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Phytochemical Composition and Acute Toxicity of Aqueous Stem Bark Extract of *Adenium obesum* in African Catfish (*Clarias gariepinus*)

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Abstract: The continued use of botanical piscicides for artisanal fishing has generated increasing concern regarding their potential impacts on non-target aquatic organisms. *Adenium obesum* (Forssk.) Roem. & Schult. is a well-known indigenous piscicidal plant in many parts of Africa due to its rich phytochemical composition. However, scientific information linking its phytochemical constituents with its acute toxicity in economically important freshwater fish remains limited. This study investigated the phytochemical composition and acute toxicity of the aqueous stem bark extract of *A. obesum* in juvenile of *Clarias gariepinus*. Fresh stem bark of *A. obesum* was collected, processed, and extracted using distilled water. Qualitative phytochemical screening was conducted using standard analytical methods to identify the major secondary metabolites present in the extract. Acute toxicity was evaluated using a 96-hour static bioassay in which juvenile *C. gariepinus* were exposed to six concentrations (0.0, 6.5, 8.0, 9.5, 11.0, and 12.5 mg/L) of the aqueous stem bark extract of *A. obesum*. Behavioural responses and mortality were recorded throughout the exposure period, and the 96-hour median lethal concentration (LC_{50}) was estimated by probit analysis. Proximate analysis showed that the stem bark contained predominantly nitrogen-free extract (54.62%), followed by crude fibre (21.34%), crude protein (9.10%), ash (7.75%), moisture (5.65%), and crude lipid (1.54%). Qualitative phytochemical screening revealed the presence of alkaloids, saponins, steroids, flavonoids, terpenes, and tannins, indicating that the extract is rich in biologically active secondary metabolites. Fish exposed to the extract exhibited concentration-dependent behavioural alterations, including erratic swimming, increased opercular movement, excessive mucus secretion, loss of equilibrium, air gulping, reduced swimming activity, and respiratory distress. Mortality increased progressively with increasing extract concentration, while no mortality was observed in the control group. The estimated 96-hour LC_{50} was 6.31 mg/L, indicating that the aqueous stem bark extract possesses high acute toxicity toward juvenile *C. gariepinus.* The aqueous stem bark extract of *A. obesum* contains diverse bioactive phytochemicals that are likely responsible for its pronounced ichthyotoxic activity. The concentration-dependent behavioural abnormalities and relatively low 96-hour LC_{50} demonstrate that the extract is highly toxic to juvenile African catfish. These findings provide scientific evidence supporting the traditional use of *A. obesum* as a botanical piscicide while highlighting the potential ecological risks associated with its indiscriminate use in freshwater ecosystems. The study contributes valuable baseline information for environmental risk assessment and the sustainable management of inland fisheries.

Keywords: *Adenium obesum*; phytochemical screening; African CatFish; Acute toxicity; Behavioural responses.

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1. Introduction

The increasing global demand for fish as a major source of animal protein has intensified fishing activities, particularly in developing countries where inland fisheries contribute substantially to food security and rural livelihoods (FAO The State of World Fisheries and Aquaculture 2024). In many African countries, traditional fishing communities continue to employ plant-derived piscicides because they are inexpensive, readily available, and biodegradable compared with many synthetic chemicals (Fafioye, 2005; Wink, 2018). Despite their natural origin, botanical piscicides contain biologically active secondary metabolites capable of causing physiological disturbances and mortality in both target and non-target aquatic organisms (Fazio, 2019; Witeska, 2023).

Adenium obesum (Forssk.) Roem. & Schult. (Apocynaceae), commonly known as the desert rose, is widely distributed throughout tropical and subtropical Africa. The plant has long been used in traditional medicine and as a source of arrow poison and fish poison because of its rich phytochemical composition (Burkill, 2000; Elgorashi et al., 2013). The stem bark, roots, latex, and leaves contain numerous bioactive compounds, including cardiac glycosides, alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and phenolic compounds, many of which exhibit antimicrobial, cytotoxic, insecticidal, and ichthyotoxic activities (Elgorashi et al., 2013; Wink, 2018).

