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RESEARCH ARTICLE

Assessing oral acute toxicity and histopathological effects of *Strelitzia reginae* Aiton leaf extracts in Zebrafish (*Danio rerio* Hamilton)

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Abstract

Strelitzia reginae, commonly known as the Bird of Paradise, is a decorative shrub endemic to southern Africa. This study marks the first comprehensive investigation into the safety of *S. reginae* leaf extract through oral acute toxicity assessments and histopathological examinations in Zebrafish (*Danio rerio*). The interest in this research arises from the historical use of *S. reginae* components by various indigenous South African societies to treat conditions like swollen glands and sexual problems. GC-MS analysis was used along with traditional methods to look at the phytochemical parts of *S. reginae.* The results showed the presence of several substances, such as eicosane, hexacosane, 1-octadecene, and neophytadiene. Notably, the analysis also identified certain chemicals with potential cytotoxic properties, such as octacosane and bis (2-ethylhexyl) phthalate. Drawing upon the biological similarities between Zebrafish and humans, who share a majority of their genes, this study represents the first attempt to evaluate the toxicity and histopathology of *S. reginae* using *D. rerio* as the test model, aligning with the OECD recommendations outlined in Article 203. The oral acute toxicity tests were done using ethanolic leaf powdered extracts of *S. reginae*. Higher concentrations (1200 mg/L) were toxic, but lower doses were less harmful to *D. rerio*. As observed in the histopathology examination, exposure to higher concentrations of *S. reginae* extract induced severe histological abnormalities in the Zebrafish's gills, liver, kidneys, intestines, and brain. This work contributes greatly to our understanding of *S. reginae's* safety profile and its potential therapeutic applications for enhancing well-being.

Keywords

Strelitzia reginae ; *Danio rerio* ; Mineral content; GC-MS ; Toxicity; Histopathology

Introduction

Plants offer a vast array of valuable chemical compounds with potential medicinal applications. Natural compounds that are both antioxidants and therapeutics increase demand by fighting the bad effects of free radicals. They are also less harmful than the synthetic antioxidants that are commonly used in drugs, cosmetics, and food (1, 2).

In many Asian and African nations, plant-based medicines serve as the primary healthcare option for about 80% of the population. In regions like Africa and India, as many as 90% and 70% of people, respectively, rely

on traditional alternative medicine. The Western world is also increasingly recognizing the holistic approach and improved patient tolerance associated with herbal remedies (3). Unlike synthetic drugs, humans have consumed herbal remedies over centuries and possess time-proven safety. However, in herbal medicine, toxicological assessment is paramount to identify adverse effects for protecting humanity (4).

While many therapeutic herbs are generally safe and have less harmful effects when used by humans, some can be toxic to both humans and animals, necessitating a comprehensive risk assessment. Compared to synthetic drug agents, which can be costly and may have adverse side effects, herbal remedies stand out for their accessibility, affordability, and potential efficacy (5). Zebrafish (*Danio rerio*), a tiny tropical bony fish with a brief life cycle and genetic similarities to humans, have emerged as an attractive model organism for nonclinical research (6). Their physiological responses and histological characteristics resemble those of mammals, making them suitable for toxicological testing. Zebrafish's small size and low body weight make them ideal for study because they only need a minimal amount of the substance being studied; they are regarded as a viable tool for preclinical studies since they have about 70% genetic similarity to humans despite their evolutionary distance from humans (7, 8).

Histopathology, the study of tissue changes, plays a crucial role in identifying substances that could harm specific organs or tissues. Zebrafish serve as an excellent model for histopathological assessments (9, 10).

Strelitzia reginae belongs to the plant family Strelitziaceae, a perennial plant commonly called the bird of paradise due to its distinct resemblance to a bird (11). The plant is adapted to warm weather and has big leaves and colorful flowers. As reported, the Abakwa Mthethwa clan in KwaZulu-Natal uses the strained decoctions from roots and inflorescence to treat venereal diseases and inflamed glands (12). Additionally, milk on the Cape is soured using seeds. Proanthocyanin polymers from the leaves and delphinidin-3-rutinoside (used for colour) have also been extracted from flower petals (13). The plant is said to be dangerous to dogs, cats, horses, and livestock, in addition to being slightly toxic to humans. According to reports, the leaves only include a small amount of hydrocyanic acid, while the fruits and seeds have deadly tannins. Clinical signs of poisoning include gastrointestinal distress, diarrhea, moderate nausea, sleepiness, and vomiting. They appear after chewing or swallowing the leaf of *S. reginae.*

Preclinical and clinical stages of toxicity evaluation in drug discovery will facilitate the identification of toxicants that can be improved or discarded into a safer alternative medicine (14). Thus, the purpose of this study was to analyze the mineral content, identify bioactive chemicals through GC-MS analysis, assess the toxicity of plant extracts, and examine their adverse effects on Zebrafish through histopathological studies.

