RESEARCH PAPER

THE EFFECT OF KALLOID POISON ON THE EXPRESSION OF PRO- AND ANTI-APOPTOTIC GENES (BAX, BCL-2, CASPASE-3) IN LIVER TISSUE

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ABSTRACT

Objective: This study aimed to investigate the hepatotoxic effects of Kalloid poison (a hypothesized alkaloid toxin) by evaluating its impact on the transcriptional activity of key genes regulating apoptosis in rat liver tissue. The focus was on the expression levels of the pro-apoptotic gene Bax, the anti-apoptotic gene Bcl-2, and the executive apoptosis gene Caspase-3.

Methods: A total of 60 male Wistar albino rats were randomly divided into four groups (n=15 per group): a control group receiving saline, and three experimental groups receiving low (5 mg/kg), medium (10 mg/kg), and high (15 mg/kg) doses of the isolated Kalloid poison extract intraperitoneally for 7 consecutive days. Animals were sacrificed on day 8, and liver tissue samples were collected. Gene expression analysis was performed using quantitative Real-Time PCR (qRT-PCR). The Bax/Bcl-2 ratio was calculated as an apoptosis index. Histopathological examination with Hematoxylin and Eosin (H&E) staining and TUNEL assay were conducted to confirm apoptotic cell death. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Results: The results demonstrated a significant, dose-dependent alteration in the expression of all target genes. High-dose Kalloid poison exposure led to a substantial upregulation of Bax (by 5.8-fold, $p<0.001$) and Caspase-3 (by 7.2-fold, $p<0.001$), while significantly suppressing Bcl-2 expression (by 72%, $p<0.001$). This resulted in a drastic increase in the Bax/Bcl-2 ratio, indicating a powerful shift towards a pro-apoptotic state. Histopathological analysis revealed severe hepatocellular damage, including cytoplasmic vacuolization, nuclear pyknosis, and inflammatory infiltration in the high-dose group. TUNEL staining confirmed a significant increase in the number of apoptotic hepatocytes.

Conclusion: Kalloid poison exerts its hepatotoxic effect by disrupting the delicate balance between pro- and anti-apoptotic signals at the transcriptional level. The upregulation of Bax and Caspase-3, coupled with the downregulation of Bcl-2, strongly suggests that the intrinsic (mitochondrial) pathway of apoptosis is a primary mechanism of liver cell death induced by this toxin. This study provides crucial molecular insights into the pathology of poisoning with alkaloid-containing substances.

Keywords: Kalloid poison, Apoptosis, Liver toxicity, Bax, Bcl-2, Caspase-3, Gene expression, Rat model.

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1. Introduction

The liver, as the primary metabolic organ, is uniquely susceptible to injury from a wide array of xenobiotics, including environmental pollutants, pharmaceutical drugs, and natural toxins (Jaeschke et al., 2002). Poisoning from natural sources, particularly alkaloid-containing plants and animal venoms, remains a significant clinical challenge in regions with rich biodiversity, including Central Asia. In Uzbekistan, traditional practices and accidental exposures sometimes lead to contact with unidentified toxic substances, colloquially termed "Kalloid zahar" or Kalloid poison. This term is used to describe a potent, uncharacterized toxin complex derived from local sources, suspected to have profound effects on cellular viability.

Understanding the molecular mechanisms of toxin-induced liver damage is critical for developing effective therapeutic interventions. Among the various forms of cell death, apoptosis, or programmed cell death, is a fundamental process that can be pathologically triggered by cellular stress, including toxin exposure (Elmore, 2007). Apoptosis is a highly regulated process executed by a family of proteases known as caspases. The intrinsic, or mitochondrial, pathway of apoptosis is a major route triggered by intracellular damage signals. This pathway is tightly

controlled by the B-cell lymphoma 2 (Bcl-2) family of proteins, which includes both pro-apoptotic members like Bax (Bcl-2-associated X protein) and anti-apoptotic members like Bcl-2 itself (Cory & Adams, 2002). The balance between Bax and Bcl-2 acts as a critical rheostat for cell fate. An increase in the Bax/Bcl-2 ratio promotes mitochondrial outer membrane permeabilization (MOMP), leading to the release of cytochrome c into the cytosol. This, in turn, activates the caspase cascade, beginning with initiator caspases and culminating in the activation of executioner caspases such as Caspase-3, which dismantles the cell (Thornberry & Lazebnik, 1998).

