factor were evaluated (, GSH (µmol/L), SOD (U/ml, MDA (nmol/ml panel C.

It correlates with the present study which revealed the increased AST and ALT activities in the serum of Cd-treated rats. These enzymes' activities are key enzymes involved in liver function, and their elevation can indicate liver damage or diseases such as liver inflammation, fatty liver disease, cirrhosis, and liver tumors (Vagvala and O'Connor, 2018).

The most important markers for liver damage detection are high levels of alanine and aspartate transaminases (Williamson *et al*., 1996).

It correlates with the present study which revealed the increased AST and ALT activities in the serum of Cd-treated rats. These enzymes' activities are liver-specific, and their changes have been used as a tool to investigate differences in cell viability and cell membrane permeability (Dasgupta *et al*., 1996).

In our study administration of bee venom (2 mg/kg) attenuated cadmium-induced hepatotoxicity as shown by the decreased levels of AST and ALT thus offering protection against Cd toxicity in rats. The above effects indicate that bee venom may offer protection by stabilizing the cell membrane in cadmium-induced hepatic damage. Consistent with the study of (Kim *et al*, 2010), studied the anti-fibrosis effects of Bee venom in mouse models of hepatic fibrosis induced by carbon tetrachloride (CCl4) and ethanol-treated hepatocytes (ETH). By suppressing pro-inflammatory cytokines (TNF-α, IL-1β) and fibrogenic genes (TGF-β1, smooth muscle actin, and fibronectin), they discovered that Bee venom significantly inhibited the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). also, the study of (Abdulmalek *et al*., 2022) suggests that supplementation of targeted bee venom-CSNPs resulted in a greater reduction in ALT and AST levels than untreated mice**,** finally with Aspartate transaminase (AST) and alanine transaminase (ALT) levels show that Bee venom improved the restoration of liver functions (Aly *et al*., 2023).

Serum Oxidative / Anti-oxidative Status

Serum reduced glutathione (GSH), superoxide dismutase (SOD) levels, and malondialdehyde (MDA) levels: To explain the toxicity of cadmium on organs, several mechanisms have been suggested, the most common is the oxidative damage caused by cadmium through increasing membrane lipid peroxidation, a harmful process only accomplished by free radicals (Agmon *et al*. 2018). Through a number of mechanisms, cadmium (Cd) exposure amplifies oxidative stress and cellular damage by activating NADPH oxidase. Because NADPH oxidase is essential for ROS overproduction, cadmium exposure causes an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses (Qu and Zheng, 2024; Singh *et al*., 2024).

The significant decline in GSH levels may be explained by cadmium's strong affinity for the thiol group of GSH, which it oxidizes to GSSG (El-Habit and Abdel Moneim 2014; Espinosa-Diez *et al*., 2015). In the present study, the decreased levels of GSH in Cd toxicity might increase the susceptibility of the liver to free radical damage. Our findings agree with the other published reports which quoted that GSH concentration is decreased during Cd intoxication (Pari and Muruga-vel, 2005). In the present study administration of bee venom (2 mg/kg) attenuated cadmium-induced hepatotoxicity as shown by the increasing level of GSH thus offering protection against Cd toxicity in rats (Table 3).

Table 3: GSH, SOD, and MDA Levels at the end of the third week and the end of the experiment.

	1st group (c)		2nd group (Bee venom)		3rd group (Cd)		(Both) cd +Bee venom	
	21 days	45 days	21 days	45 days	21 days	45 days	21 days	45 days
GSH	11.01±0.0713	10.68±0.062	11.47±0.188	11.01±0.179	6.814±0.0481	8.642±0.103 8.6%decrease	8.948±0.0293	11.49±0.264 32.95%increase
SOD	4.616±0.0089	4.537±0.025	4.795±0.0378	4.878±0.0209	3.029±0.036	2.652±0.047 41.5%decrease	4.016±0.065	3.713±0.077 40% Increase
MDA	1.175 ±0.012	1.181 ±0.008	1.119 ±0.0117	1.12 ±0.0149	2.298 ±0.0729	2.841±0.075 140.5%increase	1.637 ±0.0475	1.795±0.061 36.8%decrease

Table 4: Effects of cadmium chloride and bee venom on Serum (IL-1β),(TNF-α), and (IL-10) Levels at the end of the third week and the end of the experiment.