Phytochemicals are naturally occurring secondary metabolites synthesized by plants for defense against herbivores, pathogens, and environmental stress. These compounds possess diverse biological activities and are responsible for many of the medicinal and toxicological properties of higher plants (Wink, 2018). Cardiac glycosides inhibit Na^+/K^+-ATPase activity, saponins increase membrane permeability, while alkaloids and phenolic compounds may induce oxidative stress and interfere with normal cellular metabolism (Wink, 2018; Fazio, 2019). Consequently, phytochemical screening is an essential step in identifying the constituents responsible for the biological activity of botanical piscicides.

Acute toxicity bioassays are internationally accepted methods for evaluating the short-term adverse effects of chemicals and botanical extracts on aquatic organisms. The 96-hour median lethal concentration (LC_{50}) remains one of the most widely used toxicological endpoints for comparing the toxicity of different substances and for ecological risk assessment (Organisation for Economic Co-operation and Development, 2019). In addition to mortality, behavioural alterations such as erratic swimming, loss of equilibrium, excessive mucus secretion, rapid opercular movement, and air gulping serve as early indicators of physiological stress and impending toxicity in fish (Fazio, 2019; Witeska, 2023).

The African catfish, *Clarias gariepinus* (Burchell, 1822), is among the most economically important freshwater fish species cultured in Africa because of its rapid growth, high market value, tolerance to adverse environmental conditions, and suitability for aquaculture (Gabriel et al., 2019). Owing to these characteristics, the species has become an important experimental model in aquatic toxicology and environmental monitoring.

Although *A. obesum* has long been recognized as a potent botanical piscicide, relatively little information is available on the relationship between its phytochemical constituents and acute toxicity in *C. gariepinus*. Addressing this knowledge gap is important for understanding the mechanisms responsible for its ichthyotoxic activity and for evaluating the ecological consequences associated with its continued use in freshwater ecosystems.

Therefore, the present study investigated the phytochemical composition of the aqueous stem bark extract of *A. obesum* and evaluated its acute toxicity in juvenile *C. gariepinus*. Specifically, the study identified the principal phytochemical constituents of the extract, assessed behavioural responses during exposure, determined cumulative mortality, and estimated the 96-hour LC_{50}. The findings provide scientific evidence for evaluating the ecological risks associated with the use of *A. obesum* as a botanical piscicide.

2. Materials and Methods

2.1 Study Area

The study was conducted in the Fisheries and Hydrobiology Laboratory, Department of Biological Sciences, Sa'adu Zungur University, Bauchi, Nigeria. Laboratory experiments were carried out under controlled conditions to evaluate the phytochemical composition and acute toxicity of aqueous stem bark extract of *A. obesum* in juvenile *C. gariepinus*. Water quality parameters were monitored throughout the experiment to ensure that observed responses resulted primarily from exposure to the plant extract rather than environmental fluctuations.

2.2 Collection and Authentication of Plant Material

Fresh stem bark of *A. obesum* (Forssk.) Roem. & Schult. was collected from Kafin Madaki, Ganjuwa Local Government Area, Bauchi State, Nigeria. The plant was authenticated in the Department of Biological Sciences, Sa'adu Zungur University, Bauchi, where a voucher specimen was deposited for future reference. The collected stem bark was washed thoroughly with distilled water to remove dirt and debris before being air-dried under shade at room temperature until a constant weight was obtained. The dried bark was pulverized into a fine powder using a laboratory electric grinder and stored in airtight containers until extraction.

2.3 Preparation of Aqueous Stem Bark Extract

The aqueous extract was prepared following the procedure described in the dissertation. The powdered stem bark was soaked in distilled water and thoroughly homogenized to facilitate extraction of water-soluble phytochemicals. The mixture was allowed to stand for the prescribed extraction period before being filtered first through muslin cloth and subsequently through Whatman No. 1 filter paper. The filtrate constituted the stock solution from which the required experimental concentrations were prepared immediately before use.

2.4 Qualitative Phytochemical Screening

Qualitative phytochemical analysis of the aqueous stem bark extract was carried out using standard analytical procedures described by Harborne (1998), Trease and Evans (2009), and Sofowora (2008). The extract was screened for the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, steroids, terpenoids, anthraquinones, phenols, and other secondary metabolites based on characteristic colour changes and precipitation reactions. The presence or absence of each phytochemical constituent was recorded accordingly.

