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Research Article

Toxicological Evaluation and Central Nervous System Depressant Activities of Lizard Dung in Rat Model

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Abstract
Globally, the abuse of psychoactive substances continues to rise at astronomical levels. The use of reptiles such as the lizard or their dung for euphoric purposes is highly unconventional. A literature search revealed a lack of studies on lizard dung's toxicity or central nervous system (CNS) activity. We, therefore, assessed the toxicity and CNS activity of lizard dung in Wistar rats via oral and inhalational routes. Conventional models of tail suspension, forced swim, elevated plus maze, hole board, and sodium pentobarbital-induced sleeping time tests were used for CNS assessment. The lizard dung was orally administered at 125, 250 and 500mg/kg. The inhalational experimental groups received 0.5g, 1.0g and 2.0g of the white and black sample in a smoke chamber. Following a 28-day toxicity study, no mortality was observed for all doses of the test substance administered via both routes. Significant changes in serum urea, creatinine, bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were recorded. Mixed inflammatory infiltrates and oedema were observed in the lungs of the group that received 1.0g inhalation of the darkish part of the lizard dung. Oral and inhalational administrations of whitish and darkish parts of lizard dung altered general behavioural patterns of rats, including a dose- and route-of-administration-dependent reduction in the exploratory behaviour and potentiation of phenobarbitone-induced sleeping time. Our findings indicate depression of the CNS and support the claims about the use of lizard dung as a substance of abuse.

Keywords: lizard dung, substance abuse, toxicity, CNS, depression

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Introduction

Various news outlets and case studies have highlighted recent trends of abusing animal parts, their excretions, or other metabolic products to achieve a euphoric state. These substances of abuse of animal origin are collectively referred to as psychoactive fauna (Jimoh et al., 2022) and include snake venom, toad skin excretions, hallucinogenic fish, and ants, scorpion stings, cow dung, and human wastes (Das et al., 2017; Katshu et al., 2011; Kautilya & Bhodka, 2013; Orsolini et al., 2018). The abuse of a lizard's entire body, tail, and faeces has become increasingly popular and commonly reported in recent years (Das et al., 2020; Jimoh et al., 2022).

In Nigeria, many young people are vulnerable to substance abuse partly due to unemployment, illiteracy, peer pressure and some medical conditions like depression (Dumbili, 2020; Dumbili et al., 2021). A growing tide of substance abuse in Nigeria is being reported in the news media, many unpublished newspapers, magazines and blogs. These reports showed that the drug abusers, in trying to maintain a state of high or experience a novel euphoria, have resorted to other unimaginably bizarre alternatives that are easily available at almost no cost, and because there are no legal frameworks regulating their use (Orsolini et al., 2018; Zhang et al., 2018), this ugly trend continues to increase at unaccountable measures.

For a long time, individuals in almost all cultures have utilized a variety of psychoactive substances to achieve euphoria (WHO, 2023). Plant products (nicotine, cannabis, and opiates) and synthetic compounds (Lysergic acid diethylamide LSD, 3,4-methylenedioxy methamphetamine MDMA, and others) make up most of these substances.

The usage of reptiles like the lizard or their dung for euphoric purposes is extremely unconventional, and there is little evidence for this in scientific literature. In other climes, previous case reports have described the use of whole or different parts of a lizard as a substance of abuse. For example, the white part of the Lizard dung has been ingested and/or smoked to achieve euphoria (Chahal et al., 2016; Danjuma et al., 2015) as a single substance or in combination with other known drugs of abuse such as cannabis and tobacco.

Reports of this trend, however, have only been found in print media and newspaper articles in Nigeria, where they only speak of the supposed ecstasy and/or potentiation of euphoric experiences when mixed with other well-known psychomimetics like tobacco and cannabis. A literature search available at the time of conducting this study has revealed no original research conducted on its toxicity and untoward effects in experimental settings. And like most psychoactive faunas, there is no legal backing that asserts Lizard dung as a substance of abuse. Thus, we aim to evaluate the proximate and elemental composition of lizard dung, conduct a toxicological examination and determine the CNS activities of lizard dung to lay the basis for the inclusion of lizard dung as a substance of abuse.

Materials and Methods

Animal Handling

About one hundred and thirty-eight (138) Sprague Dawley rats (3-4weeks old) of either sex weighing 55-95g were obtained from the Institute for Medical Research and Training (IMRAT) University College Hospital (UCH), Ibadan, Oyo state Nigeria. They were then transferred to the Animal house facility at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, jointly owned by the Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences (both in the Usmanu Danfodiyo University, Sokoto). However, all procedures were conducted by approved institutional protocol and the provision for animal care and use with the scientific procedure prescribed by the Usmanu Danfodiyo University Animal and Use Committee. All ethical conduct was strictly adhered to.

Collection of Sample

Whitish and dark parts of the lizard dung were collected from the natural habitat, uncompleted/abandoned buildings and the zoological garden on the main Campus of Usmanu Danfodiyo University Sokoto.

Preparation of Sample

The dry lizard dung was crushed to fine particles using a simple mortar and pestle. A solubility test was conducted by adding the white sample to 10 ml of distilled water. This was mixed vigorously and allowed to settle for 2 hours. The same procedure was repeated for the black Sample.

The flammability of the samples was also tested by burning 1g each in an aluminium foil over a Bunsen burner. The black sample produced more fume than the white. Both samples took about 10 min each to burn completely to ash.

Proximate and Elemental Composition of Lizard Dung

This was carried out in the Central Advanced Science Laboratory Complex (CASLaC) UDUS for quantitative analysis of macro-molecules and elements present in the test sample. It was performed separately for the dark and the whitish part of the Lizard dung.

Proximate Analysis

Using chemical methods, the standard methods of analysis in a procedure described by (Aletan & Kwazo, 2019) were employed for this study. The percentage moisture content of total ash, crude protein, crude lipids, and Crude fibre was determined. The carbohydrate content was determined using the difference technique,

which included deducting the combined amount (g/100g dry matter) of crude protein, crude fat, ash, and fibre from 100%. This was performed as follows:

Moisture Content

Principle: Water undergoes evaporation at all temperatures, with the rate of evaporation increasing as the temperature rises. At 105°C, only water evaporates into the atmosphere without other volatile organic and inorganic matter.

Procedure: The crucible was first weighed (W0), and then a 2g sample was placed in the empty crucible and reweighed (W1). The sample crucible then underwent a drying process in a hot air-drying oven at a temperature of 105°C for 24 hours. After cooling in a desiccator, the crucible with the dry sample was weighed (W2). The crucible, which held the desiccated sample, was reinserted into the oven for a further 24 hours to ensure thorough drying. The sample was subjected to cooling in a desiccator, followed by several weighings until a stable weight (W2) was obtained.

$\%\text{Moisture} = \frac{W1-W2}{W1-W0} \times 100$

Where

W_0 = Empty crucible weight

W_1 = Sample and crucible weight before drying

W_2 = Sample and crucible weight after drying

Determination of Ash Content

Principle: The stability of both organic and inorganic materials is affected by temperature. At a temperature of 600°C, water and other volatile minerals undergo vaporisation, while organic molecules are combusted in the presence of oxygen in the air. Therefore, the inorganic materials are left as ash.

Procedure: The crucible was initially weighed (W_0) while empty. Then, 2g of the sample was added to the crucible and it was weighed again as W_1. The specimen was incinerated in a muffle furnace at a temperature of 600°C for 3 hours and subsequently cooled in a desiccator. The mass of the crucible and ash was measured as W_2.

$\%\text{Ash} = \frac{W2-W0}{W1-W0} \times 100$

Determination of Crude Protein

Principle: The process involves the oxidation of organic matter (Protein) with concentrated sulphuric acid (conc. H_2SO_4) and the reduction of the nutrient to ammonium sulphate. The subsequent addition of an excess amount of Sodium hydroxide (NaOH) in a closed to neutralise the acid and release ammonium which will be distilled into a boric acid solution and titrated against 0.01M HCl to the endpoint.

Procedure: This involved three (3) steps – Digestion, Distillation and Titration.

One gram (1g) of the sample was measured and placed into a 500ml Macro-Kjeldahl flask. Then, 20ml of distilled water was added to the flask, which was then rotated for a short period and left undisturbed for 30 minutes. A single tablet of mercury catalyst was introduced, followed by the addition of 10 ml of concentrated H_2SO_4. The flask was heated carefully at a low temperature on the digesting stand. Once the water has been evaporated and the foaming has stopped, the temperature is raised until the solution becomes clear, indicating that the digesting process is complete. The solution underwent boiling for 5 hours. The heating was controlled throughout the boiling process to ensure that the H_2SO_4 condenses about halfway up the neck of the flask. The flask was allowed to cool, and then distilled water was gradually added to bring the total volume of the flask to 50. 10 ml of the digest was meticulously transferred into a separate macro-Kjeldahl flask with a capacity of 750 ml. A 20ml solution of boric acid (H_3BO_3) indicator was added to a 250ml Erlenmeyer flask, which was then positioned underneath the condenser of the distillation apparatus. A 750ml Kjedahl apparatus was connected to the distillation apparatus, and 40ml of 40% NaOH was added to the distillation flask by opening the funnel stopcock. Afterwards, 40ml of the distillate was collected and NH_4-N in the distillate was determined by titrating with 0.1M H_2SO_4 using a 25ml burette graduated at 0.1ml interval. The transition from the green colour to pink signifies the endpoint.

$\%\text{Crude protein} = \frac{(a-b)\times volumemade\times 6.25}{weightofaliquot\times weightofsample} \times 100$

where a = titre value for the digested sample

b= titre value for the blank

Determination of Crude Lipid

Principle: Lipids are soluble in organic solvents but not in water. Hence, organic solvents such as petroleum ether or N-hexane can dissolve lipids, resulting in the extraction of lipids from the sample when combined with the solvent. The lipid will later be collected by evaporating the solvent.

Procedure: This was performed with the saturation method. 20ml N-hexane was added to 2g of the samples and left undisturbed overnight. The empty dish was weighed (W_1).

The solvent was carefully decanted and then the weight of the empty dish with the oil was weighed (W_2)

$\%\text{Lipid} = \frac{W2-W1}{2} \times 100$

Where W_1 = Weight of empty dish

w_2 = Weight of empty dish and oil

Determination of Crude Fibre

Principle: Following the process of boiling the sample with an acid mixture, the solid residue that remains undissolved is next isolated and subjected to combustion. The crude fibre value is determined by measuring the amount of material lost after ignition.

Procedure: Two grams (2g) of the sample was measured and placed into the 1L conical flask, denoted as W_0. 200 ml of H_2SO_4 solution with a concentration of 1.25% was heated to its boiling point and then added to the mixture. The mixture was then gently simmered for 30 minutes. The solution was filtered using a muslin cloth and then washed with hot distilled water. The specimen was carefully returned to the conical flask using a spatula, followed by the addition of 200ml of boiling 1.25% NaOH. The mixture was then gently boiled for 30 minutes. The solution underwent a second filtration using muslin cloth, and the remaining solid was

extensively rinsed with hot distilled water. Subsequently, it was washed once with a solution of 10% hydrochloric acid, twice with methylated spirit, and three times with petroleum ether (boiling point 40-60°C). Subsequently, it was allowed to completely drain, and the remaining substance was carefully collected into a crucible. The collected substance was then subjected to overnight drying at 105°C in an oven, followed by cooling in a desiccator. The specimen was measured (W_1), incinerated at a temperature of 550°C for 90 minutes in a muffle furnace, cooled in a container that prevents moisture from entering, and then measured once again (W_2).

$$\%\text{Fibre} = \frac{W1-W2}{W0} \times 100$$

Estimation of available Carbohydrate

The estimation was derived by deducting the cumulative percentages of crude protein, crude lipids, crude fibre, and Ash from a 100% moisture-free sample.