	1st group (c)		2nd group (Bee venom)		3rd group (Cd)		(Both) cd +Bee venom	
	21 days	45 days	21 days	45 days	21 days	45 days	21 days	45 days
(IL-1β)	3.928±0.0472	4.037±0.051	3.853±0.0253	3.877±0.0555	8.71±0.133	10.93±0.477 170.7% increase	5.663±0.117	6.41±0.2542 41.4% decrease
(TNF-α)	5.603±0.0335	5.67±0.0551	5.548±0.0149	5.42±0.03215	13.05±0.278	20.66±0.264 264.3%increase	8.525±0.0595	10.55±0.407 49%decrease
IL-10	68.51±0.361	67.55±0.413	69.49±0.394	66.29±0.329	44.24±0.587	39.86±0.676 41%decrease	57.54±0.479	69.37±0.533 74%increase

The impairment of the antioxidant defense system is considered a critical event in cadmium-induced hepatotoxicity as we found that GSH and SOD are significantly decreased, MDA is significantly increased in the serum of Cd-treated rats, consistent with previous studies that find A metalloprotein called SOD catalyzes superoxide radical dismutation (McCord *et al.*, 1976), The substrate is oxidized when lipid hydroperoxides, hydroxyl radicals, and MDA produced during metal intoxication react with other lipids, proteins, and nucleic acids. They might have a role in genotoxic, carcinogenic, and mutagenic effects (Kaplan *et al.*, 2011). This genotoxic effect resulting from increased MDA levels due to oxidative stress can lead to the activation of the NF-Kb signaling pathway, this activation also influences apoptosis by upregulation of the pro-apoptotic gene expression BAX showed in the liver cadmium-administrated group. It was discovered that Bee venom acupuncture reduces the quantity of free radical species (ROS) that cause oxidative damage to synovial fluid proteins (Suh *et al.*, 2006).

which agrees with the current results. Parallel to many studies, there was a significant increase in GSH levels in the bee Venom low dose treated group compared to the diabetic group. These studies suggested many mechanisms, like the antioxidant activity of Bee venom (Gawad *et al.*, 2016), Research has documented the antioxidative properties of PLA2 (Snyder *et al.*, 1985) and Bee venom samples from the USA (Rekka *et al.*, 1990) and Korea (Han *et al.*, 2010).

Regarding the antioxidant effects of Bee venom samples, they have been related to the capacity to inhibit the lipid peroxidation process (Rekka *et al.*, 1990) and to increase superoxide dismutase (SOD) activity (Han *et al.*, 2010). These studies are parallel to the results of this study as the antioxidant effect of bee venom showed in the cd-bee venom group and Bee venom group as GSH and SOD are increased but MDA is decreased in contrast with the cd-treated group.

Serum Pro-inflammatory Cytokines

Effects of cadmium chloride and bee venom on (Serum Interleukin-1Beta (IL-1β) Levels, Serum Tumor Necrosis Factor-Alpha (TNF-α) Levels, and Serum Interleukin-10 (IL-10) Levels at the end of the third week and the end of the experiment.

Another significant mechanism for Cd-induced oxidative stress is inflammation in the liver (Kayama *et al.*, 1995; Yamano *et al.*, 2000). One major source of Cd-induced inflammatory mediators like IL-1β, TNF-α, IL-6, and IL-8 is the activation of Kupffer cells, which are the resident macrophages of the liver (Kayama *et al.*, 1995; Yamano *et al.*, 2000). Additionally, small quantities of Cd-MT complexes bound to thiol-containing substances (such as GSH, L-cysteine, L-homocysteine, and N-acetyl-L-cysteine) in plasma are transported to the kidneys. They can be absorbed through the renal proximal tubule cells (Yang and Shu, 2015). The findings of these studies are parallel to our results which showed that the cd-treated group has a highly significant increase in IL-1beta and TNF-alpha with a significant decrease in IL-10 (Table 4).