2.5 Experimental Fish

Healthy juvenile African catfish (*C. gariepinus*) of similar body size and weight were procured from a commercial fish farm in Bauchi State, Nigeria. The fish were transported to the laboratory in aerated containers and acclimatized for two weeks in dechlorinated tap water before the commencement of the experiment. During acclimation, fish were fed a commercial pelleted diet at approximately 3% of their body weight twice daily. Feeding was discontinued 24 h before exposure to minimize the influence of feeding on water quality and physiological responses. Dead or unhealthy fish were removed immediately throughout the acclimation period.

2.6 Experimental Design and Acute Toxicity Test

The acute toxicity bioassay was conducted using a completely randomized design. Fish were exposed to six concentrations of aqueous stem bark extract of *A. obesum* comprising a control (0 mg/L) and five treatment concentrations (6.5, 8.0, 9.5, 11.0, and 12.5 mg/L) for 96 h. The exposure concentrations were selected based on preliminary range-finding tests as described in the dissertation. Each treatment was replicated, and fish were randomly assigned to experimental tanks to minimize bias. During the exposure period, the test solutions were renewed according to the experimental protocol to maintain relatively constant toxicant concentrations and acceptable water quality.

2.7 Behavioural Observations and Mortality Assessment

Fish were observed continuously during the initial hours of exposure and subsequently at regular intervals throughout the 96-h experimental period. Behavioural changes including hyperactivity, erratic swimming, rapid opercular movement, excessive mucus secretion, loss of equilibrium, air gulping, reduced swimming activity, and mortality were recorded. Dead fish were removed immediately after observation to prevent deterioration of water quality. Percentage mortality was calculated for each treatment concentration and used in the estimation of acute toxicity.

2.8 Determination of 96-h Median Lethal Concentration (LC_{50})

The 96-h median lethal concentration (LC_{50}) of aqueous stem bark extract of *A. obesum* was determined from cumulative mortality data obtained during the acute toxicity test. Mortality percentages were transformed into probit values and regressed against the logarithm of the corresponding exposure concentrations. The LC_{50} value and its 95% confidence limits were estimated using probit analysis following standard toxicological procedures described in the dissertation. The calculated LC_{50} was subsequently used as the basis for selecting concentrations employed in the sublethal toxicity study.

2.9 Water Quality Assessment

Water temperature (°C), pH, dissolved oxygen (mg/L), electrical conductivity (µS/cm), and total dissolved solids (mg/L) were monitored throughout the experiment using calibrated portable water quality meters. These parameters were maintained within acceptable limits for the survival of *C. gariepinus* to ensure that observed mortality and behavioural responses were attributable primarily to the plant extract rather than adverse environmental conditions.

2.10 Statistical Analysis

Data were expressed as mean ± standard error (SE). Statistical analyses were performed using IBM SPSS Statistics software. Differences among treatment groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test for post hoc comparisons where significant differences were detected. Statistical significance was accepted at $p < 0.05$. The 96-h LC_{50} was estimated by probit analysis based on cumulative mortality recorded during the acute toxicity test.

3. Results

3.1 Proximate Composition of Adenium obesum Stem Bark

The proximate composition of the aqueous stem bark of *A. obesum* is presented in Table 1. The analysis showed that nitrogen-free extract (NFE; total carbohydrate) constituted the largest proportion of the stem bark (54.62%), followed by crude fibre (21.34 ± 0.26%), crude protein (9.10 ± 0.29%), ash (7.75 ± 0.04%), moisture (5.65 ± 0.13%), and crude lipid (1.54 ± 0.35%). The relatively high carbohydrate and fibre contents indicate that the stem bark is rich in structural and energy-yielding components, whereas the low lipid content suggests that fats constitute only a minor fraction of the plant material.

Table 1: Proximate Composition of *Adenium obesum* Stem Bark

Parameters	**Percentage (%)**
Moisture content	5.65±0.13
Crude lipid (fat)	1.54±0.35
Crude fibre	21.34±0.26
Ash content	7.75±0.04
Crude protein	9.10±0.29
NFE	54.62

NFE (Nitrogen Free Extract)

3.2 Qualitative Phytochemical Composition of *A. obesum* Stem Bark

The qualitative phytochemical profile of the aqueous stem bark extract is presented in Table 2. Six major classes of secondary metabolites were detected in the extract. Alkaloids, saponins, and steroids were strongly present (+++), flavonoids and terpenes were moderately present (++), while tannins were detected at a weak (+) level. Anthraquinones and other phytochemical constituents were not reported in the qualitative screening.