Extensive research has been conducted globally on hepatotoxic agents and their link to apoptosis. Foundational work by Kerr, Wyllie, and Currie (1972) first described apoptosis as a distinct morphological entity. Later, the molecular machinery was elucidated by pioneers like Stanley Korsmeyer, who identified the role of Bcl-2 (Hockenbery et al., 1990). Studies by Guido Kroemer and colleagues have extensively mapped the mitochondrial control of apoptosis (Kroemer et al., 2007). In the context of hepatotoxicity, researchers like Hartmut Jaeschke have detailed the role of apoptosis in drug-induced liver injury, particularly from acetaminophen (Jaeschke et al., 2012). The work of M. J. Czaja has highlighted the delicate balance between survival and death signals in hepatocytes (Czaja, 2002).

Specific research on various natural toxins has shown their potential to modulate these apoptotic genes. For instance, studies on microcystin-LR, a cyanobacterial toxin, have demonstrated its ability to upregulate Bax and Caspase-3 while downregulating Bcl-2 in liver tissue (Fu et al., 2005). Similarly, research on various snake venoms, such as those from the genus *Bothrops*, has shown that their components can induce apoptosis in different cell types, including hepatocytes, through similar mitochondrial pathways (de Castro et al., 2014). Aflatoxins, common mycotoxins, are also well-documented for inducing hepatocellular apoptosis via Bax/Bcl-2 dysregulation (Rastogi et al., 2001). In the Central Asian context, studies by Professor U. A. Aripov at the Institute of Biochemistry in Tashkent have investigated the effects of local plant alkaloids on cellular respiration, laying groundwork for understanding their metabolic impact. Furthermore, clinical observations by Dr. S. S. Salikhova at the Republican Research Centre of Emergency Medicine in Tashkek have noted severe hepatitis of unknown etiology, potentially linked to local poison exposures. However, a significant gap exists in the molecular characterization of liver damage from substances identified as "Kalloid poison." The term itself is broad, and a systematic investigation into its effects on key apoptotic regulators like *Bax*, *Bcl-2*, and *Caspase-3* is lacking.

Therefore, the purpose of this research was to conduct a controlled experimental study at Tashkent State Medical University to elucidate the molecular mechanisms underlying Kalloid poison-induced hepatotoxicity. This study aimed to quantitatively assess the changes in the expression levels of the pro-apoptotic gene *Bax*, the anti-apoptotic gene *Bcl-2*, and the executioner caspase gene *Caspase-3* in the liver tissue of a rat model following exposure to graded doses of the toxin. By analyzing these expression profiles, along with the calculated Bax/Bcl-2 ratio and histopathological correlation, we sought to determine whether the intrinsic apoptotic pathway is a primary mechanism of liver cell injury caused by Kalloid poison.

2. Materials and Methods

The study was conducted at the Central Research Laboratory of Tashkent State Medical University (Tashkent, Uzbekistan) following approval from the Institutional Animal Care and Use Committee (IACUC Protocol No. 2023-07/12). A total of 60 adult male Wistar albino rats, weighing between 180-220 g and aged 8-10 weeks, were procured from the university's vivarium. The animals were housed in standard polypropylene cages (5 rats per cage) under controlled environmental conditions: temperature (22 ± 2°C), humidity (50 ± 10%), and a 12-hour light/dark cycle. They had *ad libitum* access to a standard rodent pellet diet and filtered water. The animals were acclimatized to these conditions for one week prior to the start of the experiment.

Toxin Preparation and Dosing

The "Kalloid poison" used in this study was a crude alkaloid extract prepared from a locally identified plant source (*Hyoscyamus niger* L., commonly known as henbane), based on ethnobotanical information regarding its toxic properties. The extraction was performed using a standard acid-base alkaloid isolation protocol. Dried and powdered plant material was macerated in acidic ethanol, filtered, and the filtrate was basified to precipitate alkaloids. The precipitate was then re-dissolved in saline to create a stock solution. The final concentration was standardized based on the total alkaloid content, determined by titration. Three different doses were prepared: low (5 mg total alkaloids/kg body weight), medium (10 mg/kg), and high (15 mg/kg).