%Carbohydrates = 100 - (%Ash + %Crude protein + %Crude lipids + %Crude fibre)

Elemental Analysis

To determine the mineral elements present in the test sample, The Flame photometer (Sherwood M410) was used to determine the presence of Sodium (Na) and Potassium (K). Titration using EDTA was used to determine Calcium (Ca) and Magnesium (Mg). Spectrophotometry (Baur Spectrophotometer) was used for the determination of the presence of phosphorus (P).

Flame Photometer (Na and K)

i. Ash residues of both samples were diluted with 5ml 20% HCl.
ii. This was further diluted to 50ml volume using distilled water and then filter
iii. The sample was placed into the flame photometer for aspiration.
iv. Readings were taken and recorded.

Titration using Ethylenediamine tetraacetic acid EDTA (Ca and Mg)

i. 1ml each of the sample was pipetted from the 50ml stock volume the same from which the flame spectrophotometer aspirated.
ii. Then diluted with 19ml distilled water
iii. For Ca^{2+} 1ml of 10%NaOH was added, a pinch of Murexide indicator for a pink colour was added and then titrated (using 0.01M EDTA) till the end point of purple
iv. Titre value (TV) from the biuret was measured.
v. For Mg^{2+}, 5ml of NH_3 buffer solution was added. Next, a small amount of Eriochrome black T indicator was used, resulting in the formation of a solution with a wine-red colour. This solution was next subjected to titration against 0.01M EDTA to a blue colour endpoint.

Determination of Phosphorus using the Spectrophotometer

i. 2ml of the 50ml stock volume was pipetted into a 50ml conical flask
ii. 2ml of phosphorus extraction solution was added
iii. 2ml of Ammonium molybdate was added and the total volume was made up to 30ml using distilled water
iv. 1ml of dilute stannous chloride solution was added and the total volume was made up to 50ml using distilled water
v. The sample (in a cube) was rinsed, refilled to 50ml mark and placed in the spectrophotometer
vi. Readings of the absorbance were taken at a wavelength of 660 (660λ).

Toxicity Studies of Lizard Dung in Wistar Rats

Acute toxicity Test

The acute toxicity studies were carried out through the oral and inhalational routes.

For the oral route, Lorke's 1983 (Lorke, 1983) method was employed. A total of twelve (12) animals were used in two (2) phases. During the initial phase, a total of nine animals were grouped into three, with each group consisting of three animals. The animals were then given samples at doses of 10, 100, and 1,000 mg/kg body weight. The purpose of this was to determine the range of doses that might result in any toxic effects. The mortality rate in each cohort was documented after 24 hours.

During the second phase, three dosages of the test chemicals were chosen depending on the outcome of phase 1. These doses are then administered to three separate groups, each consisting of one animal. The number of deaths was recorded after twenty-four hours, and the LD50 was determined by calculating the geometric mean of the highest non-lethal dosage (a) and the least toxic dose (b).

$$LD_{50} = \sqrt{a \times b}$$

To study acute toxicity via inhalation, Lorke's Method and the methods for inhalational administration (Blaes et al., 2019) were modified to suit the purpose of the study. The modification involved the use of two groups of two (2) adult male Sprague Dawley rats each were used air-controlled group and Lizard dung (black and white) smoke group. The animals were kept in a vivarium with controlled humidity and temperature. Each cage housed two animals. The animals followed a 12-hour reversed light-dark cycle, with the lights turning off at 8 AM. They had unrestricted access to food and water.

Rats were exposed unrestrained (whole-body exposure) to lizard dung smoke using in standard polycarbonate rodent cage (38 x 28 x 20 cm; L x W x H) with corncob bedding and wire tops, and water was freely available. Dry lizard dung (white and black) smoke was generated using an electric incense burner placed in a specially demarcated part within the smoking chamber.

In phase one, smoke was generated by burning 1000mg of samples then followed by burning graded quantities (2000, 3000, 4000 and 5000mg) of the powdered lizard dung to mimic smoking of the estimated equivalent of 1 standard cannabis cigarette (Prince et al., 2018). This was carried out continuously by adding 1000mg after each burn time averaging 10 minutes per 1000mg.

After smoke exposure, animals were observed for immediate toxic effects and 24 hours.

Table 1: Experimental Design of Acute Toxicity Study

Phase one (n=3)		Phase two(n=1)		Phase one(n=2)		Phase two(n=2)	
Route of Administration	**Dose (mg/kg)**	**Route of Administration**	**Dose (mg/kg)**	**Route of Administration**	**Dose (mg)**	**Route of Administration**	**Dose (mg)**
Oral	10	Oral	1600	Inhalation	1000	Inhalation	1000
Oral	100	Oral	2900			Inhalation	2000
Oral	1000	Oral	5000			Inhalation	3000
						Inhalation	4000
						Inhalation	5000

Sub-acute Toxicity Studies

The sub-acute toxicity study was a direct extension of the acute toxicity test. The study required ongoing monitoring of the experimental animals utilised for acute toxicity over an extended duration. All animals that received the test substance, regardless of the dosage, in the acute toxicity test did not die and were closely monitored for an additional 28 days.

1. The investigation of Oral sub-acute toxicity used the procedures outlined in (Ugwah-Oguejiofor et al., 2019). Any signs of toxicity and death were documented if noticed, similar to the acute toxicity test. When mortality occurred during the 14-day observation period, the dosage that caused the death (or the lowest lethal dose, if multiple deaths were recorded) was given to two animals and their condition was monitored for 14 days. Confirming the subacute toxicity of dosage is achieved by observing the death of any animal throughout the observation period.
   The subacute lethal dose was calculated as follows:
   Lethal Dose $_{subacute} = [M_0 + M_1] /2$
   M_0 = Highest dose of test substance without mortality after the subacute test.
   M_1 = Lowest dose of test substance that gave mortality after the subacute test.

2. For the inhalational subacute toxicity study, the same procedure for smoke exposure adopted for the acute toxicity study was repeated only for a longer smoking exposure time. Throughout the treatments for both routes of administration, the animals were observed daily to assess their overall health and to detect any signs of toxicity, such as increased salivation, impaired limb movement, uncoordinated movements, impaired righting reflex, lethality, abnormal sensations, involuntary muscle contractions, trembling, paralysis, and seizures. Additionally, changes in body weight were measured on days 0, 7, 14, 21, and 28 of the experiment. Following the conclusion of the study period, all animals had an overnight fast before the collection of blood samples. Post day 28, animals were euthanized and samples were collected for Haematological, biochemical, histological and gene expression studies.
   Under anaesthesia with ether, blood samples were obtained from the abdominal aorta using two kinds of tubes: one containing EDTA and the other without any additions. The blood that had been treated with anticoagulant (EDTA) was promptly examined for haematological parameters. To collect the serum for biochemical examination, the second tube was subjected to centrifugation at 3000 rpm at a temperature of 4°C for a duration of 10 minutes. The same approach was followed for the inhalational group.

Haematological studies

Adopting a procedure outlined in (Kharchoufa et al., 2020), haematological studies were performed using a Three Part automatic Haematology analyser (Anasys®) available in the Haematology laboratory of Medistops Diagnostics Laboratory, Mabera area Sokoto by a qualified Medical Laboratory Scientist.

The following haematological analyses were performed: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC) mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hematocrit (HCT), platelets (PLT), platelets distribution width (PDW) and Procalcitonin test (PCT).

Biochemical Studies

Clinical biochemical assessments to investigate major toxic effects in tissues and organs, notably impacts on the kidney and liver were also carried out on all experimental groups according to the methods by (Kharchoufa et al., 2020). Using an Automatic Chemistry Analyser available in the Biochemistry Department of Usmanu Danfodiyo University, Sokoto. the following parameters: Alkaline phosphatase (ALP) test, Aspartate Aminotransferase test (AST), Total Bilirubin, Direct Bilirubin, Glucose, Serum Electrolyte, Urea, Creatinine.

Histological Studies

Histological examination of the kidneys, liver, lungs and hearts was performed in the histopathology laboratory of Usman Danfodiyo University Teaching Hospital by a blinded pathologist. The fixed tissues were dehydrated using a series of increasing concentrations of alcohol, then cleared with xylene, and finally embedded in paraffin wax that melts at a temperature of 60°C. Thin sections (5 mm thick) were obtained by cutting the embedded tissue with a microtome. These sections were then placed on slides that were coated with 3-aminopropyltriethsilane and dried for 24 hours at a

temperature of 37°C. The sections on the slides were treated with xylene to remove the paraffin wax and then rehydrated using a series of decreasing concentrations of alcohol. They were then stained with Mayer's haematoxylin and eosin dyes, dried, and placed on a light microscope (at magnifications of X40 and X100) for histological evaluation.

CNS Activity of Lizard dung in Wistar rats

This was carried out by performing the Tail suspension test, forced swim test, Elevated plus maze test, Hole board test and the Phenobarbitone sleeping time test.

Tail Suspension Test

Experimental animals were randomly assigned to five experimental groups of six animals each, 10mg/kg Imipramine was administered to Group I positive control and allowed for 60 minutes for the animals to acclimatize to the test environment and for the drug to act. Graded doses of the black and white sample were administered via the oral and inhalational route for the appropriate experimental groups (II, III and IV) and distilled water to the negative control group (VIII).

Thirty (30) minutes prior to the experiment, 125mg/kg, 250mg/kg, and 500mg/kg of the white/black sample were administered respectively to the appropriate experimental groups (II, III and IV) and distilled water to group VIII via oral route.

Same was done for the inhalational group (V, VI, VII) by burning 0.5g, 1.0g and 2.0g of the white and black sample in a smoke chamber while placing all animals in the group inside the smoke chamber.

An individual rat from each experimental group was suspended on the edge of a shelf 58cm above a tabletop with adhesive tape placed approximately 1.5-2cm from the tip of the tail. Using a video camera set up in the experimental room, the time spent immobile for a 6-minute testing session was observed and recorded only the final four (4) minutes of the experimental session. Immobility time was considered as an index of depression-like behaviour (Carvalho et al., 2021).

Forced Swim Test

Thirty (30) rats were randomly divided into five groups, six each. The first group received only vehicle (distilled water) as a control group (group VIII). 10mg/kg p.o Imipramine was administered to Group 1 positive control and allowed for 60 minutes for the animals to acclimatize the test environment and for the drug to act. Graded doses of the black and white sample were administered via the oral and inhalational route for the appropriate experimental groups (II, III and IV). Thirty (30) minutes prior to the experiment, 125mg/kg, 250mg/kg, 500mg/kg of the white/black sample were administered to the appropriate experimental groups (II, III and IV) via oral route. Also 30 minutes before the experiment, graded doses were administered to the inhalational group (V, VI, VII) by burning 0.5g, 1.0g and 2.0g of the white and black sample in a smoke chamber while placing all animals in the group inside the smoke chamber. Individual rats from each group were gently immersed in an open and transparent cylindrical container measuring 12cm in diameter and 30cm in height, containing water (temperature of 21-23°C) enough to make escape impossible.

Observations were made using a video camera set up in the experimental room. For a six (6) minute testing session, the time spent immobile, or floating was recorded and only the final four (4) minutes were studied. After completion, rats were removed from the tank, allowed to dry and then placed in their home cage (Carvalho et al., 2021).

Elevated Plus Maze Test

The method of Sofidiya et al., (2022) was adopted for elevated plus maze test. The apparatus consists of two open and two closed arms that extend from a shared central platform. The entire maze was elevated to a height of 50cm above ground.

Thirty (30) animals were randomly assigned to five groups of six animals each, then 1mg/kg p.o Diazepam was administered to Group I positive control and allowed for 30 minutes for the drug to act. The black and white parts of the test sample were administered via the oral and inhalational routes to the appropriate experimental groups and distilled water 10ml/kg to the negative control group. Rats in each group were placed one at a time at the centre of the maze and observed for 5 minutes with the help of a video camera. The elevated plus maze was cleaned before placing subsequent animals. Subsequent experimental groups were subjected to a similar test 30 minutes after administering 125mg/kg, 250mg/kg, and 500mg/kg of the white/black sample and distilled water via the oral route.