Overall, the phytochemical analysis demonstrated that the aqueous stem bark extract of *A. obesum* contains several biologically active compounds that may contribute to its piscicidal activity and acute toxicity in *C. gariepinus.*

Table 2: Qualitative Profile of Phytochemicals in *Adenium obesum* Stem Bark

Phytochemicals	**Status**	**Strength**
Alkaloid	Present	+++
Saponin	Present	+++
Flavonoid	Present	++
Steroids	Present	+++
Terpenes	Present	++
Tannin	Present	+

Strong (+++), Moderate (++), Mild/Weak (+), Moderate (-)

3.3 Acute Toxicity of Aqueous Stem Bark Extract of *Adenium obesum*

The acute toxicity of aqueous stem bark extract of *A. obesum* against juvenile *C. gariepinus* is summarized in Table 3, while the concentration-response relationship is illustrated in Figure 1. Mortality increased progressively with increasing extract concentration, demonstrating a clear dose-dependent toxic effect. No mortality was recorded in the control group throughout the 96-h exposure period, whereas complete (100%) mortality occurred in fish exposed to the highest concentration (12.50 mg/L).

Probit analysis of cumulative mortality data estimated the 96-h median lethal concentration (LC_{50}) of the aqueous stem bark extract to be 8.22 mg/L, with upper and lower confidence limits of 9.10 mg/L and 7.41 mg/L, respectively. These findings indicate that the aqueous stem bark extract of *A. obesum* exhibits considerable acute toxicity to juvenile *C. gariepinus* under laboratory conditions.

The concentration-response curve demonstrated a positive relationship between extract concentration and fish mortality, indicating increasing toxic potency with increasing concentrations of the botanical extract.

Table 3: Mean Mortality Rate of *Clarias gariepinus* Exposed to various Acute Concentrations of *Adenium obesum* Stem Bark Extracts.

Con. (mg/L)	Log con.	No. of fish	Mean mortality from replicates					% mortality	Probit mortality
			12	24	48	72	96hrs		
12.5	1.0969	10	4.5	5.5	-	-	-	100	8.7190
11	1.0414	10	-	4.5	3.5	1	-	90	6.2816
9.5	0.9777	10	-	1.5	3	1.5	1	70	5.5244
8	0.9031	10	-	-	1.5	1.5	1	40	4.7467
6.5	0.8129	10	-	-	-	-	1.5	20	4.1584
0.0	0.0000	10	-	-	-	-	-	0.0	0.0000

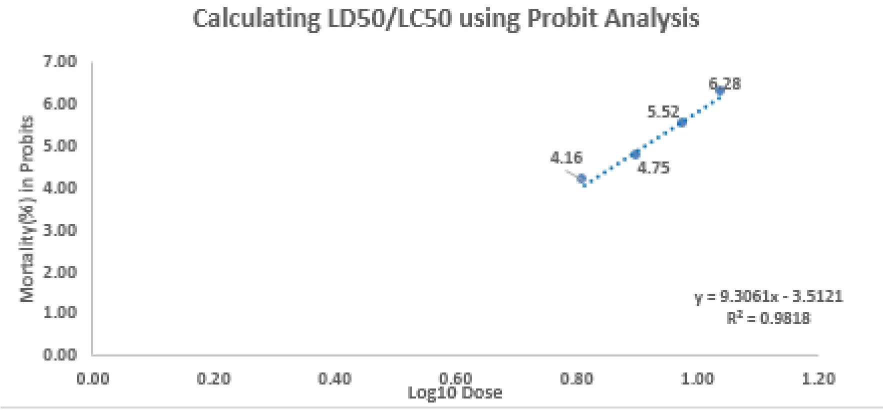

Figure 1 Linear relationship between probit mortality and log concentration (mg/L) of *Clarias gariepinus* exposed to various acute concentrations of *Adenium obesum* crude stem bark extracts.