In this study Table 4, the bee venom group and the cd-Bee venom group showed the anti-inflammatory effect of bee venom parallel with previous studies say that bee

venom (BV) exhibits significant anti-inflammatory effects, primarily through its active components that modulate immune responses and inhibit inflammatory mediators. Studies have shown that BV can scavenge free radicals and inhibit protein denaturation, which are crucial in mitigating inflammation (Florescu *et al*., 2024). Furthermore, BV has been shown to decrease the release of pro-inflammatory cytokines like TNF-α and IL-1β in a variety of cell models, suggesting that it may be used to treat inflammatory illnesses (Yun *et al*., 2021; Chung *et al*., 2016).The ability of bee venom constituents to decrease pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukins (IL-1β, or IL-6), as well as other inflammatory mediators, such as prostaglandin E2 (PGE2) and nitric oxide (NO), which are produced by cyclooxygenase (COX) and inducible nitric oxide synthase (iNOS), respectively, The production of these mediators have been demonstrated in several inflammatory tissues involved in the pathogenesis of several diseases, such as atherosclerosis, obesity, metabolic syndrome, diabetes, and several types of cancer. is demonstrated. (Nam *et al*., 2003, Jang *et al*., 2005, Janik *et al*., 2007, Karimzadeh *et al*., 2013, Mohammadi *et al*., 2015).

In vivo, experiments with Lewis rats also support the anti-inflammatory effects of Bee venom samples (Chang and Bliven, 1979; Amin and Abdel-Raheem, 2014).

Numerous mechanisms for the anti-inflammatory and/or anti-arthritis effects of Bee venom and its constituents have been reported in recent studies. The anti-arthritis effect of melittin is suggested to be related to the decrease in phospholipase (PL) A2, cyclooxygenase (COX)-2, and tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, nitric oxide (NO), and oxygen reactive species (ROS) levels (Pelletier *et al*., 1998; Yang *et al*., 1999; Amin *et al*., 1999; Cernanec *et al*., 2002; Murakami *et al*., 2017).

Real-time quantitative PCR (RT-qPCR) Results

Table 5 Effects of treatments on liver TNF-α, IL-1β, and NF-Kb Gene expression and Effects of treatments in apoptosis in rat's liver Bax. We used quantitative real-time PCR to determine changes in the mRNA levels of inflammatory genes (IL-1B - TNF-α- NK-Kb) and pro-apoptotic Bax genes. Bee venom slightly reduces mRNA level in IL-1B levels, while the toxic substance (cd) does not significantly alter IL-1B expression. However, the combination of bee venom and cd shows a more substantial reduction. Regarding TNF-α, Bee venom significantly reduces TNF-α levels. Conversely, cd increases TNF-α expression. Interestingly, the combination of bee venom and cd results in a marked increase in TNF-α. For NK-Kb, Bee venom dramatically reduces NF-Kb levels, nevertheless Cd significantly increases NF-Kb expression. However, when combined, bee venom appears to counteract the increase induced by cd, though not to the same extent as when used alone. Finally, Bee venom slightly increases Bax levels. Cd, on the other hand, causes a massive increase in Bax, The combination of both reduces Bax levels compared to cd alone but remains significantly elevated.

Table 5: Effects of treatments on liver TNF-α, IL-1β and NF-Kb Gene expression and Effects of treatments in apoptosis in rat's liver Bax.

Groups	IL-1β	TNF-α	NF-Kb	Bax
1st group(c)	1	1	1	1
2nd group (Bee venom)	0.93	0.79	0.22	1.24
3rd group (Cd)	1.01(1%)	1.09(9%)	3.86(286%)	40.57(3957%)
4thgroup (Both) cd+Bee venom	0.85 15% reduction	1.37 37% increase	0.81 19% reduction	11.74 1074% increase

Liver Histopathology

Cadmium is known to affect hepatocytic injuries directly, endothelial cell lining damage is also likely to be the cause in an in vivo system. According to Ince *et al*. (2016), damaged endothelial lining disrupts microcirculation, obstructing the liver cells' blood supply and resulting in hemorrhage, vacuolization, and sinusoidal widening in the surrounding area, or Stage I and II alterations. In the current investigation, the liver of rats treated with cadmium had different histological characteristics from those of the control group. The main alterations were disorganization of the hepatocytes' architecture with marked affection of the hepatocytes. Moreover, borderlines between the hepatocytes were destroyed, and irregular shapes and atypical positions within the hepatic tissue were observed. Some of these cells were hypertrophied and showed extensive cytoplasmic vacuolation and pyknotic nuclei of some hepatocytes were seen. In addition, the central veins and hepatic sinusoids appeared dilated and congested with areas of mononuclear cell infiltration. The sinusoidal Kupffer cells became prominent and increased in number (Figure 2C).

It could be brought on by Cd-induced lipid peroxidation and the subsequent production of extremely reactive radicals, the underlying cause of hepatocellular damage. The necrotic conditions match our biochemical findings, indicating a higher degree of lipid peroxidation.

