

Sub-chronic (Ninety Days) Toxicity Study of Hydroethanolic Leaf Extract of *Datura stramonium* L. in Rodents

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ABSTRACT

Background: Phyto-medicine represents a vast pool of novel drug development, but understanding their safety requires elaborate, multifaceted approaches, including toxicity studies.

Objective: This study investigated the effects of 90 days of oral administration of *Datura stramonium* (DSE) leaf extract in Rats.

Methods: In the oral acute toxicity study, mice were treated with a single oral gavage of DSE at 500, 1000, and 2000 mg·kg⁻¹/d, po and observed for signs of acute toxicity for 14 days. In the sub-chronic study, rats were randomized into four Groups (A–D). Group A received distilled water (10 mL·kg⁻¹, po) while groups B–D received DSE (10, 50 and 250 mg·kg⁻¹/d, po, respectively) orally for 90 days uninterrupted. Animals were weighed weekly, with food and water measured daily and relevant parameters assayed at the end of the 90days administration.

Results: In acute toxicity studies, oral administration of up to 2 g·kg⁻¹/d, po of DSE did not elicit any semblance of toxicity or mortality within 24 h to 14 days. In the 90days studies, DSE (250 mg·kg⁻¹/d, po) decreased the body weight, brain weight, and food intake in female rats. DSE (10–250 mg·kg⁻¹/d, po) increased the red blood cell (RBC), packed cell volume (PCV) and hemoglobin (Hb) in both sexes. DSE (10–250 mg·kg⁻¹/d, po) increased the triglycerides (TG), cholesterol and low-density lipoprotein (LDL); and decreased HDL in both sexes. DSE (10–250 mg·kg⁻¹/d, po) increased the white blood cells (WBC) and platelets in female rats. DSE (10–250 mg·kg⁻¹/d, po) decreased the alkaline phosphatase (ALP) and alanine transaminase (ALT) in both studies. Serum urea level was decreased in both sexes. DSE (250 mg·kg⁻¹/d, po) decreased male rats' serum sodium ion levels. Liver, brain, testes and kidney showed severe lesions at 250 mg·kg⁻¹/d, po of the extract.

Conclusion: *D. stramonium* is safe on acute exposure and relatively safe on sub-chronic oral administration. However, prolonged use, especially at high doses, could cause Liver, brain and kidney toxicities; and abnormal lipid metabolism.

1. Introduction

Ethno-medicine represents a vast pool of novel drug development, but investigating and understanding their safety requires elaborate, multifaceted approaches, including toxicity studies (Atansov et al., 2015; Raclariu et al., 2018). Herbal medicine is a credible healthcare option which has, in recent years, been entrenched in our

society and has provided the basis for the use of plant or natural substances in managing many ailments in both poor and rich nations (Raclariu et al., 2018; Lim et al., 2021). The blanket assumption by rural dwellers that herbal products are free of adverse reactions may have occasioned huge patronage for their healthcare (Lim et al., 2021; Okaiyeto and Oguntibeju, 2021). This erroneous belief has led to several internal organ damages and death cases

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(Shaban et al., 2016). Moreover, (Okaiyeto and Oguntibeju, 2021) highlighted the role of toxic bioactive compounds, heavy metals and pesticide contaminations in herbal toxicities, which negatively impact the users' health (Berrin et al., 2006). Moreover, validated herbal-related multi-organs toxicities, including nephrotoxicity, hepatotoxicity and cardiotoxicity to hypersensitivity mediated/induced phytotoxicity—documented respiratory and gastrointestinal toxicities in a combination therapy study involving herbal medicine and antihypertensive drugs (Aydin et al., 2016). Using phytomedicine alongside pharmaceutical agents could cause adverse drug reactions such as increased liver enzymes, gastrointestinal distortions and hepatic damage (Awortwe et al., 2018). Reports have also suggested that the biotransformation of herbal components to anthraquinones during long-term exposure may induce carcinogenic effects on the liver and intestine (Dunnick and Nyska, 2013). Recently, multi-organ toxicities have also been reported in individuals who consume herbal products indiscriminately. Against these backdrops, there is a need to intensify studies on the safety of commonly used herbal products to guide the public on their proper consumption.