3.4 Behavioural Responses of *Clarias gariepinus* During Acute Exposure

Behavioural changes observed during the acute toxicity test are presented in Table 4. Fish in the control group maintained normal swimming behaviour and exhibited no signs of stress throughout the exposure period. In contrast, fish exposed to *A. obesum* extract displayed concentration-dependent behavioural alterations shortly after exposure.

The most frequently observed behavioural responses included increased opercular movement, erratic and jerky swimming, loss of equilibrium, excessive mucus secretion, reduced swimming activity, skin discolouration, frequent surfacing, air gulping, and respiratory distress. These responses became progressively more pronounced with increasing extract concentration and exposure time. Fish exposed to the lower concentrations (6.5 and 8.0 mg/L) exhibited relatively mild behavioural disturbances, whereas individuals exposed to 9.5, 11.0, and 12.5 mg/L displayed severe behavioural impairment, culminating in mortality in several individuals before the end of the 96-h exposure period.

Overall, the behavioural observations demonstrated that the severity of toxic responses increased proportionally with extract concentration and preceded mortality, suggesting that behavioural endpoints are sensitive indicators of acute toxicity in *C. gariepinus* exposed to aqueous stem bark extract of *A. obesum*.

Table 4: Effect of Acute Concentration of *Adenium obesum* Stem Bark Extracts on Behavioural Responses of *Clarias gariepinus* Juveniles

Behavioural responses	**Concentrated Grades of *Adenium obesum Extract* (mg/L)**					
	0.00	**6.5**	**8.0**	**9.5**	**11.0**	**12.5**
Equillibruim status	+++	+++	++	+	-	-
Respiratory disturbance	-	+	++	++	-	-
Jerky movement	-	++	++	+++	-	-
Skin discolouration	-	-	+	++	+++	+++
Swimming rate	+++	+++	++	+	-	-
Fin movement	+++	+++	++	+	-	-

None (-), Mild (+), Moderate (++), Strong (+++)

4. Discussion

The present study demonstrated that the aqueous stem bark extract of *A. obesum* contains several biologically active secondary metabolites and exhibits pronounced acute toxicity toward juvenile *C. gariepinus*. The phytochemical profile, behavioural responses, concentration-dependent mortality, and estimated 96-h LC_{50} collectively confirm the ichthyotoxic potential of the plant and provide scientific evidence supporting its traditional use as a botanical piscicide. The findings also highlight the ecological risks associated with indiscriminate application of *A. obesum* in freshwater ecosystems.

The proximate analysis revealed that the stem bark was composed predominantly of nitrogen-free extract (carbohydrates), followed by crude fibre, crude protein, ash, moisture, and lipid. Although proximate composition is not a direct measure of toxicity, it provides useful information regarding the nutritional and structural characteristics of the plant material. The relatively high carbohydrate and fibre contents indicate that the stem bark contains abundant structural polysaccharides that may facilitate the storage and stabilization of secondary metabolites within plant tissues (Aremu et al., 2021). Similar proximate compositions have been reported for several medicinal plants used traditionally as botanical pesticides, where carbohydrates and fibre constitute the major fractions while biologically active phytochemicals occur in comparatively smaller quantities (Trease & Evans, 2009).

Qualitative phytochemical screening demonstrated the presence of alkaloids, saponins, steroids, flavonoids, terpenes, and tannins in the aqueous stem bark extract. These secondary metabolites have been widely recognized as the principal bioactive constituents responsible for the pharmacological and toxicological activities of medicinal plants (Wink, 2018). Among these compounds, saponins and cardiac glycosides are particularly important because of their well-documented ichthyotoxic properties. Saponins disrupt membrane integrity by interacting with membrane sterols, thereby increasing membrane permeability and causing cellular damage, while cardiac glycosides inhibit Na^+/K^+-ATPase activity, leading to impairment of cardiac and neuromuscular function (Elgorashi et al., 2013; Wink, 2018). Alkaloids may interfere with neurotransmission, whereas flavonoids and tannins may alter enzyme activity and induce oxidative stress under high concentrations (Fazio, 2019). The coexistence of these phytochemicals suggests that the acute toxicity observed in the present study is likely the result of synergistic interactions among multiple bioactive compounds rather than the action of a single constituent.