Similarly to the control group, the hepatic sections of rats of the Bee venom group revealed normal microanatomy with slightly dilated hepatic sinusoids (Figue 2B). The liver of Cd + Bee venom-treated rats restored normal hepatic architecture that exhibited hepatocytic improvement with

mild congestion of central veins and hepatic sinusoids (Figure 2D). According to new research, Bee venom prevents liver damage both in vivo and in vitro by acting as an anti-inflammatory and anti-apoptotic agent (Lee *et al*., 2015). The hepatic cells treated with Bee venom showed promise for improvement, as evidenced by the current microscopic examination. Matched with previous research showed that The Kupffer cell had a nearly normal nucleus, and the mitochondria and glycogen were normal. Melittin from Bee venom was found to protect against severe failure of hepatocyte functions by reducing hepatic inflammatory reactions, lowering the high rate of lethality, and inhibiting hepatocyte apoptosis. This alleviated hepatic pathological injury (Lee and Bae, 2016; Aly *et al*., 2023).

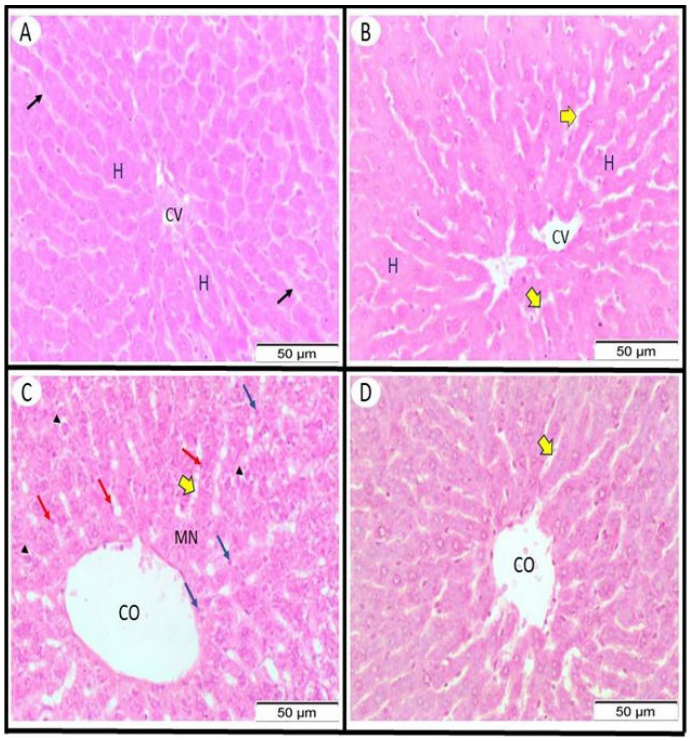

Figure 2: Representative photomicrograph of H&E-stained liver sections of control and all treated rats; control (A), Bee venom (B), Cd (C), both Bee venom + Cd (D). Central vein (CV), hepatocytes (H), hepatic sinusoids (black arrows), congested and dilated central vein (CO) and hepatic sinusoids (thick yellow arrows), pyknotic hepatocytes (blue arrows), hepatocytic vacuolization (arrowheads), Kupffer cell hyperplasia (red arrows), mononuclear cells infiltration (MN).

NF-kB signaling pathway is the most important pathway which is involved in liver inflammation. Indeed, NF-kB activation is connected to hepatocyte injury and liver fibrosis.

It was demonstrated that the administration of rat cadmium resulted in significant hepatotoxicity through the overproduction of several inflammatory mediators, including IL-6, NF-kB, and TNF-α (Lee *et al*., 2011).

Numerous research works have documented the in vivo and in vitro properties of Bee venom, including its anti-mutagenic, anti-inflammatory, anti-nociceptive, and radioprotective properties. It is shown that bee venom can protect Wistar rat lymphocytes from oxidative and basal DNA damage through radio-protective effect It does not cause oxidative damage at low concentrations and is not genotoxic (Gajski and Garaj-Vrhovac, 2009).

The hepatoprotective activity of Bee venom may have been attributed to its anti-inflammatory properties in addition to its antioxidant activity. Much evidence has documented that Bee venom modulated tissue inflammation in various experimental models. Also, recent research revealed that melittin inhibits IκB phosphorylation, which in turn inhibits the DNA-binding activity of NF-κB, a crucial transcriptional factor controlling the expression of inflammatory genes (Park *et al*., 2004, 2007).