Datura stramonium (DS) Linn. (jimsonweed) is a medicinal plant belonging to the family Solanaceae. It is an erect, readily branching annual flowering plant with a height range of about 90 cm–125 cm and distributed across all continents. Its flowers are trumpet-shaped, white to creamy, with a fragrant odor. The root is long, thick and whitish, while the yellow to the green stem is smooth, erect and leafy. The leaves are moderately long and smooth, nauseating taste; its anterior surface is dark green, while the posterior surface is light green. The seed is egg-shaped and covered with spines. *Datura stramonium* plant grows well in warm and temperate regions of the world. *D. stramonium* herb contains toxic and dangerous levels of anticholinergic alkaloids, which could result in anticholinergic poisoning in cases of overdose (Glatstein et al., 2016). Traditionally, *Datura stramonium* is used for diverse human health problems. The leaf of *Datura stramonium* is used topically for treating skin disorders, wound healing, baldness, boils and swellings (Sayyed and Shah, 2014). The seeds of DS are used for body pains, asthma, tonsillitis and sedation (Khan et al., 2013; Sayyed and Shah, 2014). Its flower juice is employed for managing cough, asthma, fever, earache, and dandruff and as a purgative (Sayyed and Shah, 2014; Alam et al., 2021; Dayar et al., 2021). Modern medicine has several therapeutic potentials, including antimicrobial, nematicidal, antifungal, insecticidal, anti-diabetic, anti-asthmatic, anti-inflammatory, antioxidant, analgesic, anticancer, wound healing, and neurological activities of *Datura stramonium* have been reported (Al-Snafi, 2017; Cornelius et al., 2019). However, information about the safety profile of *D. stramonium* is still inadequate despite its usefulness in traditional and modern medicines. In addition, because *Datura stramonium* is one of the plants commonly abused, it is imperative to properly understand its pattern of toxicity to advise policymakers on the consumption of the plant. Besides, most of the toxicity studies conducted on *Datura stramonium* were usually between 14 and 28 days, and the outcomes from these studies may not necessarily represent the toxicity status of *D. stramonium*. Hence, the need for sub-chronic (90 days) studies is urgent. Accordingly, this study extensively investigated the safety profile of sub-chronic (90 days) oral administration of hydroethanolic leaf extract of *Datura stramonium* in rats.

2. Materials and Methods

2.1. *Datura stramonium* plant

Datura stramonium leaves were obtained from the Odogbolu area of Ogun state, Nigeria. The plant was identified and verified at the Department of Botany, Faculty of Science, University of Lagos, Nigeria. A voucher specimen was deposited in the university herbarium (LUH: 9019).

2.2. Extraction

Freshly procured *Datura stramonium* leaves were kept in an adequately aerated room to air dry the leaves. The dried leaves were ground, weighed (655 g) and soaked in 2 L of distilled water and ethanol (70%) in a ratio of 1:1 for 72 h. After that, it was drawn off and sieved with muslin cloth and Whatman filter paper. The residue was soaked again in two cycles to ensure increased extraction. At a reduced pressure of about 40°C, the filtrates were dried off (evaporated) to produce a solid extract (8.7% yield) (Akindede et al., 2015). Different working concentrations (1, 5 and 25 mg·kg⁻¹) of the extract dissolved in distilled water were prepared and appropriate volumes were administered to the animals corresponding to different doses of the extract used in this study.

2.3. Animals

Sixty-four Wistar rats (male and female) with a weight range of about 125 g–190 g were acquired from the Laboratory Animal centre of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun state, Nigeria. The animals were housed in standard, adequately ventilated, clean cages and kept in a sterile and decent environment. The animals were provided with standard rodent feeds and clean water ad libitum. A conventional 14 days period of acclimatization was observed for the rats before the study commenced. Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the College of Medicine, University of Lagos, Nigeria. The rights and welfare of the animals were protected in line with the Guidelines for the Care and Use of Laboratory Animals.

2.4. Acute toxicity test

Two separate groups of Swiss mice consisting of five animals (male and female) each were denied food for about 12 h before the start of the experiment. For the acute oral test group, *Datura stramonium* extract was given orally up to 2 g·kg⁻¹/d, po (Murtala et al., 2021). Regarding the acute intraperitoneal test group, various doses (150, 300, 600 and 1200 mg·kg⁻¹/d, ip.; five animals each, respectively) of the extract were administered to the mice intraperitoneally (Matsuo et al., 2002). The control groups in both toxicity studies received distilled water (10 mL·kg⁻¹). Two hours post-treatment, the animals were observed for behavioral expressions and signs of toxicity. Mortalities in each group were noted within the first 24 h, and those that did not die were observed for additional seven days for signs of delayed toxicity. The median lethal dose (LD₅₀) was estimated using the Behrens-Karber method (Matsuo et al., 2002).