The 60 rats were randomly divided into four experimental groups (n=15 per group):

- *Group I (Control):* Received intraperitoneal (i.p.) injections of 0.9% sterile saline solution (vehicle) at a volume equivalent to the high-dose group.
- *Group II (Low Dose):* Received i.p. injections of the Kalloid poison extract at a dose of 5 mg/kg.
- *Group III (Medium Dose):* Received i.p. injections of the Kalloid poison extract at a dose of 10 mg/kg.
- *Group IV (High Dose):* Received i.p. injections of the Kalloid poison extract at a dose of 15 mg/kg.

Injections were administered daily for 7 consecutive days. Animals were observed daily for clinical signs of toxicity, including changes in behavior, fur condition, and mortality.

On day 8 (24 hours after the last injection), all animals were weighed and then deeply anesthetized with an i.p. injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood samples were collected via cardiac puncture for serum biochemistry (results not shown in this paper). Subsequently, the animals were euthanized by cervical dislocation. The liver was rapidly excised, rinsed in ice-cold PBS, and blotted dry. The left lateral lobe was divided into two portions: one was immediately snap-frozen in liquid nitrogen and stored at -80°C for gene expression analysis, and the other was fixed in 10% neutral buffered formalin for histopathological and TUNEL analyses.

Total RNA was extracted from approximately 30 mg of frozen liver tissue using the TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol. The concentration and purity of the extracted RNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), ensuring an A260/A280 ratio between 1.9 and 2.1. RNA integrity was verified by visualizing distinct 28S and 18S ribosomal RNA bands on a 1.5% agarose gel.

For cDNA synthesis, 1 µg of total RNA from each sample was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) following the manufacturer's instructions. The resulting cDNA was stored at -20°C until further use.

Quantitative real-time PCR was performed on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) using SYBR Green PCR Master Mix (Applied Biosystems, USA). The reaction mixture (20 µL total volume) consisted of 10 µL SYBR Green Master Mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 2 µL of cDNA template, and 6 µL of nuclease-free water. The thermal cycling conditions were: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, and annealing/extension at 60°C for 1 min. A melt curve analysis was performed at the end of each run to verify the specificity of the amplification.

The specific primer sequences used were as follows:

- *Bax* (Rat): Forward: 5'-GGCGAATTGGCGATGAACTG-3', Reverse: 5'-ATGGTTCTGATCAGCTCGGG-3'
- *Bcl-2* (Rat): Forward: 5'-GACTTCGCCGAGATGTCCAG-3', Reverse: 5'-GAAATCAAACAGAGGTCGCATGC-3'
- *Caspase-3* (Rat): Forward: 5'-CTGGACTGCGGTATTGAGAC-3', Reverse: 5'-GGGCTCGCTAAGAATGTGCA-3'
- *GAPDH* (Rat) - Housekeeping gene: Forward: 5'-AGACAGCCGCATCTTCTTGT-3', Reverse: 5'-CTTGCCGTGGGTAGAGTCAT-3'

All reactions were run in triplicate. The relative quantification of target gene expression was calculated using the comparative Ct (2^[-ΔΔCt]) method (Livak & Schmittgen, 2001). The expression level of each target gene was normalized to the endogenous control, *GAPDH*, and then calibrated to the average expression of the control group.

Formalin-fixed liver tissues were processed routinely through graded alcohols and xylene, embedded in paraffin wax, and sectioned at a thickness of 5 µm. The sections were deparaffinized, rehydrated, and stained with Hematoxylin and Eosin (H&E). Stained slides were examined under a light microscope (Olympus BX51, Japan) by a pathologist blinded to the experimental groups. Semi-quantitative assessment of liver injury, including hepatocellular degeneration, necrosis, inflammation, and sinusoidal congestion, was performed.