The present study found that concurrent treatment with Bee venom had significantly decreased the serum IL-1β, IL-10, TNF- α, ALT, and AST levels, indicating the hepato-protective effect of Bee venom, which might be explained by the reduction of NF-kB expression in the liver of bee venom administrated group and the 3rd group that administrated both cadmium and bee venom compared to the cadmium administrated group that has high significant elevation of NF-kB expression Figure 3. This was further confirmed by histological examination of the liver Figure 2. These results are consistent with other studies that showed the potent hepato-protective effect of Bee venom by inhibiting the secretion of pro-inflammatory cytokines, such as TNF-a, and decreasing the elevated serum amino-transferase enzymes (Park *et al*., 2010; Kim *et al*., 2010).

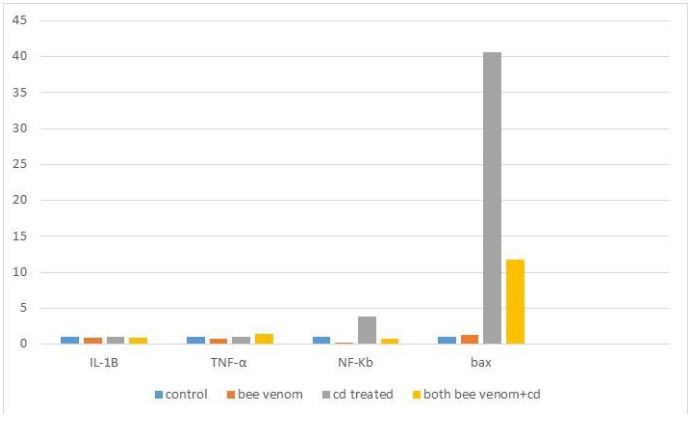

Figure 3: Effect of treatments on IL- 1β, TNF-α, NF-κB, and Bax genes expression in rat liver.

In the present study, we also found up-regulation expression of IL-1β, TNF-α, and NF-kB genes in rat liver, which confirms the results of previous studies on Cd's ability to induce inflammation.

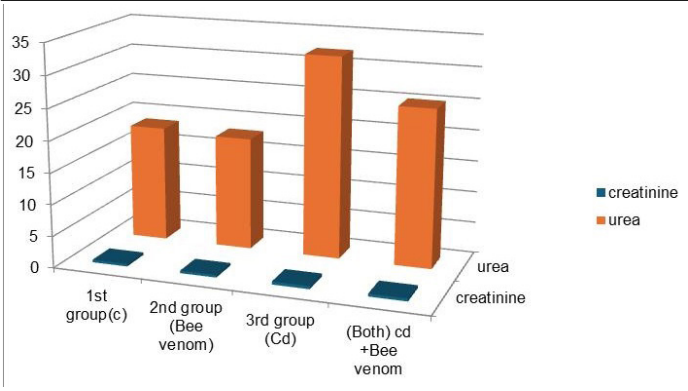

Figure 4: Effect of Bee venom and Cadmium chloride treatment in the activity of urea and Cr (mg/dl) in the serum of rats at the end of the experiment.

By inducing IL-1β and TNF-α overproduction, excessive ROS generation can activate the canonical pathway of NF-kB (Song *et al*., 2018). Cadmium exposure upregulates proinflammatory gene expression, including interleukin-1, interleukin-6, tumor necrosis factor-α, and interferon-γ, while downregulating anti-inflammatory cytokine IL-10 in rat testes (Sivaprakasam and Nachiappan, 2016). which is consistent with this study showing upregulation of both IL- 1β and TNF-α gene expression in cadmium administered group.

Kim *et al*., 2010 suggest that Bee venom possesses anti-fibrogenic properties that are mediated by the suppression of pro-inflammatory cytokines and fibrogenic gene expression like IL-1beta and TNF-alpha. which is consistent with this study showing down-regulation of both IL- 1β and TNF-α gene expression in Bee venom administered group.