Also, the Inhalational groups were subjected to the same test thirty (30) after animals in the groups were exposed to the smoke of the test sample. This was achieved by burning 0.5g, 1.0g and 2.0g of the white and black sample respectively in the smoke chamber while placing all animals in the group inside the chamber.

The following parameters were observed and recorded - number of entries into the open arm, number of entry into the closed arm and time spent in the arms.

Hole-board Test

The method provided by Takeda et al., (1998) was utilised. The hole-board is a wooden box with sixteen evenly distributed holes arranged in a grid pattern on the floor. The equipment was raised to a height of approximately 50cm above the ground. A total of thirty (30) rats were divided into five groups, with each group consisting of six rats, for the experiment. Group I was designated as the positive control. Each rat in the group was administered 0.5mg/kg of diazepam orally and given a 30-minute period for the drug to take effect. Also administered were the black and white samples via the oral and inhalational routes to the appropriate experimental groups and then 10ml/kg distilled water for the negative control group. Individual rat in the group were placed at the centre of the hole board one at a time and observed for 5 minutes. Subsequent groups of

rats were subjected to similar test 30 minutes after administering 125mg/kg, 250mg/kg, 500mg/kg of the white/black sample and distilled water via oral route.
The inhalational experimental groups were subjected to similar test thirty (30) after animals in the group were exposed to smoke of the test sample. This was achieved by burning 0.5g, 1.0g and 2.0g of the white and black sample respectively in the smoke chamber while placing all animals in the group inside the chamber. Observations were made with the help of a video camera set up in the experimental room and the numbers of head dips were recorded.

Phenobarbitone-induced Sleeping time Test (PBST)
The methods described in Sofidiya et al., (2022) were adopted. Thirty (30) rats were randomly assigned to five experimental groups of six animals each. Group I (negative control) received 10ml/kg distilled water. Graded doses of the black and white sample were administered via the oral and inhalational route for the appropriate experimental groups (II, III and IV) and Group V received phenobarbitone sodium (50 mg/kg, i.p). 125mg/kg, 250mg/kg, 500mg/kg of the white/black sample were administered to the experimental groups (II, III and IV) and distilled water to group VI via oral route.
The experimental groups that received inhalational administration of the samples were subjected to similar test thirty (30) minutes after animals in the group were exposed to smoke of the test sample. This was achieved by burning 0.5g, 1.0g and 2.0g of the white and black sample respectively in the smoke chamber while placing all animals in the group inside the chamber. After 1 hour, phenobarbitone sodium (50 mg/kg, i.p.) was administered to each rat in Groups I-IV. The onset and the duration of sleep for animals in the various groups were observed and recorded using a video camera set up in the experimental room.

Statistical Analysis
Shapiro-Wilk Normality Test and homogeneity of variance test (Levene's Test) were conducted. Data were seen to be normally distributed (parametric). One-way analysis of variance (ANOVA) was performed followed by Tukey's multiple comparison test and results were deemed significant at $p < 0.05$. SPSS® Software V.19 was used for the analysis. Data were presented in percentages, tables and charts, and were summarized as Mean ± Standard Deviation.

Results
Proximate Analysis of Lizard Dung
The results of proximate composition revealed the presence of more crude protein (64.3%) in the whitish sample than in the darkish part of the lizard dung (10.5%); also showing no presence of crude lipids and fibre in the whitish part. The reverse was the case for ash content, where the results revealed ash contents of 49.0% and 15.0% respectively for the darkish and whitish part of the lizard dung (Table 2).

Table 2: Proximate Composition of Lizard dung

Parameter	**Composition (%)**	
	Sample W	Sample B
Moisture content	3.5	3.0
Ash content	15.0	49.0
Crude Protein	64.3	10.5
Crude lipids	N/A	1.5
Crude fibre	N/A	17
Carbohydrate	17.2	19.0

Sample W: Whitish part of the Lizard Dung; Sample B: Darkish part of the Lizard Dung

Elemental Analysis
Elemental analysis revealed the presence of Sodium, Potassium, Calcium, Magnesium and Phosphorus in the lizard dung. Higher mg/kg quantities of Potassium (220,30), Calcium (59,47) and Magnesium (115,90) were seen in the whitish part than in darkish part (whitish, darkish). Similar quantities of phosphorus (2.34, 2.31) were found in both samples but there was more sodium in the darkish part of the lizard dung (220mg/kg) than in the whitish part (190mg/kg) (Table 3)

Table 3: Mineral Composition of Lizard dung

Element	Composition (mg/kg)	
	Sample W	Sample B
Sodium (Na)	190	220
Potassium (K)	220	30
Calcium (Ca)	59	47
Magnesium (Mg)	115	90
Phosphorus (P)	2.34	2.31

Sample W: Whitish part of the Lizard Dung; Sample B: Darkish part of the Lizard Dung

Toxicity Studies

Acute Toxicity Studies

After 24 hours, there was no visible sign of toxicity or mortality in the animals during both phases of the acute toxicity study, upon oral administration of the white and dark parts of lizard dung (10–5000 mg/kg). The oral LD_{50} for lizard dung in Sprague Dawley rats was determined to be greater than 5000 mg/kg.

In the first phase of the inhalational administration, both samples produced similar effects. Animals were calm and sedated immediately after they inhaled the smoke made from 1000mg of both samples. Grooming was observed and activity of animals were not regained until after about 17minutes and 13minutes after burning for the white and dark sample respectively. Animals slept off at exposure of 3000mg of smoke for both samples and sleeping was maintained as exposure was increased (4000mg and 5000mg. After 1 hour, the animals were awake, and activity fully regained (same for both samples). There was no mortality observed.

Subacute Toxicity studies

All the rats treated via both routes of administration of all doses survived the 28 days of treatment. There were no discernible indicators of toxicity observed in the rats administered with the extract, as compared to the control group.

Effect of Lizard Dung on Haematological Parameters

The results revealed that compared to the negative control, there is no significant difference in levels of all the haematological parameters tested irrespective of the experimental group and dose of the whitish or darkish part of the lizard dung administered. This was the same for both oral and inhalational administration (Supplementary Table S1 and S2)

Effect of Lizard Dung on Biochemical parameters

The oral administration of the whitish part of lizard dung resulted in a significant decrease (6.43±0.66; 7.34±1.49 and 7.29±0.36) in the levels of serum urea (Figure 4) for all the tested doses (125, 250, and 500 mg/kg) respectively. A conforming significant decrease was seen for the oral administration of 125mg/kg (7.02±2.12) and 250mg/kg (7.89±0.88). There was no significant difference in the serum urea levels after 28-day inhalation administration of all test doses of lizard dung (Table 5). Only the group given 2.0g of the whitish part showed a significant increase (15.24±1.46) in comparison to the negative control (12.16±1.30) of serum urea levels.

When compared to the negative control group (12.57±1.07), creatinine levels in majority of the experimental groups were not affected by oral administration of the whitish and darkish parts of lizard dung after 28 days, except for a significant decrease (5.63±0.70) seen in the group administered 500mg/kg of the whitish sample (Table 4). Except for the group which received 0.5g of the darkish dung which was unchanged, the inhalational administration of the whitish and darkish parts of lizard dung led to an increased serum level of creatinine (Table 5)

Bilirubin levels in majority of the treatment groups, doses administered via the both routes of administration were not significantly different from the control upon 28-day administration of whitish and darkish parts of lizard dung (Tables 4 and 5). However, a significant increase (6.15±0.94) was recorded in the bilirubin levels of the group which received 1.0g of the whitish dung via inhalation compared to the control (3.56±2.13) (Tables 5)

Serum alkaline phosphatase (ALP) levels were unaffected in the groups that received 2.0g of the whitish part (163.18±28.49) and 1.0g of the darkish part (111.78±29.43) of lizard dung via inhalation. All other treatment groups via the oral and inhalational routes, showed a significant decrease (Tables 4 and 5) in serum levels of the ALP in comparison to the negative control group (159.53µ/L ±5.53).

Oral treatment of the whitish and darkish parts of the lizard led to a statistically significant decline in the serum levels of Alanine aminotransferase (ALT) for all tested doses after 28 days. Similar decline in µ/L levels of serum ALP was recorded for inhalational administration of the whitish part of lizard dung (Table 5). The groups that received the darkish part via inhalation showed no significant difference (82.86±9.41; 92.12±13.76 and 101.65±11.2) when compared to the control (98.08±11.88) (Figure. 5).

In comparison to the levels seen in the negative control (359.05±1.13), a significant decrease in levels of Aspartate aminotransferase (AST) was recorded for the groups that received oral administration of the 125mg/kg (164.09±19.85), 500mg/kg (123.90±0.60) of whitish part as well 125mg/kg (156.23±21.48) of the darkish part (Table 4). Similar significant decrease was seen after inhalational administration of 1.0g (228.59±1.84) and 2.0g (108.64±1.36) of the darkish sample; as well the group that inhaled 2.0g (104.55±3.54) of the whitish part (Table 5). A significant increase in serum AST levels was observed upon sub-acute inhalational administration of 0.5g (416.98±2.66) of the whitish part, 0.5g (447.90±8.64) of the darkish part as well as an

increase after oral administration of 500mg/kg (394.51±2.55) of the darkish part of lizard dung.

Table 4: Effect of Oral administration of Lizard dung Biochemical on parameters

Sample	Parameter	Dose(mg/kg) -Oral Route			
		125	**250**	**500**	**Control**
W	Urea (mmol/L)	6.43±0.66*	7.34±1.49*	7.29±0.36*	12.16±1.30
	Creatinine (µmol/l)	11.06±0.43	11.49±0.16	5.63±0.70*	12.57±1.07
	Bilirubin (mg/dL)	1.38±0.65	1.72±0.73	1.53±0.65	3.56±2.13
	ALP (µ/L)	53.81±3.54*	75.24±13.42*	29.67±2.64*	159.53±5.53
	ALT (µ/L)	29.10±5.59*	65.77±8.28*	57.92±4.68*	98.08±11.88
	AST (µ/L)	164.09±19.85*	370.75±1.34	123.90±0.60*	359.05±1.13
B	Urea (mmol/L)	7.02±2.12*	7.89±0.88*	10.17±1.59	12.16±1.30
	Creatinine (µmol/l)	10.55±4.26*	9.56±1.40*	12.75±0.06	13.50±1.94
	Bilirubin (mg/dL)	1.23±0.40	2.01±0.74	1.09±0.54	3.56±2.13
	ALP (µ/L)	30.08±1.51*	92.24±30.47*	106.26±1.59*	159.53±5.53
	ALT (µ/L)	28.30±5.63*	34.21±5.20*	32.43±2.71*	98.08±11.88
	AST (µ/L)	156.23±21.48*	372.88±1.16	394.51±2.55*	359.05±1.13

*Values are presented as Mean± S.D, n=7; Significant in relation to control at *p < 0.05 compared to the control for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung Sample B=Darkish part of the Lizard Dung. ALP=Alkaline phosphatase, ALT=alanine amino transferase, AST=Aspartate aminotransferase.*

Table 5: Effect of Inhalational administration of Lizard dung on Biochemical parameters

Sample	Parameter	Dose(mg/kg) -Inhalational Route			
		0.5	**1.0**	**2.0**	**Control**
W	Urea (mmol/L)	13.93±0.97	15.01±1.62	15.24±1.46*	12.16±1.30
	Creatinine (µmol/l)	25.80±0.91*	23.84±1.55*	18.90±1.15*	13.50±1.94
	Bilirubin (mg/dL)	5.03±0.78	6.15±0.94*	2.46±0.86	3.56±2.13
	ALP (µ/L)	7.57±6.10*	55.15±5.06*	163.18±28.49	159.53±5.53
	ALT (µ/L)	22.18±0.31*	57.10±0.39*	74.78±0.98*	98.08±11.88
	AST (µ/L)	416.98±2.66*	360.54±1.34	104.55±3.54*	359.05±1.13
B	Urea (mmol/L)	10.89±0.28	10.67±0.50	11.05±0.89	12.16±1.30
	Creatinine (µmol/l)	12.65±0.16	26.50±0.58*	25.37±1.32*	13.50±1.94
	Bilirubin (mg/dL)	1.86±1.21	1.10±1.01	3.50±1.28	3.56±2.13
	ALP (µ/L)	67.62±38.94*	111.78±29.43	46.93±22.18*	159.53±5.53
	ALT (µ/L)	87.26±9.41	97.12±13.76	101.65±11.42	98.08±11.88
	AST (µ/L)	447.90±8.64*	228.59±1.84*	108.68±1.36*	359.05±1.13

*Values are presented as Mean± S.D, n=7; Significant in relation to control at *p < 0.05 compared to the control for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard DungSample B=Darkish part of the Lizard Dung. ALP=Alkaline phosphatase, ALT=alanine amino transferase, AST=Aspartate aminotransferase.*

Effect of Lizard Dung on the Histological assessments

Similar to the haematological parameters, no changes were seen compared to the control upon histological evaluations of the kidneys, lungs and hearts from groups given the whitish and darkish parts of the lizard dung via oral and inhalational routes. The sections of the kidneys showed normal glomeruli, tubules and interstitium (Figure 1a and 1b). Normal hepatocyte, portal triad and central vein were also observed on the sections of the liver while the cardiac myocytes were normal (Figure 1a and 1b).