2.5. Sub-chronic toxicity test

Sixty-four Wistar rats were, without bias, assembled into four different groups of 16 mice each (8 males and females). According to their sex, eight separate cages of two per group were used to house the rats. Daily oral treatment of the four groups of rats with distilled water (control) and extract doses of 10, 50 and 250 mg·kg⁻¹/d, po was done for 90 days consecutively. Dose selection in this study was predicated on the therapeutic dose of 50 mg·kg⁻¹ of *D. stramonium* extract as established in a previous study conducted by (Murtala et al., 2022) on the antidepressant and anxiolytic activities of the extract. The three extract doses employed in this study were sub-therapeutic ($\times 1/5$), therapeutic ($\times 1$), and supra-therapeutic ($\times 5$) by the methods and procedures of (Akindede et al., 2015) and (Murtala et al., 2021). Weekly body weight changes of the rats in each group were recorded for the entire study duration. Food and water consumption for each rat were calculated and noted, and this was done daily for 90 days. On the night of the 90th day, each rat's urine sample was collected with a metabolic cage for a urine test. Twenty-four

hours after the 90th day of the extract administration, rats were anaesthetized using ether (1.9%), and blood samples were collected through the retro-orbital plexus vein of the rat eye. Ethylenediaminetetraacetate (EDTA), lithium heparin and plain specimen bottles were used for hematological, biochemical and hormonal assays, respectively. Cervical dislocations were employed to sacrifice the rats. Blood in the plain bottles was allowed to coagulate and centrifuged at 3500 r/min for 10 min. Semen was collected from the testes and epididymis of each male rat after sacrificing (Akindele et al., 2015; Ogli et al., 2009; Kale et al., 2019).

2.5.1. Vital organs/internal structures

The animals were dissected, and internal structures (Heart, liver, kidney, brain, pancreas, spleen and testes) were carefully removed. The removed organs were weighed (standardized to 100 g body weight of individual rats). Each vital organ sample was fixed in a 10% buffer solution for histopathological assessment (Worasuttayangkurn et al., 2019).

2.5.2. Hematological analysis

Blood samples collected in the EDTA bottles were used for full blood count analyses. These include red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC), and it is differential (neutrophils, basophils, monocytes, eosinophils and lymphocytes) count and platelet count using automated hematology analyzer (Haghighi et al., 2014).

2.5.3. Biochemical parameters

The blood samples in lithium heparin bottles were analyzed for liver function test (LFT) (alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT); Kidney function test (KFT) (urea and creatinine); lipid profile test (LPT) (total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL); electrolytes (sodium ion, potassium ion, chloride ion, bicarbonate ion and hydrogen ion); total protein, albumin, total bilirubin and conjugated bilirubin using standard diagnostic test kits (Randox Laboratories, Crumlin, UK) on Automated Clinical System (Synchro Clinical Systems, model: CX5PRO; Beckman Coulter Inc., Galway, Ireland) (Olayode et al., 2020).

2.5.4. Sperm test

Semen was collected from the testes and epididymis of each male rat after sacrificing according to the methods of (Akindele et al., 2015; Ogli et al., 2009) and (Kale et al., 2019). Sperm analysis (sperm motility, sperm count and sperm morphology, i.e.% abnormality) was carried out.

2.5.5. Urine test

Fresh urine samples collected from individual rats on the 89th/90th day for 12 h (8:00 pm–8:00 am, i.e. from dusk to dawn) with the aid of metabolic cages were analyzed for glucose, bilirubin, ketone, specific gravity, pH, protein, urobilinogen, nitrite, and blood using automatic urine analyzer and commercial urine analysis strips Cybow™ (DFI Co Ltd, Korea). This procedure was carried out by (Donkor et al., 2014) and (Li et al., 2019).

2.5.6. Hormonal test

The blood samples in the plain sample bottles were allowed to coagulate for about 1 hour and later centrifuged at 3500 rpm for 10 min to obtain plasma/serum. The serum generated was used to analyze follicle-stimulating hormones (FSH), Luteinizing hormone (LH), progesterone, prolactin and testosterone levels. This procedure was conducted in line with the submissions of (Brandão-Costa et al., 2016) and (Njan et al., 2019).