In this study, we demonstrated that cadmium significantly increases the expression of the pro-apoptotic *Bax* gene in the liver. Mitochondria play a crucial role in regulating the intrinsic pathway of apoptosis, with Bax protein being a key regulator of this process. Excessive cadmium exposure in mitochondria triggers Bax activation. Bax interacts with components of the mitochondrial permeability transition pore (MPTP), causing the pores to open and allowing the release of cytochrome C (Cyt C) into the cytoplasm. This release ultimately activates caspase-3, leading to cell death. This is following prior studies that showed modulation of the same gene in apoptosis. (Alian, *et al*., 2018; Ghajari *et al*., 2019; Arab-Nozari *et al*., 2020; Mahdavi *et al*., 2018). Cadmium exposure has been shown to significantly impact BAX gene expression in various tissues.

Results showed that Bax expression was downregulated in the group administered bee venom with cadmium chloride compared to animals exposed to Cadmium only. Interestingly, bee venom administration suppressed apoptosis in the liver tissues of rats via down-regulating pro-apoptotic Bax. These findings were consistent with the data from (Park *et al*., 2012), which showed that in animals with GalN/LPS-induced acute hepatic failure, melittin (0.1 mg/kg) prevented acute hepatic failure by blocking NF-κB activation, caspase and Bax protein expression levels, and cytochrome c release.

The protective effect of bee venom from a molecular view mentioned by previous studiesThe principal BVT mechanisms. By controlling microglia activity, NF-κB transcription, the MAPK pathway, and the LC-descending coerulospinal pathway, BVT may have an anti-inflammatory effect. BVT reduces pro-apoptotic Bax and caspase-3 activity and increases apoptotic Bcl-2 expression to modify mitochondrial function. BVT may shield hepatocytes from harm in liver fibrosis by inhibiting pro-inflammatory cytokines, pro-apoptotic genes, and the fibrogenic gene. Blood vessel lipoid deposition is inhibited by BVT in the circulatory system through the suppression of pro-inflammatory cytokines, adhesion cytokines, anti-apoptosis genes, and fibrogenic genes like TGF-β. Additionally, in mice with atherosclerosis, BVT inhibits the migration and proliferation of vascular smooth muscle cells (VSMC) through the Akt pathway. (Zhang *et al*., 2018). Furthermore, it has been demonstrated that PLA2, a major component of bee venom, inhibits inflammatory reactions, protects against cisplatin-induced acute kidney injury (AKI), and regulates the expression of interleukin (IL)-10 and Tregs by binding to CD206 (Kim *et al*., 2015, Deng *et al*., 2001; Sinuani *et al*., 2013).

Park *et al*. (2014) showed that melittin prevented DNA damage to hepatocytes by preventing the activation of caspase, the bcl-2 family of proteins, and poly ADP-ribose polymerase (PARP)-1 via the NF-κB pathway. By suppressing p38/JNK and NF-B expression, bee venom successfully reduced PMA-induced MMP-9 gene expression, reducing MCF-7 cell invasion and migration (Cho *et al*., 2010).

Kidney Function Evaluation

An increasing amount of research has demonstrated that apoptosis plays a significant role in Cd-induced nephrotoxicity (Fujiwara *et al*., 2012; Erboga *et al*., 2016; Almeer *et al*., 2019; Zhuang *et al*., 2019). Significant renal damage was caused by cadmium exposure, as evidenced by decreased body and kidney weights, elevated serum BUN, and creatinine levels, histopathological lesions, inflammatory markers, and oxidative stress in the kidney. These results showed a significant increase in serum urea and creatinine in the Cd-administrated group compared with the Bee Venom-Cd group (Figure 4) The kidneys are a target organ for Cd toxicity, and the renal dysfunction seen in

this study may be caused by exposure to the metal during normal excretion (Ibrahim *et al*., 2018). parallel with previous studies that suggest a protective effect of bee venom on kidney function (Kim *et al*., 2020) that showed Melittin inhibited creatinine excretion and BUN. According to Clark, (1999), the current study discovered that after bee venom treatment, uric acid increased and kidney function, including urea and creatinine, decreased (Figure 4).