Materials and Methods

Plant Material and Extract Preparation

Strelitzia reginae (Authentication/SMPU/CARI/BNG/2013-24/1095) was collected from Lalbagh Botanical Garden, Mavalli, Bengaluru, Karnataka. The plant's leaves were powdered after being dried and ground. About 10 g of the dry powder were extracted using the Soxhlet method in 150 mL of ethanol. To filter the extract, a rotary evaporator was utilized, and several test solution concentrations were made (15).

Atomic Absorption Spectroscopy (AAS)

A dried leaf sample of about 0.5 grams was extracted and treated overnight with HNO_3 (4 mL) and H_2O_2 (2 mL). The material was then heated for digestion at 220 °C for 5 minutes before being chilled. Distilled and deionized water were used to provide a sample volume of 25 ml (14). Following filtering, analyses of several minerals, including Ca, Fe, Mg, Zn, Mn, Cd, Cu, and Pb, were performed using a SHIMADZU AA-6880 flame atomic absorption spectrometer with a wavelength range of 185-900 nanometers, an optical double beam photometric method with a photomultiplier tube as a detector, and an air-C_2H_2 flame (16).

Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectroscopy (GC-MS) was used to identify volatile components in an ethyl acetate extract of *S. reginae* leaves. A 1 g plant sample was soaked in ethyl acetate (10 mL) and shaken for 24 hours in a rotary shaker. The extract was then collected in a test tube and dried in a hot air oven. The bioactive chemicals in *S. reginae* leaf were analyzed using a GC-MS QP2010SE system (Shimadzu) provided with an improved scan speed procedure for high-speed data collection and processing. For the spectroscopic detection method of the GC-MS, an electron ionization system with high-energy (70 eV) electrons was employed. A column-flow rate of 1.25 ml/min was employed for the carrier gas, which was pure helium gas. The temperature increased from its initial setting of 100 °C by 3 °C/min after a holding period of roughly 10 minutes. The temperature was eventually raised to 280 °C at a 10 °C/min rate. The range of 1.5-1000 mass-to-charge (m/z) was used to get the mass spectrum. A spitless injection of one microliter of 1% extract diluted with the appropriate solvents was made. The relative quantity of the chemical constituents contained in each extract was recorded as a percentage of the chromatogram's peak area. To identify bioactive compounds, GC retention lengths and computer techniques were utilized to compare the spectra to standard values (17).

Oral Acute Toxicity of Strelitzia reginae Ethanolic Leaf Extracts

To assess the oral acute toxicity of ethanolic leaf extracts derived from *S. reginae* plant samples, the study employed Zebrafish (*D. rerio*) following the guidelines outlined in OECD 203 (18). A total of seven Zebrafish were utilized in the experiment. The Zebrafish were initially housed in a tank filled with 100-liter of water equipped with a

circulation device, maintained under a 16-hour light/8-hour dark cycle, and provided with a diet consisting of pellets at 1% of their body weight. Following a seven-day habituation period, the Zebrafish were transferred to individual fish bowls, each with a volume of 10 liters. A 24-hour acclimatization period was allowed before commencing the toxicity tests. Throughout the experiments, dechlorinated tap water was used. To ensure consistent conditions, the Zebrafish were subjected to a 48-hour fast. After the fast, the toxicity tests with various doses of *S. reginae* leaf extracts at concentrations of 600, 700, 800, 900, 1000, and 1200 mg/L were introduced in the tanks and subsequently monitored for mortality of the Zebrafish at intervals of 24, 48, 72, and 96 hours. Seven healthy Zebrafish were promptly introduced into each of the prepared concentrations. A control group consisting of seven fish was maintained for each treatment. The experiment recorded mortalities at 24, 48, 72, and 96 hours after exposure. Subsequently, LC_{50} values, indicative of the concentration at which 50% mortality occurred, were calculated as part of the analysis (19).