Sections of the lungs from all inhalation groups showed presence of mixed inflammatory infiltrates and edemas were seen within the alveolar spaces (Figure 2a-2f). Sections of lungs from the group that received 1.0g of the whitish part of the lizard dung showed hemorrhage within the alveolar spaces (Figure 2b).

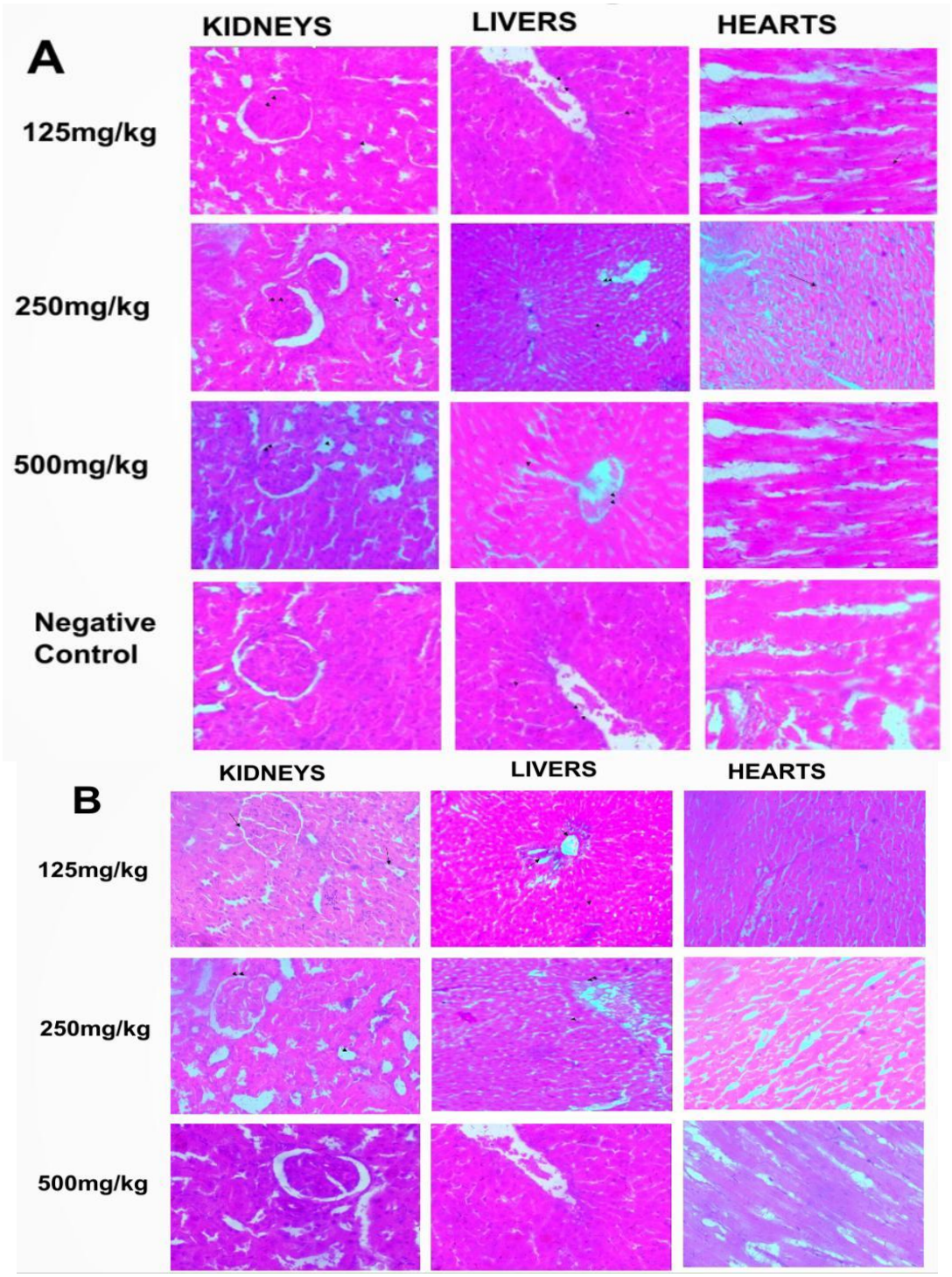

Figure 1a: Photomicrograph of sections (H & E X 100) of Kidneys, Livers and Hearts in control and oral treated groups of (W) Whitish and (B) Darkish part of lizard dung. All organ sections were similar to the control.

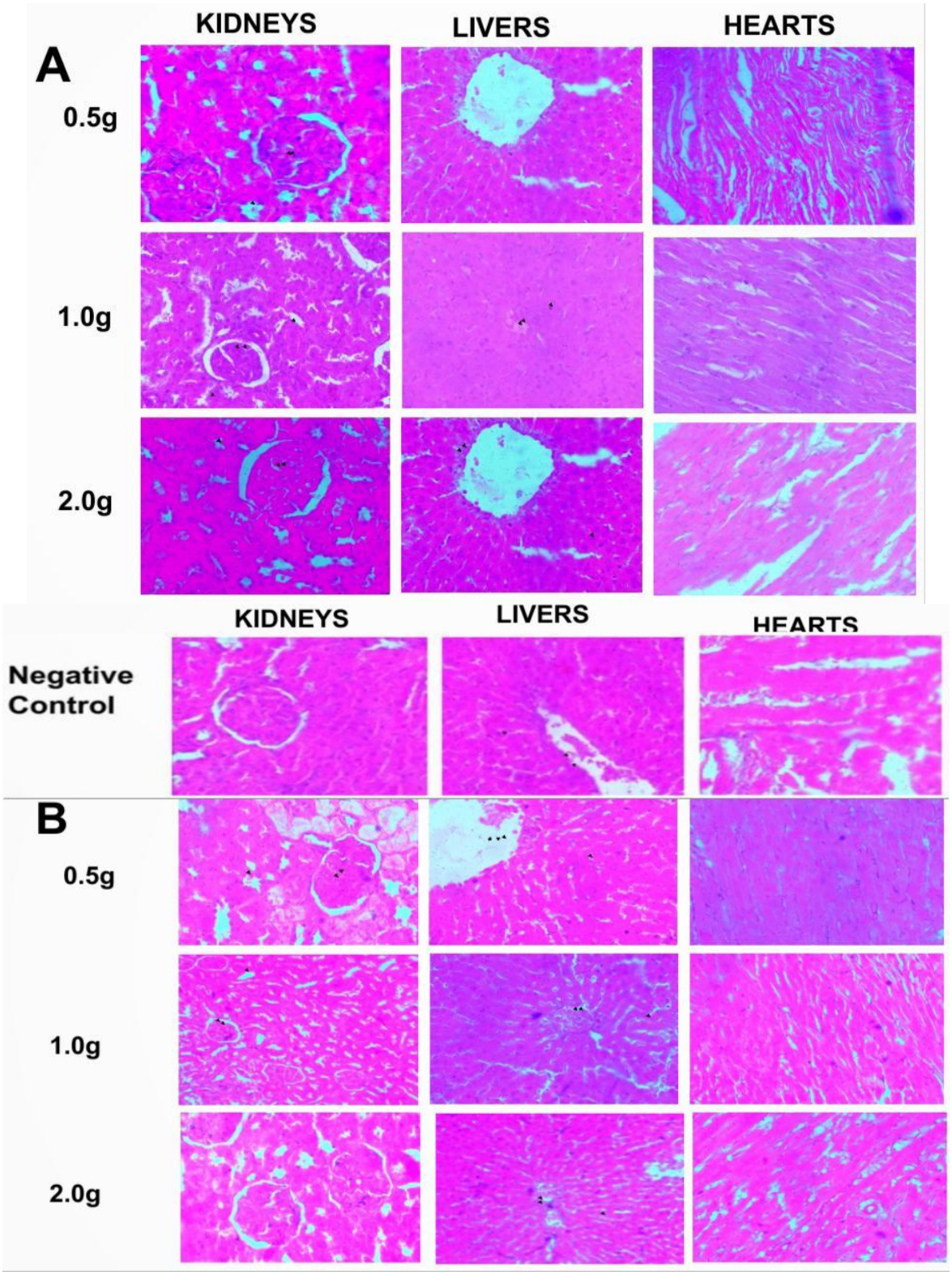

Figure. 1b: Photomicrograph of sections (H & E X 100) of Kidneys, Livers and Hearts in control and Inhalation treated groups of (W) Whitish and (B) Darkish part of lizard dung. All organ sections were similar to the control.

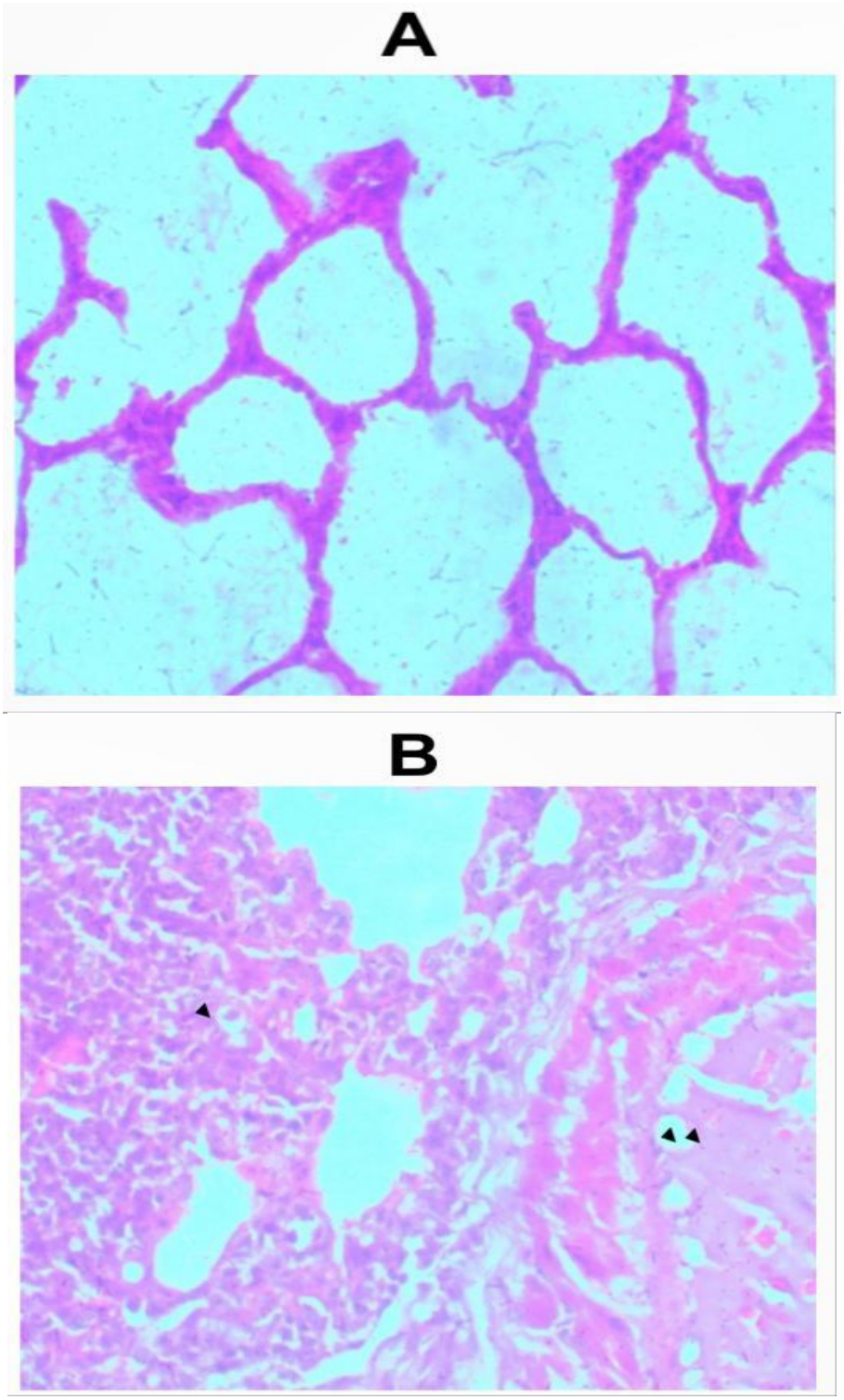

Figure. 2a: Photomicrograph of sections (H & E X 100) of lungs in (A) control and (B) 0.5g inhalation treated group of whitish part of lizard dung. Mixed inflammatory infiltrates (arrow) and oedema (arrows) are seen within the alveolar spaces of (B)