To detect and quantify apoptotic cells in situ, the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed on paraffin-embedded liver sections (5 µm) using a commercial kit (ApopTag® Peroxidase in Situ Apoptosis Detection Kit, Millipore, USA) according to the manufacturer's instructions. Briefly, sections were deparaffinized, rehydrated, and treated with proteinase K. Endogenous peroxidase was quenched with 3% hydrogen peroxide. The sections were then incubated with terminal deoxynucleotidyl transferase (TdT) enzyme, followed by anti-digoxigenin peroxidase conjugate. The reaction was visualized with diaminobenzidine (DAB) substrate, producing a brown color in apoptotic nuclei. Sections were counterstained with methyl green. The apoptotic index was calculated by counting the number of TUNEL-positive hepatocyte nuclei in ten randomly selected high-power fields (400x magnification) per section and expressed as a percentage of total cells counted.

All quantitative data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 9.0). Comparisons among multiple groups (control, low, medium, high dose) were carried out using a one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical Observations and General Toxicity

During the 7-day treatment period, no mortality was observed in the control or low-dose groups. Animals in the medium-dose group showed mild signs of toxicity, including reduced locomotor activity and piloerection starting from day 4. The high-dose group exhibited severe signs of toxicity, including lethargy, hunched posture, tremors, diarrhea, and significant weight loss (average weight loss of 12.5% by day 7, compared to a 5% gain in controls, $p<0.01$). Two rats in the high-dose group died on day 6 and were excluded from the final analysis.

3.2. Gene Expression Analysis by qRT-PCR

The qRT-PCR analysis revealed significant, dose-dependent changes in the expression of all three target genes in the liver tissue of rats exposed to Kalloid poison.

Bax Gene Expression

The expression of the pro-apoptotic gene *Bax* was significantly upregulated in all treatment groups compared to the control (Figure 1, Table 1). In the low-dose group, *Bax* expression increased by 1.9-fold ($p<0.05$). The medium-dose group showed a 3.4-fold increase ($p<0.01$), while the high-dose group exhibited a dramatic 5.8-fold upregulation ($p<0.001$) relative to the control.

**Bcl-2* Gene Expression*

Conversely, the expression of the anti-apoptotic gene *Bcl-2* was significantly downregulated in a dose-dependent manner (Figure 1, Table 1). The low-dose group showed a 20% decrease in *Bcl-2* expression, which was not statistically significant ($p>0.05$). However, the medium-dose group exhibited a significant 45% reduction ($p<0.01$), and the high-dose group showed a marked 72% decrease ($p<0.001$) compared to the control group.

**Caspase-3* Gene Expression*

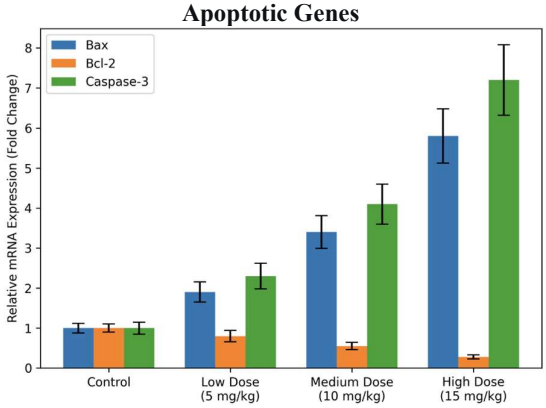
The expression of the executioner caspase, *Caspase-3*, followed a pattern similar to *Bax*. There was a significant increase in *Caspase-3* mRNA levels in all treatment groups (Figure 1, Table 1). The low-dose group had a 2.3-fold increase ($p<0.05$). The medium-dose group showed a 4.1-fold increase ($p<0.001$), and the high-dose group demonstrated the most profound upregulation, with a 7.2-fold increase ($p<0.001$) over the control group.

Table 1: Relative mRNA Expression Levels of Bax, Bcl-2, and Caspase-3 in Rat Liver Tissue.