Histopathology of Zebrafish (D. rerio) from Oral Acute Toxicity Test

The dead Zebrafish had their gills, liver, kidneys, intestines, and brain extracted and stained with (H&E) haematoxylin and eosin in accordance with predetermined procedures. Testing for behavioral and motor toxicity will be done in the diving tank and swim motion. After the 96-hour exposure period, the organs (gills, liver, kidney, intestines, and brains tissues) of the experimental fish from the tank with the highest mortality based on the LC_{50} will be removed and preserved in neutral buffered formalin (10%). Embedded in a block using melted paraffin, sectioned at a thickness of 5 m utilizing a microtome device, and stained with haematoxylin and eosin. Using a microscope and a digital camera, the glass slides will be studied for specific histopathological abnormalities in the gills, liver, kidney, intestines, and brain tissues (15).

Results

Atomic Absorption Spectroscopy for Mineral Analysis

The macro and micro components of *S. reginae* leaf extract are shown in Table 1. The results demonstrate that leaf extracts had a higher Ca concentration, followed by manganese, iron, magnesium, zinc, copper, lead, and cadmium in the list of minerals.

Gas Chromatography-Mass Spectrometry Analysis

GC-MS was used to figure out what volatile secondary metabolites were in the leaf extract of *S. reginae* (Table 2). Fig. 1 depicts the chromatogram of the ethyl acetate leaf extract. Bioactive substances such as squalene, neophytadiene, octacosane, hexacosane, nonadecane, and eicosane are found to be present in the plant extract. Other compounds like bis (2-ethylhexyl) phthalate and octacosane are also present. The retention time, compound name, area percentage, chemical formula, molecular weight, and bioactivity of secondary metabolites are also presented in the Table 2.

Oral Acute Toxicity

The oral acute toxicity studies were conducted with *D. rerio* (Zebrafish) using ethanolic leaf extracts of *S. reginae* according to OECD guidelines under 203. In this toxicity investigation, concentration-dependent changes in fish mortality were seen after exposure to plant sample leaf extract for 24, 48, 72, and 96 hours (Table 3). For 24, 48, 72,

Table 1. Mineral assay in the leaf extract of *S. reginae*

Types of Elements	Samples	Concentration in PPM
Macro-elements		
Ca	Leaf	45.34±0.45
Fe	Leaf	2.65±0.016
Mg	Leaf	1.40±0.0006
Zn	Leaf	0.45±0.004
Micro-elements		
Cd	Leaf	0.002±0.0003
Cu	Leaf	0.005±0.01
Mn	Leaf	4.34±0.10
Pb	Leaf	0.06±0.006

Table 2. Bioactive compounds of *S. reginae* leaf ethyl acetate extract

S.N	R. Time	Compound Name	Area %	Chem. Formula	Mol. Weight	Bioactivity
1	23.625	Squalene	26.81	$C_{30}H_{50}$	410	Antioxidant, antitumor
2	16.605	Neophytadiene	11.57	$C_{20}H_{38}$	278	Analgesic, antipyretic, antioxidant, and anti-inflammatory properties; treat skin diseases, rheumatism, and headache
3	22.537	Octacosane	7.94	$C_{28}H_{58}$	394	Larvicidal and cytotoxicity potential
4	24.471	Hexacosane	9.90	$C_{26}H_{54}$	366	Antimicrobial
5	17.159	1-Octadecene	3.54	$C_{18}H_{34}$	250	Antifungal
6	20.632	Nonadecane	1.36	$C_{19}H_{40}$	268	Antimicrobial
7	19.574	1-Tricosanol	1.16	$C_{23}H_{48}O$	340	Potent antimicrobial
8	21.499	Bis (2-ethylhexyl) phthalate	0.40	$C_{24}H_{38}O_4$	390	Cytotoxicity potential
9	23.875	(E, E, E)-3,7,11,15-Tetratmethylhexadeca-1,3,6	0.74	$C_{20}H_{32}$	272	Aromatic compound (pheromones)
10	20.113	Eicosane	0.71	$C_{20}H_{42}$	287	antifungal compound