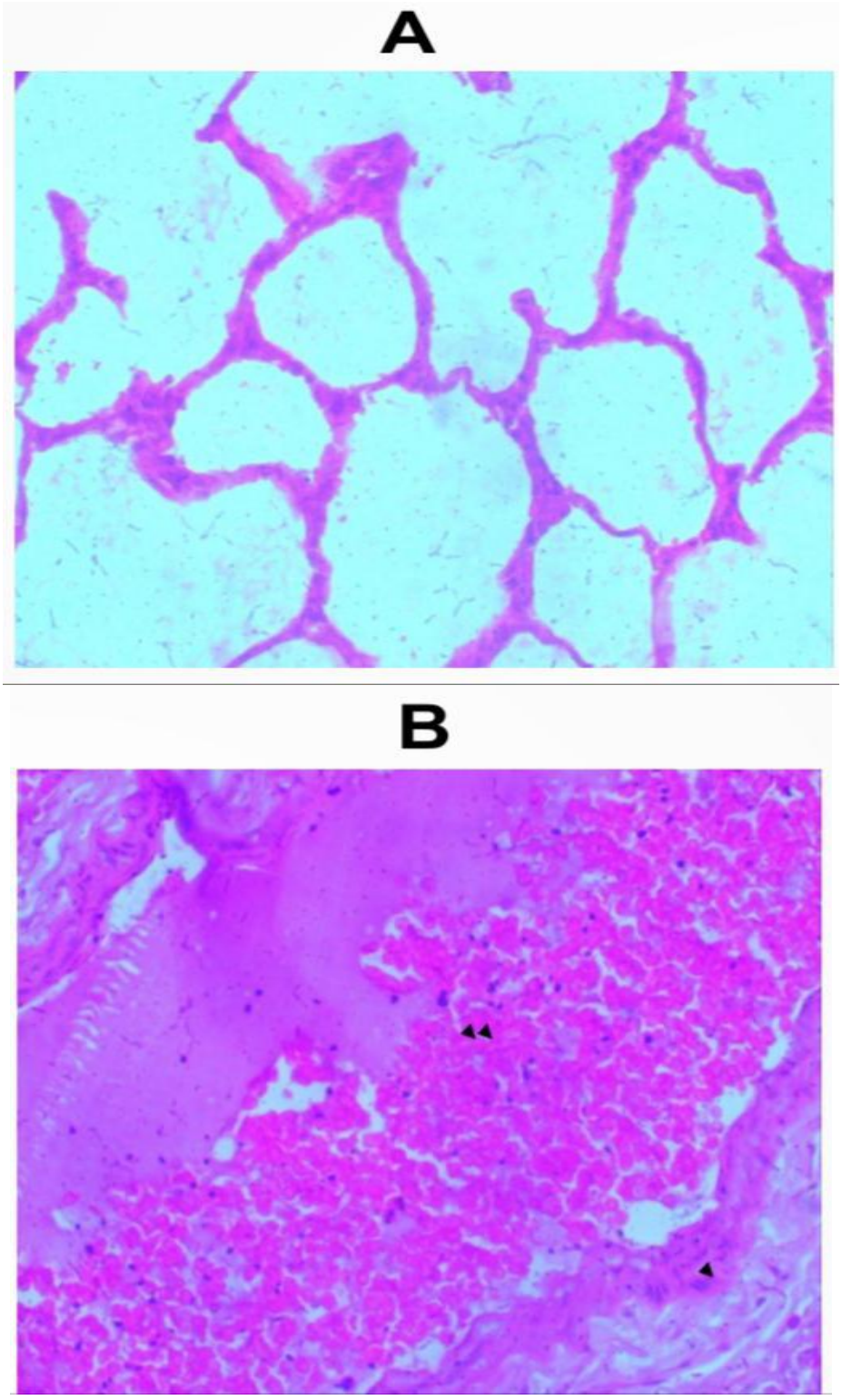

Figure 2b: Photomicrograph of sections (H & E X 100) of lungs in (A) control and (B) 1.0g inhalation treated group of whitish part of lizard dung. Mixed inflammatory infiltrates (arrow) and Haemorrhage (arrows) seen within the alveolar spaces of (B)

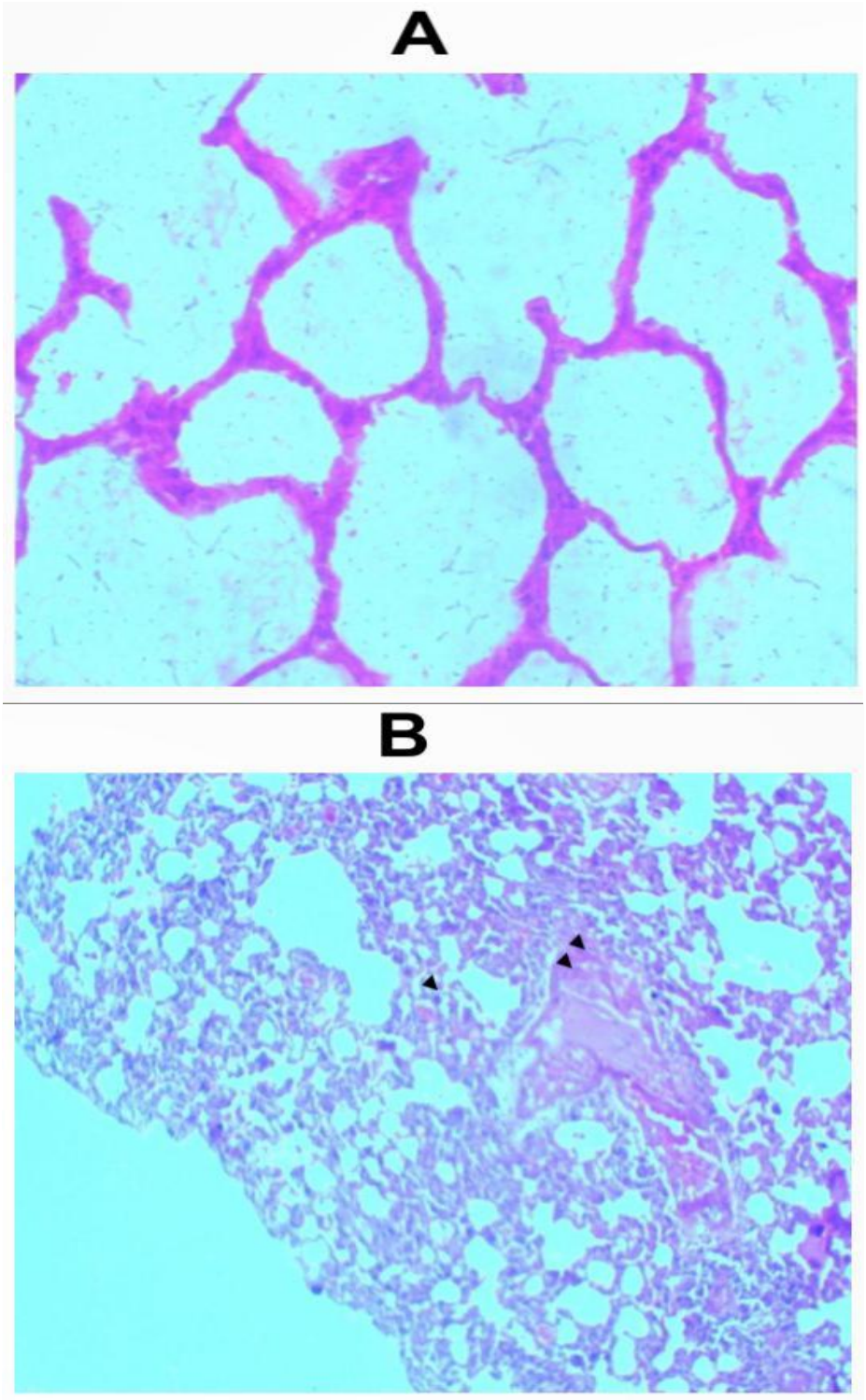

Figure 2c: Photomicrograph of sections (H & E X 100) of lungs in (A) control and (B) 2.0g inhalation treated group of whitish part of lizard dung. Mixed inflammatory infiltrates (arrow) and oedema (arrows) are seen within the alveolar spaces of (B)