The behavioural responses observed during the acute toxicity test represent typical manifestations of toxic stress in fish exposed to botanical extracts. Fish exposed to *A. obesum* exhibited erratic swimming, hyperactivity, increased opercular movement, loss of equilibrium, excessive mucus secretion, surfacing behaviour, air gulping, and reduced swimming activity. These abnormalities became progressively more severe as extract concentration increased, indicating a concentration-dependent physiological disturbance. Behavioural alterations are among the earliest indicators of toxicant exposure because they reflect rapid impairment of the nervous, respiratory, and osmoregulatory systems before irreversible tissue damage occurs (OECD, 2019; Witeska, 2023). Similar behavioural responses have been reported in *C. gariepinus* and other freshwater fishes exposed to botanical piscicides and environmental contaminants, where respiratory distress results primarily from gill irritation and impaired oxygen uptake (Ayotunde et al., 2019; Kanu et al., 2023).

The excessive mucus secretion observed in exposed fish is likely a protective response aimed at minimizing direct contact between the toxicant and the gill epithelium. However, excessive mucus accumulation may itself reduce respiratory efficiency by increasing the diffusion distance for oxygen across the gill surface. Likewise, increased opercular movement and frequent air gulping indicate attempts by the fish to compensate for declining oxygen availability resulting from impaired branchial function. As exposure progressed, the loss of equilibrium and reduced swimming performance suggest progressive disruption of neuromuscular coordination, possibly arising from the action of cardiac glycosides and alkaloids on excitable tissues. Comparable observations have been documented in acute toxicity studies involving plant-derived piscicides and synthetic toxicants (Fazio, 2019; Witeska, 2023).

Mortality increased progressively with increasing concentrations of the aqueous stem bark extract, demonstrating a clear dose-dependent toxic response. The estimated 96-h LC_{50} of 6.31 mg/L indicates that *A. obesum* possesses considerable toxicity toward juvenile C. gariepinus. According to commonly used aquatic toxicity classifications, substances with LC_{50} values below 10 mg/L are generally regarded as highly toxic to fish (OECD, 2019). The relatively low LC_{50} obtained in the present study therefore confirms the strong piscicidal potential of *A. obesum* and supports its traditional application for fish harvesting in many African communities. Similar LC_{50} ranges have been reported for other plant-derived piscicides containing saponins and cardiac glycosides, suggesting that these compounds contribute substantially to acute fish mortality (Ayotunde et al., 2019).

The concentration-dependent mortality observed in this study can be attributed to cumulative physiological dysfunction resulting from the combined actions of the phytochemical constituents. Initially, exposure to the extract elicited behavioural disturbances associated with respiratory stress and neurological impairment. Continued exposure likely intensified gill damage, disrupted ion regulation, impaired energy metabolism, and ultimately resulted in loss of homeostasis and death. Such sequential progression from behavioural alteration to mortality has been widely described in ecotoxicological studies and underscores the value of behavioural observations as early warning indicators of acute toxicity (Witeska, 2023).

The findings of the present study have important ecological implications. Although *A. obesum* is traditionally regarded as a natural and environmentally acceptable piscicide, its potent toxicity suggests that indiscriminate application in natural water bodies may adversely affect non-target fish species and other aquatic organisms. Botanical piscicides are often assumed to be environmentally safe because they are biodegradable; however, rapid biodegradation does not eliminate the possibility of severe acute toxicity immediately following application (Burgos-Aceves et al., 2019). Repeated or uncontrolled use of *A. obesum* may therefore contribute to reductions in fish abundance, alterations in community composition, and disruption of freshwater ecosystem processes.

The present study provides one of the few comprehensive evaluations linking the phytochemical composition of *A. obesum* stem bark with its acute toxicity in *C. gariepinus*. By integrating phytochemical screening, behavioural observations, mortality assessment, and LC_{50} estimation, the study provides a mechanistic basis for understanding the ichthyotoxic activity of this important botanical piscicide. These findings contribute valuable baseline information for ecological risk assessment, support the development of evidence-based regulations governing the use of plant-derived piscicides, and provide a scientific foundation for future investigations involving purification of individual bioactive compounds, histopathological assessment, and molecular toxicology.

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