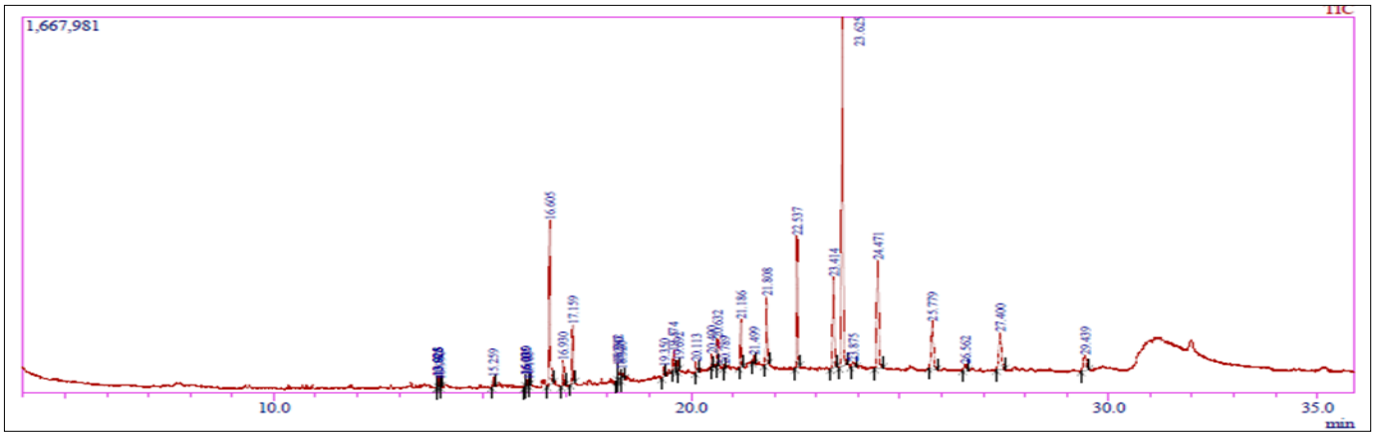

Fig. 1. Chromatogram of *S. reginae* ethyl acetate leaf extract

Table 3. Concentration-dependent change in the mortality during 24, 48, 76, and 92 hrs exposure to *S. reginae* leaf extract

Group (mg\kg bw)	Number of fishes/groups	Number of dead fish	Erratic behavior	Loss of equilibrium	Mortality ratio (%)	LC_{50} (mg\kg bw)
Observation period: 24 hours						
Control	7	0	0	0	0	3717.555
600 mg/L	7	0	0	0	0	
700 mg/L	7	0	0	0	0	
800 mg/L	7	0	0	0	0	
900 mg/L	7	0	0	0	0	
1000 mg/L	7	1	0	0	14.29	
1200 mg/L	7	1	0	0	14.29	
Observation period: 48 hours						
Control	7	0	0	0	0	2305.395
600 mg/L	7	1	0	0	14.29	
700 mg/L	7	1	0	0	14.29	
800 mg/L	7	1	0	0	14.29	
900 mg/L	7	1	0	0	14.19	
1000 mg/L	7	2	0	0	28.71	
1200 mg/L	7	2	0	0	28.71	
Observation period: 76 hours						
Control	7	0	0	0	0	1075.041
600 mg/L	7	2	0	0	28.71	
700 mg/L	7	2	0	0	28.71	
800 mg/L	7	1	0	0	14.29	
900 mg/L	7	2	0	0	28.72	
1000 mg/L	7	3	0	0	42.86	
1200 mg/L	7	5	0	0	71.43	
Observation period: 92 hours						
Control	7	0	0	0	0	1075.041
600 mg/L	7	2	0	0	28.71	
700 mg/L	7	2	0	0	28.71	
800 mg/L	7	1	0	0	14.29	
900 mg/L	7	2	0	0	28.71	
1000 mg/L	7	3	0	0	42.86	
1200 mg/L	7	5	0	0	71.43	

and 96 hours of exposure, the concentration-dependent in vitro LC_{50} values changed based on the time exposure of *S. reginae* leaf extracts to 3717.555, 2305.395, 1075.041 and 1075.041 mg/L (Table 4). All quantities of the plant extract were shown to be less harmful within 24 hours of the trial, but a higher concentration of 1200 mg/L was found to be more hazardous, and five fish started to die between 76 and 92 hours after exposure. The exposure period and the LC_{50} showed a perfect negative connection (Table 5). When there was no discernible movement and the caudal peduncle was touched, the fish was deemed dead. When mortalities were recorded, dead fish were removed. The lethal concentration (LC_{50}) was established using plant extract test dosage concentrations in water that killed 50% of test fish over a given exposure duration. Toxic effects of the *S. reginae* plant sample were identified (Table 3). After 24 hours of exposure to different test doses of concentrations of the extracts ranging from 600 mg/L to -800 mg/L, less than 50% mortality in *D. rerio* was observed. After 48–76 hours of exposure, 60% mortality was observed at 900 mg/L- 1200 mg/L leaf extract doses, respectively. After 96 hours of exposure, 80% mortality was observed at the 1200 mg/L dose. When ≤600 mg/L of ethanolic extract was used, the results showed that it did not cause toxicity. Based on the oral acute toxicity test results, the ethanolic leaf extracts of *S. reginae* were found to be less toxic to *D. rerio* at lower doses but toxic at three concentrations: 900, 1000, and 1200 mg/L. During the experiment, there was no mortality in the control group. At 96 hours after Zebrafish exposure to plant leaf extracts, variations were seen in acceptable values calculated using various methodologies, as indicated in Table 6. The most commonly used foundation for the oral acute toxicity test is the median lethal concentration (LC_{50}).