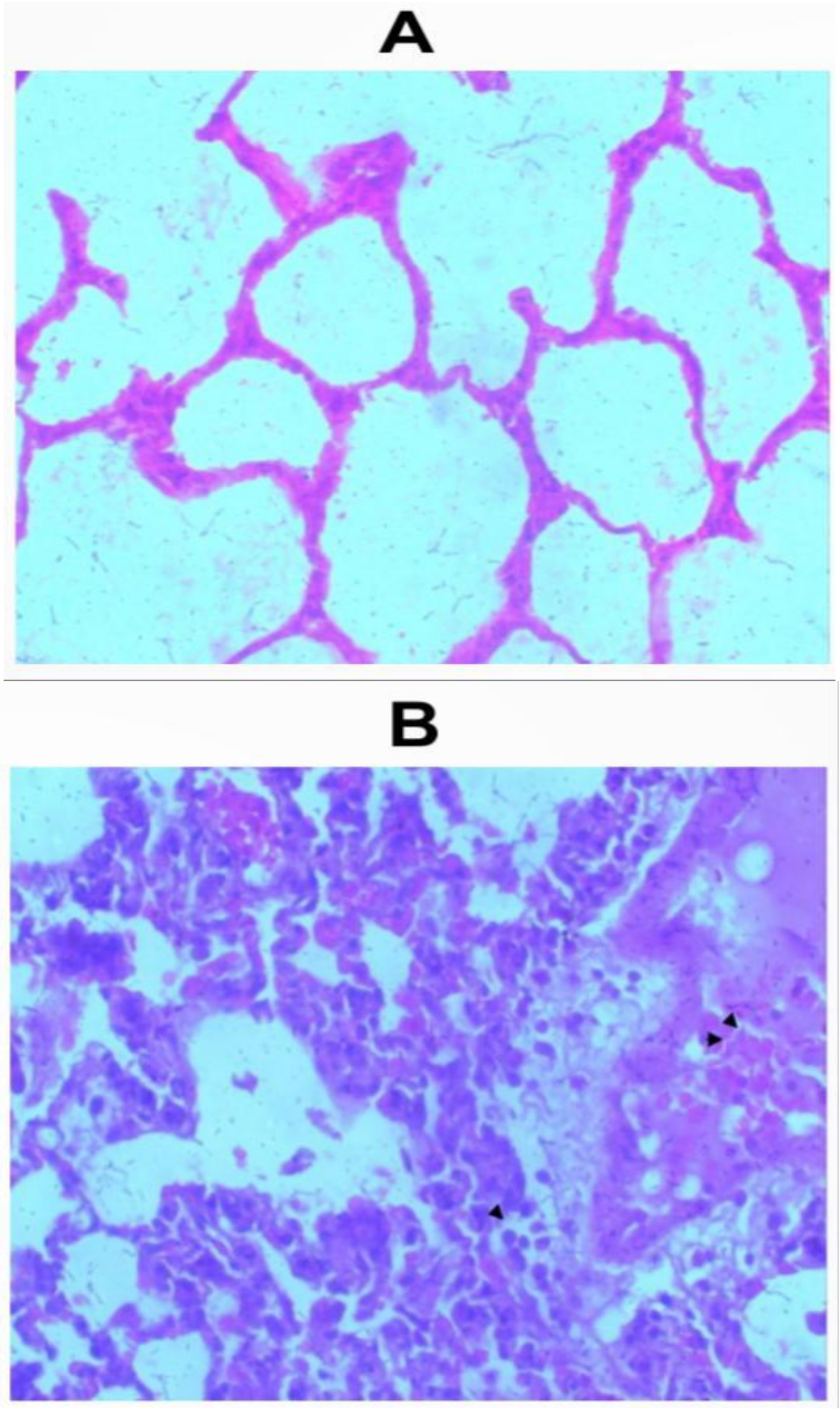

Figure 2d: Photomicrograph of sections (H & E X 100) of lungs in (A) control and (B) 0.5g inhalation treated group of darkish part of lizard dung. Mixed inflammatory infiltrates (arrow) and oedema (arrows) are seen within the alveolar spaces of (B)

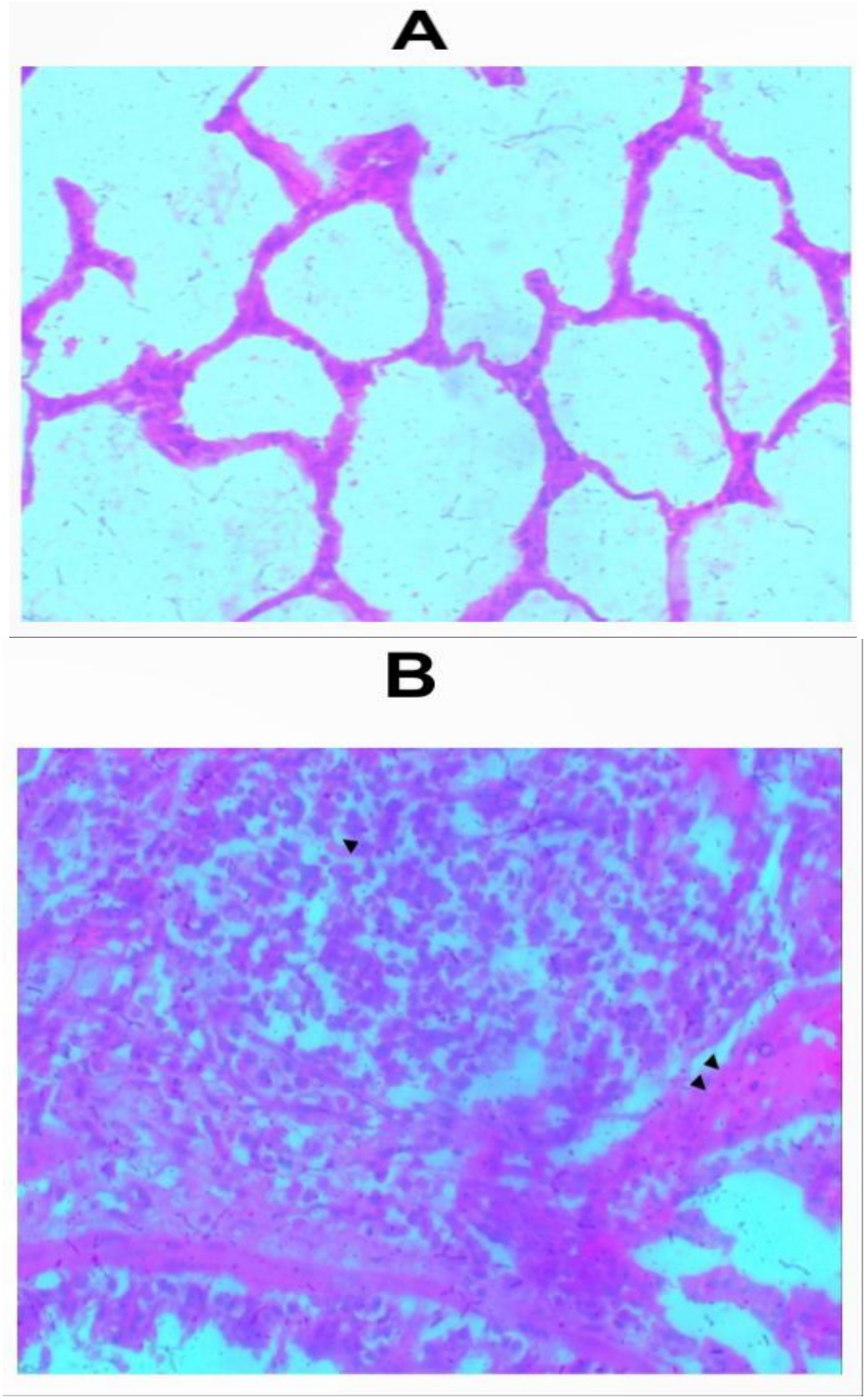

Figure 2e: Photomicrograph of sections (H & E X 100) of lungs in (A) control and (B) 1.0g inhalation treated group of darkish part of lizard dung. Mixed inflammatory infiltrates (arrow) and oedema (arrows) are seen within the alveolar spaces of (B)

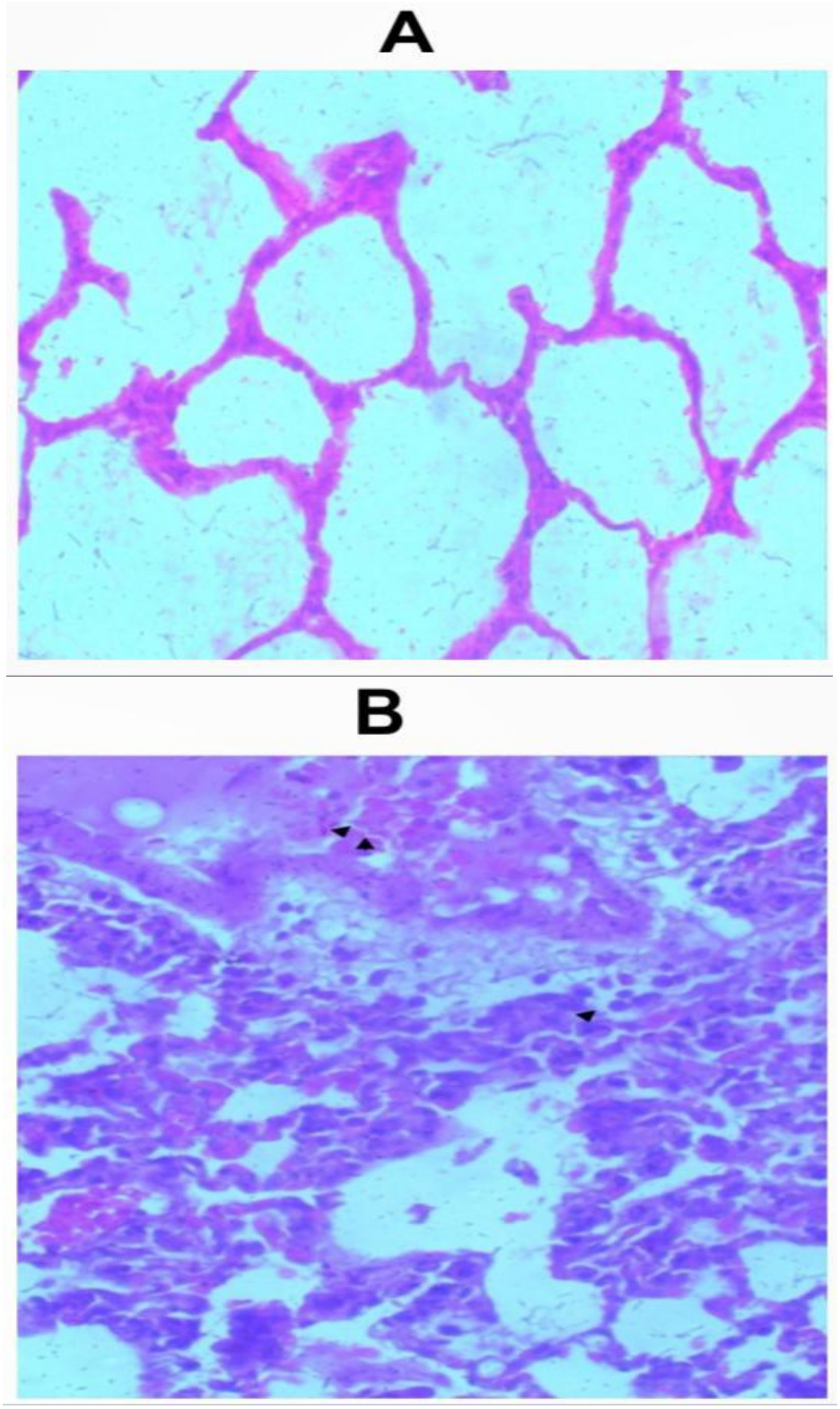

Figure 2f: Photomicrograph of sections (H & E X 100) of lungs in (A) control and (B) 2.0g inhalation treated group of darkish part of lizard dung. Mixed inflammatory infiltrates (arrow) and oedema (arrows) are seen within the alveolar spaces of (B)

CNS Activity of Lizard Dung in Wistar Rats

Tail Suspension Test

All tested doses significantly reduced the duration of immobility (Tables 6a and 6b) of the rats during the tail suspension tests ($p < 0.05$) compared to the negative control (204.00±4.32), except for the group that received 0.5g of the black sample via the inhalational route. The reduction in duration of immobility seen in the groups that inhaled 1.0g (61.00±4.76) and 2.0g (35.00±2.58) of the whitish part of lizard dung was also significantly different (Table 4.8) from the reduction by imipramine standard (91.75±5.32).

Table 6a: Effect of Oral administration of Lizard dung on Time spent immobile during Tail suspension Test

Sample	Parameter	Dose(mg/kg) -Oral Route				
		125	250	500	Control	Imipramine
W	Time Spent Immobile (secs)	152.75±10.05*	142.75±0.96*	106.50±2.89*	204.00±4.32	91.75±5.32*
B	Time Spent Immobile (secs)	159.00±6.68*	132.25±6.24*	116.25±7.68*	204.00±4.32	91.75±5.32*

*Values are presented as Mean± S.D, n=5; Significant about control at *p < 0.05, for each row. One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung*

Table 6b Effect of Inhalational Administration of Lizard Dung on Time Spent Immobile During Tail Suspension Test

Sample	Parameter	Dose(g) -Inhalational Route				
		0.5	1.0	2.0	Control	Imipramine
W	Time Spent Immobile (secs)	113.75±7.68*	61.00±4.76*	35.00±2.58*	204.00±4.32	91.75±5.32*
B	Time Spent Immobile (secs)	198.50±5.45	167.50±4.35*	161.75±6.45*	204.00±4.32	91.75±5.32

*Values are presented as Mean± S.D, n=5; Significant about control at *p < 0.05, for each row. One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung; Sample B=Darkish part of the Lizard Dung*

Forced Swim Test

Except for the groups that were orally administered 125mg/kg (44.50±3.42) and 500mg/kg (55.00±2.58) of the darkish part of lizard dung, all other treatment groups significantly reduced the immobility time of the animals in comparison to the control (52.00±3.65). Specifically, oral and inhalational administration of the whitish part of the lizard dung significantly reduced the duration of immobility in comparison to imipramine (31.75±6.24). The experimental groups that received inhalation of 1.0g (9.00±0.82) and 2.0g (6.00±0.82) of the darkish part of lizard dung also significantly reduced the duration of immobility in comparison to the negative control and imipramine group (Tables 7a and 7b).