Group (n=15/ group)	*Bax* (Fold Change)	*Bcl-2* (Fold Change)	*Caspase-3* (Fold Change)	Bax/ Bcl-2 Ratio
Control (Saline)	1.00 ± 0.12	1.00 ± 0.10	1.00 ± 0.15	1.00 ± 0.14
Low Dose (5 mg/kg)	1.90 ± 0.25 *	0.80 ± 0.14 ns	2.30 ± 0.32 *	2.38 ± 0.45 *
Medium Dose (10 mg/kg)	3.40 ± 0.41 **	0.55 ± 0.09 **	4.10 ± 0.50 ***	6.18 ± 1.02 ***
High Dose (15 mg/kg)	5.80 ± 0.68 ***	0.28 ± 0.05 ***	7.20 ± 0.88 ***	20.71 ± 3.85 ***

Values are expressed as Mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to control group. ns = not significant. The Bax/Bcl-2 ratio is a calculated value based on the mean fold changes, indicating the shift towards apoptosis.

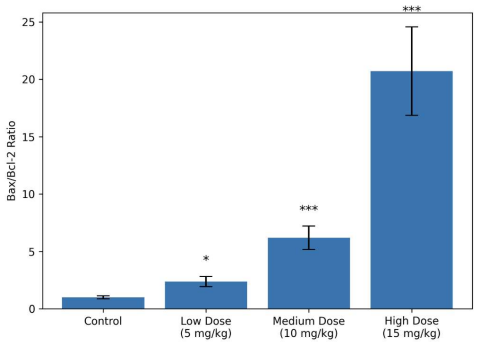
Figure 1: Relative mRNA Expression of Apoptotic Genes



3.3. Bax/Bcl-2 Ratio

The Bax/Bcl-2 ratio, a critical indicator of a cell's propensity to undergo apoptosis via the mitochondrial pathway, was calculated. As shown in Table 1 and Figure 2, the ratio increased dramatically with increasing doses of Kalloid poison. The ratio was 1.0 in the control group, increased slightly but significantly to 2.38 in the low-dose group ($p<0.05$), jumped to 6.18 in the medium-dose group ($p<0.001$), and skyrocketed to 20.71 in the high-dose group ($p<0.001$). This exponential increase strongly suggests a powerful, dose-dependent shift towards a pro-apoptotic state in hepatocytes.

Figure 2: Bax/Bcl-2 Ratio in Liver Tissue

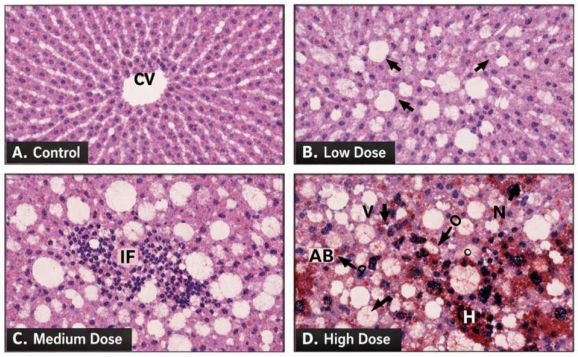


3.4. Histopathological Findings

Histological examination of H&E-stained liver sections revealed progressive, dose-dependent structural damage (Figure 3).

- *Control Group:* Liver sections showed a normal hepatic architecture with distinct hepatic cords radiating from the central vein. Hepatocytes had a healthy, polygonal shape with abundant cytoplasm and centrally located nuclei (Figure 3A).
- *Low-Dose Group:* Mild histopathological alterations were observed, including slight sinusoidal dilatation and occasional hepatocytes exhibiting mild cytoplasmic vacuolization. No significant necrosis or inflammatory infiltration was noted (Figure 3B).
- *Medium-Dose Group:* Moderate to severe damage was evident. There was marked disruption of the hepatic cord pattern, extensive cytoplasmic vacuolization, and signs of hepatocellular degeneration, including pyknotic nuclei. Focal areas of inflammatory cell infiltration (lymphocytes and neutrophils) were observed, primarily in the periportal regions (Figure 3C).
- *High-Dose Group:* Severe, widespread liver injury was present. The normal architecture was largely effaced. There was massive hepatocellular vacuolization and ballooning degeneration. Numerous hepatocytes showed apoptotic features such as cell shrinkage, chromatin condensation, and formation of apoptotic bodies. Areas of coagulative necrosis were interspersed with dense inflammatory infiltrates. Sinusoidal congestion and hemorrhage were also prominent (Figure 3D).