Table 4. In vitro Lethal concentration (LC_{50}) of leaf extracts of *S. reginae* depending on exposure time (hr).

Exposure (hr)	LC_{50} (mg/L)	Calculated chi-square (X^2)
24	3717.555*	4.053**
48	2305.395*	0.377**
76	1075.041*	2.561**
92	1075.041*	2.561**

*n = 7; ** no significant difference observed.

Table 5. Correlation analysis between exposure time and LC_{50}.

		LC_{50}	Exposure time
LC_{50}	Pearson correlation	1	-0.969*
	Sig. (2-tailed)		0.031
	N	4	4
Exposure time	Pearson correlation	-0.969*	1
	Sig. (2-tailed)	0.031	
	N	4	4

*Correlation is significant at 0.05 level (2-tailed).

Table. 6. Estimation of safe levels of the *S. reginae* leaf extracts at 96 hr of exposure

96 hr LC_{50} (mg/L)	Method	AF	Safe level (mg/L)
1075.041	Hart *et al.**		30.3288
	Sprauge	0.1	107.5041
	CWQC	0.01	10.705041
	NAS/NAE	0.1-0.00001	107.5041-0.01075041
	CCREM	0.05	53.75205
	IJC	5% of 96 hr LC_{50}	53.75205

*C= 48 hr LC_{50}×0.03/S^2 where C is the presumable harmless concentration and S= 24 hr LC_{50}/48 hr LC_{50}

Histopathology of Strelitzia reginae Leaf Extract-Treated Zebrafish with Control

The fish exposed to 1200 mg/L had their gills, liver, kidneys, intestines, and brain histopathologically examined. Zebrafish exposed to highly poisonous plant extracts experienced cell degeneration in the lamellae and displacement of lamella epithelial cells in the gills (Fig. 2b). The liver tissue in the treated samples also revealed that cytoplasmic vacuolization, a frequent tissue change in hepatocytes, was seen (Fig. 3b). In the tubular cells of the structural tissue, tubular disorganization and tubular degeneration have been seen in the kidneys (Fig. 4b). Figures show that the intestine's tissues (Fig. 5b) exhibit displacement in the lamina propria, the villi structure is ruptured, and it is separated from the basal membrane. The brain subjected to test materials revealed an impingement in the tissue of the brain parenchyma (Fig. 6b). The control samples were observed to have normal tissues, as seen in Fig. 2a- 6a.

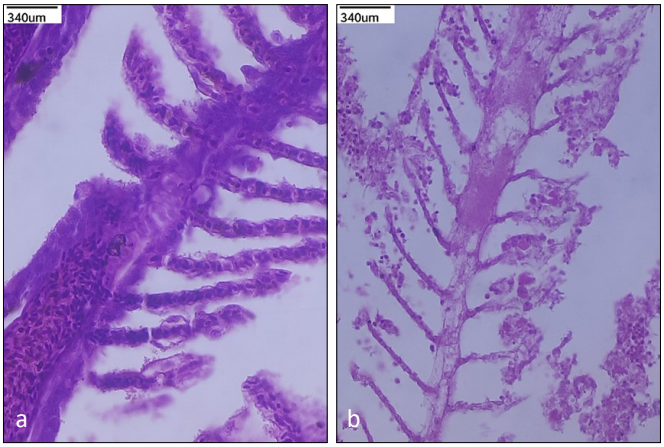

Fig. 2. a) Control- gill; **b**) Gill treated with leaf extract.

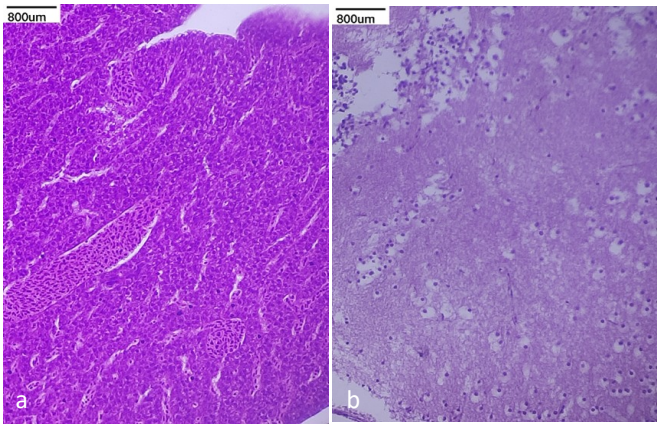

Fig. 3. a) Control- liver; **b**) Liver treated with leaf extract.