Table 7a Effect of Oral administration of Lizard dung on Time spent immobile during Forced Swim Test

Sample	Parameter	Dose(mg/kg) -Oral Route				
		125	250	500	Control	Imipramine
W	Time Spent Immobile (secs)	18.75±2.99*	11.50±1.29*	14.00±1.41*	52.00±3.65	31.75±6.24*
B	Time Spent Immobile (secs)	44.50±3.42	41.75±2.06*	55.00±2.58	52.00±3.65	31.75±6.24*

*Values are presented as Mean± S.D, n=5; Significant about control at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung.*

Table 7b Effect of Inhalational Administration of Lizard Dung on Time Spent Immobile During Forced Swim Test

Sample	Parameter	Dose(g) -Inhalational Route				
		0.5	1.0	2.0	Control	Imipramine
W	Time Spent Immobile (secs)	10.50±1.92*	7.50±2.08*	2.75±0.96*	52.00±3.65	31.75±6.24*
B	Time Spent Immobile (secs)	31.75±6.24*	9.00±0.82*	6.00±0.82*	52.00±3.65	31.75±6.24*

*Values are presented as Mean± S.D, n=5; Significant in relation to control at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung.*

Elevated Plus Maze

In comparison to the control group, the majority of the treatment groups did not significantly increase the number of entries into the open arm of the elevated plus maze apparatus. Exceptions were seen as significant increase in number of entries into the open arm upon the oral administration of 500mg/kg of the darkish part (3.0±1.73), the inhalational administration of 0.5g of the whitish part (7.75±1.89) of the lizard dung and the inhalational administration of 2.0g of darkish part of the lizard dung (4.00±1.83).

The whitish and darkish parts of Lizard dung showed a significant activity ($p < 0.05$) in increasing the time spent in the open arms compared to control. Post hoc

results of the comparison of the control group (0.00±0.00) to other experimental groups revealed significant increases in time spent (Tables 8a and 8b) after the oral administration of 250mg/kg of the whitish part (157.50±164.55). The same was seen after inhalation of 0.5g of the whitish part (140.00±14.72) and 2.0g of the darkish part of lizard dung (213.75±82.60).

Table 8a Effect of Oral administration of Lizard dung on Number of entries and Time spent in open arms of the Elevated Plus Maze

Sample	Parameter	Dose(mg/kg) -Oral Route				
		125	250	500	Control	Diazepam
W	Number of entries into the open arm	2.00±1.16	1.00±0.00	1.00±1.16	0.00±0.00	1.00±1.16*
	Time Spent in open arm (secs)	68.75±22.87	157.50±164.55*	25.00±28.87	0.00±0.00	18.75±13.15
B	Number of entries into open-arm	2.00±0.00	0.50±0.58	3.50±1.73*	0.00±0.00	1.00±1.16*
	Time Spent in open arm (secs)	81.25±8.54	5.00±5.77	120.00±4.08	0.00±0.00	18.75±13.15

*Values are presented as Mean± S.D, n=5; Significant in relation to control at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung*

Table 8b: Effect of Inhalational administration of Lizard dung on Number of entries and Time spent in open arms of the Elevated Plus Maze

Sample	Parameter	Dose(g) -Inhalational Route				
		0.5	1.0	2.0	Control	Diazepam
W	Number of entries into open-arm	7.75±1.89*	1.50±1.29	2.00±1.41	0.00±0.00	1.00±1.16*
	Time Spent in open arm (secs)	140.00±14.72*	68.75±47.50	73.75±33.51	0.00±0.00	18.75±13.15
B	Number of entries into open-arm	2.00±0.00	2.50±0.58	4.00±1.83*	0.00±0.00	1.00±1.16*
	Time Spent in open arm (secs)	65.25±18.72	122.50±19.14	213.75±82.60*	0.00±0.00	18.75±13.15

*Values are presented as Mean± S.D, n=5; Significant in relation to control at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung.*

Hole Board Test

Results of the hole board test (Tables 9a and 9b) present a significant reduction when compared to the control (9.25±1.71) in the number of head dips in all the groups that received the whitish part (0.5g,1.0g and 2.0g) of the dung via inhalation (2.00±1.16, 3.50±2.89 and 2.00±1.16 respectively). All tested doses of the oral administration of the whitish part and 500mg/kg administration of the darkish part of the lizard dung showed no significant difference in the number of head dips of the animals compared to the observation seen in the control group. On the contrary, a significant increase in the number of head dips (16.5±2.89) was observed for the animals that were exposed to inhalation of 1.0g darkish part while the 0.5g inhalation of the darkish part of lizard dung also demonstrated a marked reduction in the number of head dips (3.5±0.58) in the hole board test.

Table 9a Effect of Oral administration of Lizard dung on Number of Head dips in the Hole Board Test

Sample	Parameter	Dose(mg/kg) -Oral Route				
		125	250	500	Control	Diazepam
W	Number of Head Dips	6.50±1.73	6.50±0.58	4.50±1.29	9.25±1.71	11.50±4.80
B	Number of Head Dips	7.50±0.58	3.00±1.16*	6.50±0.58	9.25±1.71	11.50±4.80

*Values are presented as Mean± S.D, n=5; Significant in relation to control at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung.*

Table 9b Effect of Inhalational Administration of Lizard Dung on the Number of Head Dips in the Hole Board Test

Sample	Parameter	Dose(g) -Inhalational Route				
		0.5	1.0	2.0	Control	Diazepam
W	Number of Head Dips	2.00±1.16*	3.50±2.89*	2.00±1.16*	9.25±1.71	11.50±4.80
B	Number of Head Dips	3.50±0.58*	16.50±2.89*	8.25±0.96	9.25±1.71	11.50±4.80

*Values are presented as Mean± S.D, n=5; Significant in relation to control at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung, Sample B=Darkish part of the Lizard Dung.*

Phenobarbitone-induced Sleeping Test

In relation to the administration of the phenobarbitone alone, co-administration of all the tested doses of whitish and darkish parts of lizard dung showed a significant difference in the duration of sleep. Oral co-administration of the higher dose (500mg/kg) of the whitish sample significantly increased the duration of sleep (9052.25±55.81) in comparison to the administration of phenobarbitone alone (7544.75±30.77). In the same vein, significant increases in the duration of sleep were seen in all the groups that inhaled 0.5, 1.0 and 2.0g of the whitish part (7937.50±35.00, 9428.50±23.00 and 9225.00±311.29 respectively) in addition to phenobarbitone (Table 10b). Animals in the groups that received oral co-administration of (125mg/kg and 250mg/kg) as well as inhalational of 2.0g (in addition to phenobarbitone) of the darkish sample also exhibited a significant increase in the duration of sleep compared to the animals in the group that received only phenobarbitone (Table 10a and 10b).

Table 10a: Effect of Oral administration of Lizard dung on Sleeping time in the Phenobarbitone-induced Sleeping Test

Sample	Parameter	125+PB	250+PB	500+PB	PB
W	Sleeping Time(secs)	6154.25 ±104.44*	6412.00 ±36.72**	9052.25 ±55.81*	7544.75 ±30.77
B	Sleeping Time(secs)	9198.25 ±53.52*	9562.50 ±59.09*	6854.500±50.21*	7544.75±30.77

*Values are presented as Mean± S.D, n=5; Significant in relation to the standard (PB) at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung Sample B=Darkish part of the Lizard Dung PB=Phenobarbitone.*

Table 10b: Effect of Inhalational administration of Lizard dung on Sleeping time in the Phenobarbitone-induced Sleeping Test

Sample	Parameter	0.5+PB	1.0+PB	2.0+PB	PB
W	Sleeping Time(secs)	7937.50 ±35.00*	9428.50 ±23.00*	9225.00±311.29*	7544.75±30.77
B	Sleeping Time(secs)	4367.50 ±45.74*	4964.50 ±30.35*	10620 ±16.36*	7544.75±30.77

*Values are presented as Mean± S.D, n=5; Significant in relation to the standard (PB) at *p < 0.05 for each row, One-way ANOVA followed by Tukey's post hoc test. Sample W=Whitish part of the Lizard Dung Sample B=Darkish part of the Lizard Dung PB=Phenobarbitone.*

Discussion

From the foregoing, this research is an attempt to provide a scientific basis for the purported use of Lizard dung as a substance of abuse. This study became expedient because available evidence in literature at the time of embarking on the research is low on the hierarchy of evidence in scientific research (Borgerson, 2009) and is not sufficient to drive a strong argument. There are only case reports as well as articles in print and electronic media, describing the various methods of using lizard dung as a substance of abuse. Oral and inhalational routes of administration were reported as how these abusers use whole dung or only the whitish part of the lizard dung to achieve euphoria (Jimoh et al., 2022).

In achieving the aim of this study, a quantitative analysis of the macromolecules in the whitish and darkish parts of the lizard dung was carried out. The proximate study indicated that the whitish lizard faeces are primarily composed of crude protein and are even higher in

comparison to quantities found in the darkish part. This agrees with findings of high urea content previously reported, asserting that the whitish part of the lizard dung is a composition of crystallized uric acids which is a means of excretion of nitrogenous wastes analogous to urine (Tan et al., 2020). It becomes plausible, therefore, to consider the urate content as a possible psychoactive substance. This is subject to experimental verification even though enhancement of cognitive functions and motivations upon administration of uric acid in different human population samples had previously been confirmed (Tovchiga & Shtrygol', 2014). Uric acid and its analogues can cross the blood-brain barrier. In comparison to the breakdown of catecholamines and dopamine, urates can be produced and broken down in the central nervous system (CNS). As a result, they demonstrate strong neuroprotective and antioxidative effects (Tovchiga & Shtrygol', 2014). These findings suggest that the whitish part of lizard dung is likely more effective in eliciting effects relating to CNS activities.

This study also revealed higher ash content in the darkish part than in white and a total absence of crude fibre in the whitish part of the dung. This is in line with previous findings that lizards feed on a broad spectrum of arthropods majorly ants, beetles and highly mobile flying insects like wasps because lizards are predators that ambush their prey (Burghardt, 1971; Tan et al., 2020). The presence of fibre in the darkish part is supportive of previous studies that showed the presence of herbaceous materials and sand particles in the stomachs of lizards (Tan et al., 2020). Previous studies have established the indispensable role of Potassium in the propagation of nerve impulses (Awwad et al., 2019; Holland et al., 2019). Potassium, in conjunction with sodium, controls the water and the acid-base balance in the blood and tissues. Additionally, it has a vital function in the transmission of electrical signals in the heart. The process of actively transporting potassium in and out of cells is essential to cardiovascular and nerve function (Holland et al., 2019). The sufficient elemental potassium present in the whitish part of the lizard dung is indicative of its potential to serve as a source of experimental potassium, whereby its administration would lead to a concomitant increase in serum potassium levels. A study on the link between the administration of the whitish part of lizard dung and increased nerve impulses becomes reasonable.

Analyses of haematological parameters are used to evaluate the level of toxicity of drug substances (Ibrahim et al., 2016) including psychoactive faunas like lizard dung. Understanding the alterations in the biological system responsible for blood cell formation (haematopoiesis) is more effective in predicting human toxicity when data from animal research are extrapolated (Olson et al., 2000). An erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet) is believed to be derivable from an immature pluripotential stem cell (Ugwah-Oguejiofor et al., 2019). In this study, the oral and inhalational administration of whitish and darkish parts of lizard dung in rats over 28 days did not result in any notable alteration in any of the investigated blood parameters.