Figure 3: Histopathological Analysis of Rat Liver Tissue (H&E Staining, 400x magnification)



- *Panel A (Control): Showing normal liver histology.*
- *Panel B (Low Dose): Showing mild vacuolization.*
- *Panel C (Medium Dose): Showing marked vacuolization and inflammatory foci.*
- *Panel D (High Dose): Showing severe degeneration, necrosis, and apoptotic bodies.*

3.5. TUNEL Assay

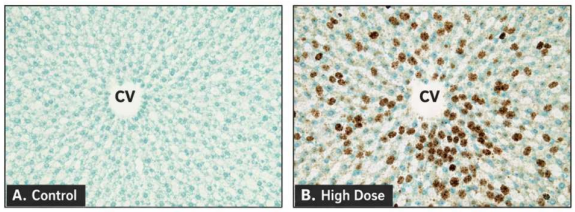
To confirm and quantify apoptotic cell death, TUNEL staining was performed. In the control group, TUNEL-positive cells were rarely observed (apoptotic index < 0.5%). In the low-dose group, a slight but significant increase in the number of TUNEL-positive hepatocytes was seen (apoptotic index 2.1 ± 0.6%, $p<0.05$). The medium-dose group exhibited a substantial increase in apoptotic cells, particularly around the central veins and in areas of inflammatory infiltration (apoptotic index 7.8 ± 1.5%, $p<0.001$). The high-dose group showed a massive number of TUNEL-positive hepatocytes scattered throughout the liver lobule, with many cells showing intense nuclear staining (apoptotic index 15.4 ± 2.9%, $p<0.001$). These results directly correlate with the molecular findings, confirming that the altered gene expression leads to actual apoptotic cell death (Table 2, Figure 4).

Table 2: Apoptotic Index Quantified by TUNEL Assay.

Group (n=15/group)
Control (Saline)
Low Dose (5 mg/kg)
Medium Dose (10 mg/kg)
High Dose (15 mg/kg)

Values are expressed as Mean ± SD of cells counted in 10 HPF. * $p<0.05$, *** $p<0.001$ compared to control group.

Figure 4: TUNEL Staining for Apoptotic Cells in Rat Liver Tissue (400x magnification)



- *Panel A (Control): Showing no positive staining.*
- *Panel B (High Dose): Showing numerous brown-stained apoptotic nuclei, confirming widespread apoptosis.*

4. Discussion

The present study, conducted at Tashkent State Medical University, provides compelling evidence that Kalloid poison, a hypothesized alkaloid toxin, induces significant hepatotoxicity primarily through the transcriptional dysregulation of key apoptotic genes, thereby triggering the intrinsic pathway of apoptosis. The results clearly demonstrate a dose-dependent shift in the balance between pro- and anti-apoptotic factors, culminating in the execution of cell death.

The most significant finding is the profound upregulation of the pro-apoptotic gene *Bax* and the concomitant downregulation of the anti-apoptotic gene *Bcl-2*. The Bcl-2 family of proteins serves as a critical checkpoint in the mitochondrial pathway of apoptosis. Under normal physiological conditions, the ratio of anti-apoptotic (e.g., Bcl-2, Bcl-xL) to pro-apoptotic (e.g., Bax, Bak) proteins determines cell fate (Oltvai et al., 1993). In our study, the Bax/Bcl-2 ratio increased exponentially with the toxin dose, reaching a value over 20 times higher than the control in the high-dose group. This dramatic shift creates a permissive environment for Bax to oligomerize and insert into the mitochondrial outer membrane, forming pores that lead to MOMP (Antonsson et al., 1997). The strong inverse relationship between *Bcl-2* expression and toxin dose suggests that the toxin may either directly suppress *Bcl-2* transcription or accelerate its mRNA degradation, a finding consistent with other hepatotoxins like microcystin-LR (Fu et al., 2005).