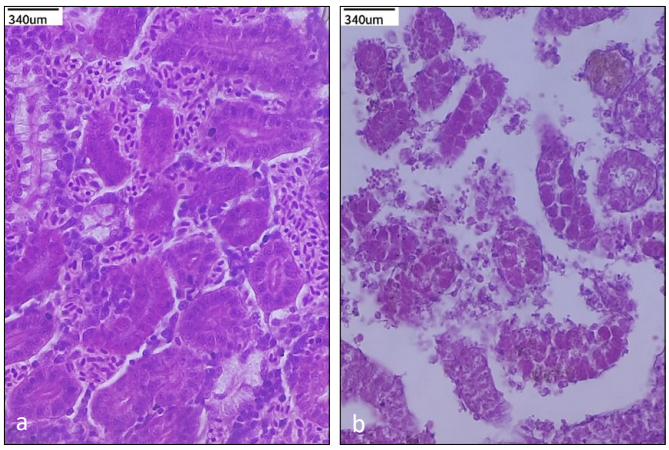

Fig. 4. a) Control- kidney; **b**) Kidney treated with leaf extract

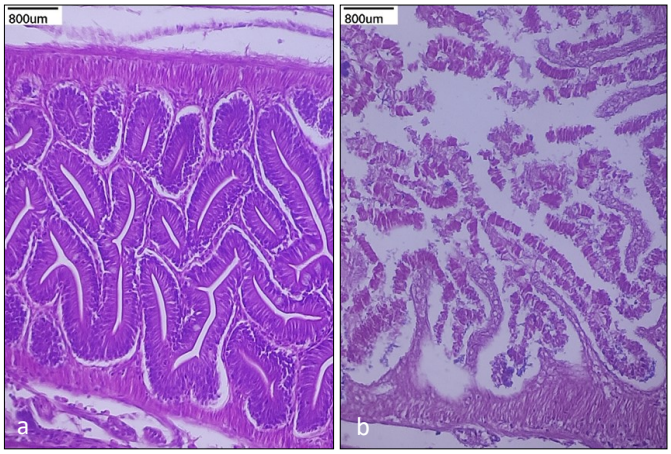

Fig. 5. a) Control- intestine; **b**) Intestine treated with leaf extract

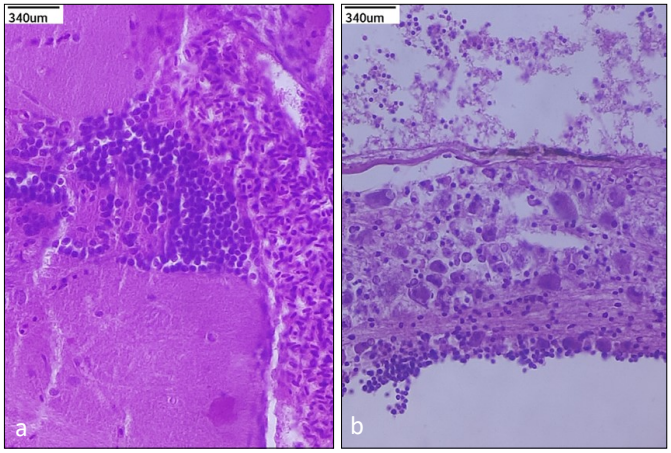

Fig. 6. a) Control- brain; **b**) Brain treated with leaf extract.

Discussion

The present research focused on the macro and micro-elements, GC-MS analysis, toxicity, and histopathology study of *Strelitzia reginae* leaf extract on *Danio rerio*.