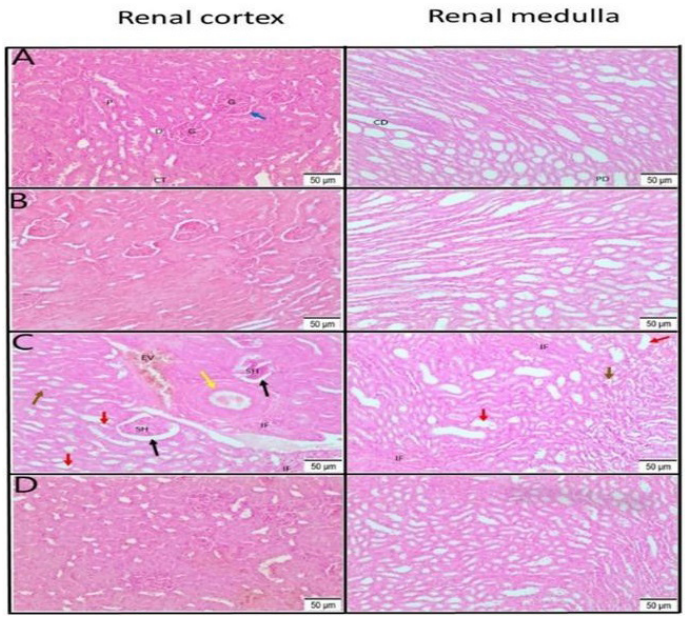

Figure 5: Representative photomicrograph of HandE-stained renal tissue sections (cortex and medulla) of control and all treated rats. control (A), Bee venom (B), Cd (C), both Bee venom + Cd (D) Glomerulus (G), Bowman' capsule (blue arrow), proximal convoluted tubule (P), distal convoluted tubule (D), collecting tubules (CT), collecting duct (CD), papillary duct (PD), shrunken glomeruli (SH) with wide subcapsular capsular spaces (black arrows), interstitial inflammatory cells infiltration (IF), vascular congestion (yellow arrow), extravasated red blood cells (EV), dilatation of tubular lumen (red arrows), fragmented and sloughed epithelial cells (brown arrows).

In contrast to previous reports, our study found that exposure to Cd resulted in tissue damage not only in the renal cortex but also in the renal medulla. (Figure 5) This was evidenced by pyknotic nuclei in the epithelial cells of collecting ducts and clear hyperemia in the renal medulla. Furthermore, it was discovered by (Nazima *et al*., 2015) that glomerular atrophy, renal capsule dilatation, tubular degeneration, and necrosis were altered in albino Wistar rats that were given intragastric administration of CdCl2 (5 mg/kg) for four weeks. according to Imed *et al*. (2008) Observing that the reports of Cd-induced alterations in renal histopathology varied, it is possible that variations in Cd exposure occurred due to variations in exposure time, dosage, route, and animal strain. In our study, rats in the Bee venom- Cd administrated group showed significantly lesser histological damage in the kidney than the Cd administrated group. Furthermore, bee venom reduced cadmium-induced renal tissue damage in a dose-dependent manner. Consistent with previous studies that suggest Melittin prevented renal tubular damage in mice that was caused by cisplatin (Kim *et al*., 2020). These findings imply that bee venom protects against functional and structural damage caused by lipopolysaccharide (LPS) using renoprotection (Kim *et al*., 2020) additionally, it was reported that the administration of bee venom improved the renal damage caused by unilateral ureteral obstruction in terms of both structure and function (An *et al*., 2015). According to Hyunseong *et al*. (2013), Bee venom can prevent renal tubular injury (epithelial necrosis) Table 6.

Table 6: Effect of Bee venom and Cadmium chloride treatment in the activity of urea and Cr (mg/dl) in the serum of rats at the end of the experiment.

	Urea	Creatinine
1st group(c)	18.53±0.4256	0.4133±0.01202
2nd group (Bee venom)	18±0.3606	0.3967±0.008819
3rd group (Cd)	32.13±0.9939 73.4%increase	0.4833±0.003333 16.9 % increase
4th group (Both) cd +Bee venom	25.17±1.049 21.7% decrease	0.45±0.005774 7.8% decrease

The obtained results of the brain histological lesions in the Cd-treated group cerebrum revealed pronounced congestion in both cortical blood vessels and pia mater. Moreover, pronounced degenerated neurons with pyknotic nuclei, and vacuolar spaces around the pyramidal cells were noted. The pyramidal cells were more affected than the cerebellar blood capillaries and also showed marked congestion, in addition to degenerated and shrunken Purkinje cells with the appearance of intracellular vacuoles. Most Purkinje cells have irregular outlines with deep homogenous cytoplasm with the absence of Nissl granules and darkly stained pyknotic nuclei (Figure 6). parallel with previous studies Concerning the cadmium-induced pathological lesions observed, in the cerebellum, medulla oblongata, and cerebrum, chromatolysis, pyknosis, and neuronal degeneration have all happened by cadmium treatment (Maodaa *et al*., 2016).