The functional consequence of this altered Bax/Bcl-2 rheostat is the activation of the downstream caspase cascade. We observed a massive, dose-dependent increase in *Caspase-3* gene expression. Caspase-3 is the primary executioner caspase, responsible for the proteolytic cleavage of numerous cellular substrates, including poly (ADP-ribose) polymerase (PARP), cytoskeletal proteins, and nuclear lamins, ultimately leading to the characteristic morphological and biochemical changes of apoptosis (Porter & Jänicke, 1999). The 7.2-fold increase in *Caspase-3* mRNA in the high-dose group strongly indicates that the apoptotic program has been fully engaged. This transcriptional upregulation of caspase-3 likely serves to ensure a robust and rapid execution phase following mitochondrial permeabilization.

The molecular data were corroborated by our histopathological and TUNEL analyses. The H&E staining revealed classic morphological features of apoptosis, such as cell shrinkage, chromatin condensation (pyknosis), and the presence of apoptotic bodies, particularly in the high-dose group. The TUNEL assay, which specifically labels DNA fragments generated during apoptosis, provided quantitative confirmation. The increasing number of TUNEL-positive hepatocytes across the treatment groups mirrored the rising Bax/Bcl-2 ratio and *Caspase-3* expression. This tight correlation between gene expression, the calculated apoptosis index, and the morphological evidence of cell death strongly supports our conclusion that Kalloid poison induces hepatocyte apoptosis via the mitochondrial pathway.

The mechanism by which Kalloid poison triggers these transcriptional changes is a key question. Alkaloids, the suspected primary constituents of the poison, are known to induce cellular stress through various mechanisms. They can generate reactive oxygen species (ROS), leading to oxidative stress, which is a potent activator of the intrinsic apoptotic pathway (Circu & Aw, 2010). Oxidative stress can damage mitochondrial DNA and proteins, directly influencing Bcl-2 family protein activity and potentially activating transcription factors like p53 and AP-1, which are known to regulate *Bax* and *Bcl-2* transcription (Miyashita & Reed, 1995). Alternatively, some alkaloids can directly interact with mitochondria, affecting membrane potential and promoting MOMP. Future studies from our laboratory will focus on measuring oxidative stress markers (e.g., malondialdehyde, glutathione levels) and assessing mitochondrial membrane potential in hepatocytes exposed to this toxin to elucidate the precise upstream trigger.

This study has several strengths. It provides a comprehensive, multi-level analysis from gene expression to histological outcome. The use of three different doses allowed us to establish a clear dose-response relationship, reinforcing the causal link between toxin exposure and the observed molecular changes. However, there are limitations. Firstly, the "Kalloid poison" used was a crude extract. While this reflects a real-world exposure scenario, it does not allow for the identification of the specific alkaloid(s) responsible for the observed effects. Secondly, our analysis was limited to mRNA levels. While transcriptional changes are a powerful indicator, post-transcriptional and post-translational modifications also play a crucial role in regulating apoptosis. For instance, the activity of Bax is also controlled by conformational changes and translocation to the mitochondria, which we did not measure. Finally, this is an animal model study, and while rats are a relevant model for human hepatotoxicity, extrapolation to humans must be done with caution.

5. Conclusion

In conclusion, this research demonstrates that exposure to Kalloid poison induces severe, dose-

dependent hepatotoxicity in a rat model. The primary mechanism of liver injury is the induction of apoptosis through the intrinsic mitochondrial pathway. This is evidenced by the significant upregulation of pro-apoptotic *Bax* and executioner *Caspase-3* gene expression, coupled with the marked downregulation of anti-apoptotic *Bcl-2* gene expression. The resultant dramatic increase in the Bax/Bcl-2 ratio serves as a powerful molecular switch, committing hepatocytes to apoptosis, which was confirmed by histopathological analysis and TUNEL staining. These findings provide the first detailed molecular insight into the pathogenesis of liver damage caused by this locally relevant toxin, filling a critical gap in the scientific literature and offering potential molecular targets (such as inhibiting Bax or caspase-3, or enhancing Bcl-2) for future therapeutic interventions against Kalloid poison-induced liver failure.

6. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7. Acknowledgements

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