Micro and macro elements were found in *S. reginae's* mineral composition. Trace element concentrations have an impact on numerous organs in both humans and animals. The concentration of calcium was higher, and that of cadmium was lower. These elements were present in good amounts in *S. reginae*, with Ca being the most abundant metal. Ca is present in numerous bones, extracellular fluids, and human blood. It is also essential for the heart's proper operation, blood clotting, and milk clotting, as well as for controlling cell permeabi-lity, nerve impulse transmission, and the neuromuscular system's mechanism (20). The necessity of maintaining Ca ion homeostasis for cell functions is highlighted by the possibility that excessive levels of intracellular Ca ion and mitochondrial Ca ion overload may cause apoptosis (21). The element magnesium occurs naturally in human bodies and is frequently found in food. However, excessive magnesium intake, whether from overconsumption or underexcretion, can have a negative impact on a patient's health (22). According to Sloup *et al*. (23), zinc acts as an enzyme cofactor and prevents complement activation and toxin release from damaging cell membranes. In the past, zinc toxicosis has been reported in people as well as in numerous big, tiny, exotic, and wild species. Unspecific splenomegaly, hepatomegaly, reddish-brown kidneys, pancreatic nodules, edema, inflammation, and ulceration of the gastrointestinal mucosa are examples of gross pathology. Haemoglobin nephropathy, pancreatic interstitial fibrosis, pancreatic acinar cell necrosis, and hepatic necrosis with pigment amassing are among the histopathology findings (24). Copper is a common component of human blood and is involved in copper-iron metabolism in the blood. regulates hormone production and functions as a cofactor and activator of numerous enzymes. However, high concentrations of copper can be toxic to plants and animals and even stunt their growth (25). A risk might also arise from having too much copper in the body; according to Wang (26), acute Cu poisoning can cause a number of diseases, including mortality in extreme situations, Wilson's illness, and progesterone interference caused by too much copper in the body, which can both impact ovulation and fertility. In humans, manganese (Mn) plays a critical role in numerous enzyme creation and activation processes as well as the control of lipids and glucose (27). According to studies, excessive Mn exposure results in the generation of reactive oxygen species and severe liver, heart, and neurological system damage (28). According to He WL (29), iron is a crucial micronutrient involved in energy metabolism and oxygen transport in the flesh. Lead (Pb) and cadmium (Cd) are two persistent, pervasive heavy metals that have been related to a variety of harmful consequences in animal systems (30, 31). The toxicity of lead exposure, whether acute or chronic, can have numerous harmful cellular consequences that eventually lead to various disorders (32). Lead is a carcinogenic toxin that affects organs, including the skin, lungs, kidneys, urinary bladder, prostate, liver, and tissues (33).

The GC-MS test showed that the ethyl acetate leaf extract of *S. reginae* contained many important bioactive compounds for medicine, such as squalene, a triterpenoid that can fight cancer and free radicals (34). Neophytadiene has numerous activities, like analgesic, antipyretic, antioxidant, and anti-inflammatory, for treating skin diseases, rheumatism, and headaches (35). Hexacosane has antimicrobial properties (36). 1-octadecene and eicosane have antifungal compounds (37). Similarly, a study conducted on the leaf of Sri Lankan Alpinia species, a close relative of Strelitzia species, showed the potential to be used as an antioxidant and antidiabetic agent (38). Since they generate numerous beneficial compounds helpful in the

food business for flavoring and in the medical industries as antioxidant and antibacterial agents, Curcuma species, a related family of Strelitizia species, have drawn a lot of interest (39). Other substances with cytotoxic potential were discovered, including bis (2-ethylhexyl) phthalate (BEHP), octacosane, and triacontane. Previous research suggests that these chemicals may work against cells that are trying to grow (40, 41). They are also the main chemicals found in callus volatile extract that can cause cancer. Along with the testes, the liver is also recognized as the primary target of phthalate poisoning. Furthermore, the findings are consistent with previous studies in the liver that found that BEHP (at a 500-fold greater dosage) increased the activity of glutamic pyruvic transferase (GPT), which is a crucial indication of hepatic injury and the extent of cell membrane damage (40).

The sensitivity of *S. reginae* leaf extracts in Zebrafish was investigated in this work for the first time, and it was found that Zebrafish were most susceptible to the leaf extract's deadly effects, which peaked after 96 hours of exposure at 1200 mg/L but was less toxic at lower concentrations. The acute cytotoxic action of *Centella asiatica* leaf ethanolic extract on adult wild-type Zebrafish generated the same results. Zebrafish were subjected to extract test concentrations of 156.5 and 312.5 mg/L for up to 96 hours with no mortality. The mortality of the zebrafish model, on the other hand, was affected at higher test doses. 50% of the Zebrafish died at the 1250 mg/L test dose, whereas 100% died at the highest quantity tested (2500 mg/L) (42). Also, the LC50 for the Zebrafish in this study was 1075.041 mg/L after 96 hours, which was higher than the LC50 for the test with Enydrafluctuans, which was 92.95 mg/L (15). It was also higher than the 96-hour LC50 value of Kandhamalhaladi for D. rerio, which was 173.516 µM (43). Bis (2-ethylhexyl) phthalate was found in the leaf of S. reginae. Studies show that this chemical is toxic in different ways depending on how it is structured in the thyroid, kidneys, liver, and testosterone glands, which adds to its overall toxicity. In addition, they have been classified as hormone-wise medications since they can disrupt the human endocrine system and can cause cancer. Given the wide spectrum of phthalate toxicities in humans, animals, and aquatic organisms, the uptake of phthalates in many portions of plants, primarily herbal medicine and agriculture, may pose a significant problem (44, 45). As supported by previous data, bis (2-ethylhexyl) phthalate is the possible main compound contributing to the toxicity results in the present study.