Interestingly, the Cd + Bee venom-treated group showed a marked improvement in the histological structures of cerebral and cerebellar cortical tissues compared to that obtained from the Cd-treated group. Most cerebral and cerebellar neuronal cells had a similar appearance to the control group (Figure 6) and with the explanations According to Abd El-Hameed *et al*. (2021), Bee venom has a neuroprotective effect. Its anti-inflammatory and anti-apoptotic characteristics corroborate this in the experimental models' hippocampal tissues (Lee and Bae, 2016). In male rats ex-

posed to MMC sub-acute intoxication, Bee venom has an in vivo neuroprotective effect by modifying the MMC-induced neurobehavioral deviations, inflammation, oxidative stress, altered TJPs, and TGF-β gene relative mRNA expression at the BBB of the exposed rats (Abu-Zeid *et al.*, 2021). According to the points mentioned before of the kidney and brain lesion evaluation, we suggest that the control negative group score is (0) no lesion present, the bee venom administrated group score is (0) no lesion present, the cadmium administered group score is (4) very severely affected tissue, finally the group administrated both bee venom and cadmium score is (1) mildly affected tissue.

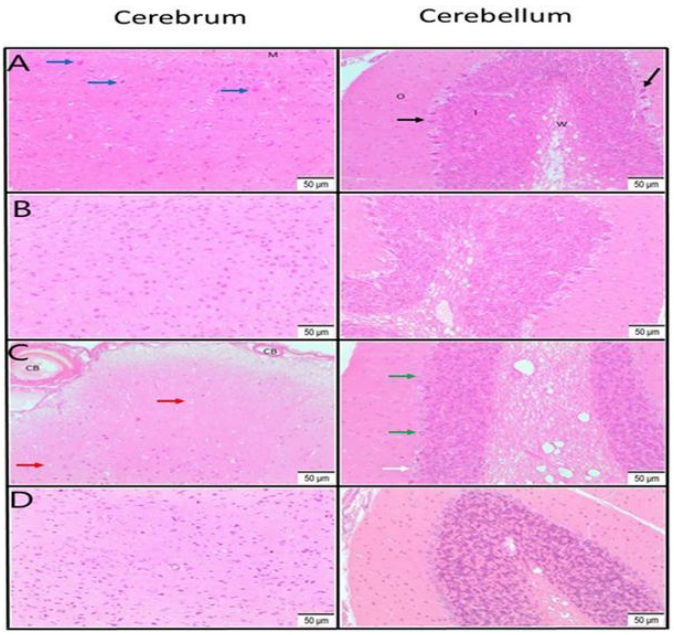

Figure 6: Representative photomicrograph of HandE-stained cerebral and cerebellar tissue sections of control and all treated rats; control (A), Bee venom (B), Cd (C), both Bee venom + Cd (D)... Cerebral molecular layer (M), different-sized pyramidal neuronal cells (blue arrows), congested blood vessels (CB), and vacuolar spaces around the pyramidal cells (red arrows). The cerebellar folium consists of molecular (O), Purkinje cell layer (black arrows), granular layer (I), and the inner white matter (W). Shrunken Purkinje cells (green arrows), intracellular vacuoles (white arrows).

CONCLUSIONS AND RECOMMENDATIONS

For the first time, Bee venom showed that has a beneficial effect against hepatotoxicity, nephrotoxicity, and neurotoxicity induced by Cd exposure. As a possible mechanism, Bee venom could ameliorate Cd hepatotoxicity, nephrotoxicity, and neurotoxicity owing to its antioxidant, anti-inflammatory, and antiapoptotic properties.

NOVELTY STATEMENT

This study provide novel insights into the combined effects of bee venom and cadmium on key biochemical ,oxidative ,and inflammatory markers in rats, focusing on the unique potential of BV to mitigate cad-induced toxicity ,while previous studies have examined the effects of either BV or Cd individually.

AUTHOR'S CONTRIBUTIONS

Prof. Rania Helmi Abdou and Prof. Kawthar Abdelwahed contributed to the experiment design and revision of the manuscript,Dr Mary Ayad sargious handled the molecular part of experiment (RT-PCR) and wrote it.Dr.Omnia Mohammed khattab contributed to manuscript`s writing,editing and revising. Dr. Mariam Ismail Ali contributed to the performing of the experiment ,writing ,editing,and revising the manuscript.

Conflict of Interest

All authors have no conflict of interest to disclose.

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