It is already established that the kidney and liver play significant roles in digestion, metabolism, detoxification, and elimination of substances from the body. Therefore, testing for derangement in normal serum levels of their biochemical biomarkers provides an important guide into the health of the liver and kidney; and is crucial in the toxicological evaluation of available xenobiotics (Bariweni et al., 2018; Ugwah-Oguejiofor et al., 2019). Serum electrolytes, urea and creatinine are widely requested blood tests to ascertain the functionality of the kidneys. In the current study, only the serum urea and creatinine levels were analyzed as a source of insight into kidney health status. Results from this study showed that out of a total of twelve (12) experimental groups (of both routes of administration), six (50%) of the experimental groups (doses) demonstrated significant differences ($p < 0.05$) in serum urea levels compared to the negative control. Over 83% of significantly different observations were a significant reduction of serum urea levels in comparison to the negative control group. Reduced plasma/serum urea is less common and usually of less clinical significance than its increase (Higgins, 2016). Acute liver failure or overhydration and malnutrition might result in decreased urea levels (Ugwah-Oguejiofor et al., 2019). The animals were fasted before euthanasia and collection of blood samples and hence urea levels are

The major reason for conducting safety studies in any experimental sample is to outline the nature and magnitude of adverse effects and to determine the amount of exposure that causes this effect (Ibrahim et al., 2016; Ugwah-Oguejiofor et al., 2019). Acute toxicity study shows that the aqueous preparation of whitish and darkish parts of lizard dung administered through oral route to rats at 1600, 2900 and 5000 mg/kg using Lorke's (1983) method of acute toxicity testing revealed no evidence of toxicity or death in the animals (Lorke, 1983). The OECD criteria for testing chemical substances and mixtures, under its Globally Harmonised Classification System (GHS), substances with LD_{50} >5000 mg/kg are categorised as unclassified or category 5 (OECD, 2001). These findings indicate that the oral LD50 of lizard dung, which exceeds 5000 mg/kg, is considered safe. A similar suggestion could be made for the inhalation administration of whitish and darkish parts of lizard dung. Results from acute toxicity studies often have a limited application in clinical practice. Hence, we proceeded to a sub-acute toxicity study.

Substances of abuse, (like psychoactive faunas) are similar to drugs administered in chronic disease conditions; often need repeated application and hence, toxicological evaluation (sub-acute toxicity study) since daily use may result in accumulation in the body with progressive effects on vital tissues and organs (Bariweni et al., 2018; Ugwah-Oguejiofor et al., 2019). It is important to test for sub-acute toxicity when looking at the effects of test samples on target organs and their haematological or biochemical effects since these effects aren't readily apparent from data from acute toxicity studies. Also, it is a progressive step required in establishing the safety of such substances in humans.

expected to be elevated as reported previously by Hassan et al. in 2018 (Hassan et al., 2018). Only the inhalation of 2.0g of the darkish part of the lizard led to elevated serum urea levels. Creatinine clearance is a measure of glomerular filtration rate that assesses kidney function. Significant elevation in levels of serum creatinine was observed after 28-day inhalation of whitish and darkish arts of lizard dung. The oral administration led to no significant change. Although the histology of the kidneys in rats that received inhalation did not produce any pathological changes and supported the safety of the sample in the kidney, elevated serum creatinine levels observed in this study could be a pointer to degeneration in the glomerular filtration rate.

Primary biochemical liver function tests provide information about the status of the liver (Kumar et al., 2020). Bilirubin is a widely recognized marker that is regularly included in biochemical testing for individuals with liver disease or any other medical issue. Nevertheless, bilirubin lacks sensitivity and specificity as a liver function measure, thus requiring a meticulous analysis of test findings for precise deductions (Guerra Ruiz et al., 2021). Alkaline phosphatase is an enzyme that is found in many different parts of the body, such as the liver, bile ducts, intestine, bone, kidney, placenta, and leukocytes. It functions by catalysing the release of orthophosphate from ester substrates under alkaline conditions (Prasad, 2004). Aminotransferase includes the Aspartate aminotransferase (AST) and the alanine aminotransferase (ALT) which are the biomarkers of hepatocellular damage. These enzymes facilitate gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid, resulting in the production of oxaloacetic acid and pyruvic acid, respectively (Kumar et al., 2020). AST exists as both cytosolic and mitochondrial isoenzymes and is located in various organs including the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, white blood cells, and red blood cells (Kalas et al., 2021; Kumar et al., 2020; Robles-Diaz et al., 2015). AST is less sensitive and selective for detecting liver damage compared to ALT. An increase in AST levels may be observed due to non-liver-related factors (Robles-Diaz et al., 2015). By implication, high serum levels of liver enzymes may indicate hepatocellular toxicity (Kumar et al., 2020) whereas a decrease could result from enzyme inhibition (Kazeem, 2013). Bilirubin (Total and Direct), ALP, ALT and AST were analyzed as the biomarkers of liver health condition after a 28-day sub-acute study.

Overall, there was no significant change in the serum levels of Bilirubin after the study. Heme catabolism results in bilirubin, of which 80% is derived from haemoglobin (Kumar et al., 2020). This finding conforms to the insignificant change in serum haemoglobin already reported in this study. Oral and inhalation application of lizard dung showed a decrease in serum levels of ALP. This is less common, moreover, alkaline phosphatase assays can be affected by negative interference due to the denaturation of haemoglobin by alkali, which can result in a decrease in absorbance measurements (Kumar et al., 2020). The sub-acute toxicity study showed a significant decrease in serum levels of ALT which in conjunction with the histological results, suggests no significant change in the health state of the liver. The result of the AST assay also showed a significant reduction in serum levels compared to the negative control.

Available case reports on the abuse of lizard dung (Chahal et al., 2016; Danjuma et al., 2015) did not specify durations of usage. It becomes noteworthy, therefore, to assert that a typical substance abuser would not stop after 28- days. The usage of lizard dung could persist for months and years especially as these substances are easily available at almost no cost and are no legal framework militating against the usage (Orsolini et al., 2018; Zhang et al., 2018). The lack of indications of toxicity observed (after this 28-day study) in haematological parameters and after histological studies of the kidney, livers and hearts may not be conclusive on the overall safety of the oral and inhalational administration of lizard dung because the longer duration of toxicity study (say 120-180days), would appropriately replicate its real-life duration of usage, and as such, more likely to provide stronger evidence about the toxicity profiles of the use of lizard dung as a substance of abuse.

Previous studies have established chronic inflammation as a major player in lung cancer (Cho et al., 2011; Gomes et al., 2014; O'Callaghan et al., 2010; Yanbaeva et al., 2007). Histological examination of sections of the lungs of the groups that received the whitish and darkish part of the lizard dung via the inhalational route revealed the presence of mixed inflammatory infiltrates, acute or chronic inflammation with associated oedema and occasional haemorrhage in keeping with chronic irritation. This could be suggestive of the initiation of lung fibrosis and a possible progression of lung cancer.

Animal models for central nervous system activities are an attempt to reproduce features of human CNS behaviours in laboratory animals. Assumptions are often made in theory that a model should reproduce all the features of the CNS being studied. Unfortunately, this is hardly achieved due to the complex manifestation of psychiatric responses and the huge disparity between human and laboratory animal cognition. The whitish and darkish parts of lizard dung were evaluated (after oral and inhalational routes) for CNS activities in rats using tail suspension and forced swim tests (antidepressant-like effect), hole board and elevated plus maze tests (anxiolytic-like effect) and sodium pentobarbital-induced sleeping time test (sedative effect).

The forced swim and tail suspension studies subjected rats to an inescapable and stressful circumstance, resulting in a mental state characterized by feelings of hopelessness and despair. This behaviour can be measured by the duration of immobility (Sofidiya et al., 2022). The immobility or despair behaviour shown in both the forced swim and tail suspension experiments is considered a model of depression that mirrors the behavioural despair observed in individuals with depression. Typically, the immobility response is diminished by the use of antidepressant medications and

other therapies that are proven to be successful in treating depression (Lucki, 2010). The test sample reduced immobility time in the two tests after oral and inhalational administrations suggesting CNS depression. In the tail suspension test, the reduction in duration of immobility seen in the groups that inhaled 1.0g and 2.0g of the whitish part of lizard dung was also significantly different from and comparable to the reduction by imipramine standard. In a similar observation, the experimental groups that received inhalation of 1.0g and 2.0g of the darkish part of lizard dung before the forced swim test, significantly reduced the duration of immobility in comparison to the negative control and comparable extent to the imipramine group. This suggests anti-depressant activity and that inhalational administration is more effective than oral administration, therefore explaining why abusers usually smoke/inhale the lizard dung (Jimoh et al., 2022).

In the elevated plus maze, anxiety can be indicated by an animal's tendency to avoid the open arms of the maze. The increase in the amount of time animals spend in the open arms indicates a calming impact, which is a result of CNS depression with an anxiolytic-like effect. In this experiment, the rats were pre-treated with oral and inhalational dosages of the extract, which significantly increased their entry and time spent in the open arms, suggesting an anxiolytic-like effect (Sofidiya et al., 2022). The Hole board test evaluates an individual's levels of anxiety, emotionality, and reaction to stress (Wegener & Neigh, 2021). A significant reduction in head dips during the test is used to assess CNS depressant activity (Wegener & Neigh, 2021). Oral and inhalational administration of lizard dung, especially the whitish part significantly reduced the number of head dips during the exploratory behaviour of the rats in the hole board apparatus, also suggesting CNS depression.

One can use the potentiation of phenobarbital-induced sleepiness to assess the potential sedative-hypnotic properties of substances (Sofidiya et al., 2022). The depression of the CNS was also evident by the potentiation of phenobarbital-induced hypnosis in a dose-dependent manner after oral and inhalational co-administration of the whitish and darkish parts of lizard dung and phenobarbitone in the rats. The observed effects of the lizard dung may be mediated through central mechanisms implicated in sleep regulation or through inhibition of phenobarbital metabolism (Hudu et al., 2017).

Conclusion

The whitish and Darkish part of lizard dung poses no visible acute toxicity in rats upon oral and inhalational administration. Although alterations in the kidney and liver biomarkers were observed after 28 days, the oral and inhalational administration of the whitish and darkish part of the lizard dung led to no visible change in the physiology of the kidney, liver and heart. Oral and inhalational administrations of whitish and darkish parts of lizard dung altered general behavioural patterns of rats, including a dose- and route-of-administration-dependent reduction in the exploratory behaviour and potentiation of phenobarbitone-induced sleeping time. These observations may suggest depression of the CNS and support the claims about the use of lizard dung as a substance of abuse.

Recommendation

Subsequent research may focus on characterizing the exact crude proteins in the whitish part of the lizard dung. A longer duration of toxicity study (about 120-180 days) would appropriately replicate its real-life duration of usage, more likely to provide stronger evidence about the chronic toxicity profile of the use of lizard dung. Additionally, subsequent studies may explore the underlying mechanisms of CNS depression by the lizard dung and isolate the compound(s) responsible for the observed effects.

Declarations:

Ethical Approval

The study was conducted by the ARRIVE guidelines [47]. The Departmental Research and Ethics Committee approved the research protocol and assigned it a reference number UDUS/DREC/2022/019.

Competing interests

The authors declare that they have no conflict of interest.

Author's Contributions

Conceptualization, Abdulgafar Jimoh and Shuaibu Hudu; **Formal analysis,** Sydney Okwor; **Funding acquisition**, Abdulgafar Jimoh; **Investigation,** Abdulgafar Jimoh and Sydney Okwor; **Methodology**, Sydney Okwor, Umar Tukur and Abubakar Bilyaminu; **Project administration**, Abdulgafar Jimoh and Sydney Okwor; **Resources,** Shuaibu Hudu, and Zuwaira Sani; **Supervision**, Umar Tukur and Abubakar Bilyaminu; **Visualization**, Umar Mohammed and Muhammad Haruna; **Writing original draft,** Sydney Okwor; **Writing review & editing**, Kehinde Adeshina.

Competing interests

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Data Availability

Data are available upon reasonable request from the corresponding author.

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