In the present work, the histopathology of control and plant extract-treated Zebrafish was compared. Histopathological examinations of the treated fish's gills, liver, kidneys, intestines, and brain revealed abnormalities in comparison to the control. The gills are in charge of gas exchange in the water as well as waste excretion. The displacement of lamella epithelial cells was one of the first modifications noticed, believed to be an effort by the aquatic organism to adjust to the new changes in environmental settings. Histological investigations of the gills revealed epithelial cell detachment and displacement. These factors—healthy tissue in gills and substantial tissue alterations brought on by dangerous substances—can lead to the animal's death (46, 47). Hepatocyte cytoplasmic vacuolization, which results in a reduction in glycogen storage and lipid buildup, is a typical tissue modification in the liver that may be brought on by toxic substances. It was found in a study that bis (2-ethylhexyl) phthalate was present in *S. reginae* leaf samples. This suggests that phthalates have a big effect on the liver as well as the testicles. These two organs have been identified as the principal targets of phthalate poisoning (48). The most common tissue changes in the kidney after exposure to hazardous substances were seen in the tubules. The tubules showed tubule cell hypertrophy and a change caused by the dryness of the epithelial cells enclosing the tubules, brought on by changes in the kidney's function following exposure to harmful substances. This mechanism is thought to impair the liver's natural function. Tubular disorder, deterioration, and cytoplasmatic degradation are common structural tissue abnormalities in tubular cells (6). The treatment caused the villi structure to rupture and separate from the intestine's basal membrane, as a result of which the fish died, according to histological investigations done on the gut of the treated fish. The villi that make up the intestine of Zebrafish are encased in a mucous coating. The lamina propria, which houses defense cells, is located in the center of these villi (46). Villi expand the interior surface area of the intestinal walls, increasing the amount of surface area available for absorption. Damage from noxious chemicals in the colon frequently exhibits a characteristic aspect of inflammation or lamina propria displacement (49). While the control is standard without brain impingement, the histological findings of brain tissues exposed to test extracts indicated considerable impingement in the brain parenchyma. Following the study on the acute test of the plant extract from *Enydra fluctuans* (16), histological analysis of the brain tissue of Zebrafish indicated the same dramatic changes from *S. reginae* extract exposure.

Conclusion

Toxicity assessment is a fundamental aspect of both quality control and pharmacological research on health products sourced from plants. This study specifically investigated the standardized ethanolic leaf extract of *S. reginae* by subjecting it to adult Zebrafish for a duration of up to 96 hours, using concentrations exceeding 900 mg/L. The primary objective was to determine the lethal concentration, a critical step before its utilization in further experimental pharmacological investigations. The findings from this concentration-dependent experiment revealed that higher concentrations of *S. reginae* pose a substantial risk to Zebrafish. This highlights the importance of exercising caution and utilizing the plant extract in more controlled, lower quantities to avoid potential toxicity. Furthermore, only the highest concentration of 1200 mg/L was considered for histopathological examinations since more than fifty percent of the Zebrafish died, and at lower doses, less

than fifty percent died. The protocol is based on OECD Guidelines 203. The results provided evidence that increased doses of *S. reginae* ingestion can lead to organ damage and even prove fatal. These new insights into the toxicity of *S. reginae* offer valuable information that can contribute to increased awareness regarding plant toxicity and the need for vigilance in its future utilization. This knowledge is essential for ensuring the safe and responsible application of plant-derived health products and pharmaceutical research.

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Authors contributions

The research has significantly benefitted from the contributions of all authors. All have carried out the experiments, processed the results, and written the paper.

Compliance with ethical standards

Conflict of interest: The authors state that they do not have competing interests.

Ethical issues: None.

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