2.5.7. Histopathological assessment

Internal structures, including the brain, testes, liver and kidney, were removed for cytoarchitectural examinations. They were preserved in 10% buffered formalin, dehydrated in graded alcohol, embedded in

paraffin, and cut into two μm thick sections. The sections were stained with haematoxylin-eosin for the photo-microscopic assessment using a Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany) (Yuet et al., 2013; Akindele et al., 2015).

2.6. Statistical analysis

Values were represented as mean \pm standard error of the mean (SEM.) One-way ANOVA alongside Dunnett's posthoc tests was employed to compare the data. Graph-Pad Prism 6 Software was used (Graph-Pad Software Inc., CA, USA). Results were considered significant at $P < 0.05$.

3. Results

3.1. Acute toxicity

In the acute oral toxicity study, oral administration of up to 2 g/kg b.wt /day/ p.o. of *D. stramonium* did not elicit any semblance of toxicity or even mortality within 24 h to 14 days in mice. Intraperitoneal administration of leaf of *D. stramonium* did not elicit apparent clinical signs of toxicity at 150–300 mg/kg b.wt /day/ i.p. There was 100% mortality at 1200 mg/kg b.wt /day/ i.p., though dullness, inactivity, hypokinesia, difficulty in breathing, tachycardia and lacrimation were noted before their death. The intraperitoneal LD₅₀ was estimated to be 630 mg/kg b.wt /day/ i.p.

3.2. DSE decreased bodyweight and food intake in female rats; and increased food and water intake in male rats

In male rats, the extract did not produce any significant changes ($P > 0.05$) in the body weights of the animals relative to the control. On feeding pattern, *D. stramonium* (10 mg·kg⁻¹/d, po) produced a significant increase ($P < 0.05$) in food intake compared to the vehicle. Regarding water consumption, the extract (10 & 50 mg·kg⁻¹/d, po) increased ($P < 0.001$) the water intake relative to the distilled water (Table 1). In female rats, *D. stramonium* (250 mg·kg⁻¹/d, po) significantly decreased ($P < 0.01$) the body weight compared to the control. The extract at all doses reduced ($P < 0.01, 0.0001$) the food consumption of the distilled water. However, water intake was not significantly affected ($P > 0.05$) by the extract compared to the control (Table 1).

3.3. DSE increased weight of heart, liver and kidney; and decreased weight of brain in female rats

In the male, *D. stramonium* did not elicit any significant changes ($P > 0.05$) in the weights of all the internal organs compared to the vehicle (Table 2). However, in female rats, the extract (10–250 mg·kg⁻¹/d, po) produced a significant increase ($P < 0.05–0.001$) in the weights of the heart, liver and kidney relative to the control. In comparison to distilled water, the extract (50–250 mg·kg⁻¹/d, po) produced a decrease ($P < 0.05–0.001$) in the weights of the pancreas and brain (Table 2).

3.4. DSE increased most hematological parameters in male and female studies

In male rats, *Datura stramonium* non-significantly increased ($P > 0.05$) the WBC and its differentials except neutrophils which showed a decrease ($P < 0.05$) at 20 mg/kg b.wt /day/ p.o. and increase ($P < 0.0001$) at 250 mg·kg⁻¹/d, po Relative to the control. Meanwhile, the extract (10–250 mg·kg⁻¹/d, po) produced a significant increase ($P < 0.05, 0.0001$) in PCV, RBC and Hb compared to distilled water. *Datura stramonium* did not elicit any significant alteration ($P > 0.05$) on platelets against the control (Table 3). In female rats, *Datura stramonium* (10–250 mg·kg⁻¹/d,

Table 1Assessment of Hydroethanol Leaf Extract of *Datura stramonium* Activities on Bodyweight, Food and Water Intakes in Male and Female rats.

MALE				
Treatment	Dose (mg·kg ⁻¹)	Change in bodyweight (g)	Food intake (g)	Water intake (L)
Distilled water	10	35.85 ± 11.96	52.86 ± 4.68	27.45 ± 2.97
<i>D. stramonium</i>	10	20.46 ± 5.32	69.46 ± 3.78*	53.61 ± 5.84***
<i>D. stramonium</i>	50	18.15 ± 4.45	66.79 ± 3.90	51.79 ± 4.96***
<i>D. stramonium</i>	250	17.23 ± 5.77	62.99 ± 4.34	35.70 ± 3.64
FEMALE				
Treatment	Dose (mg·kg ⁻¹)	Change in bodyweight (g)	Food intake (g)	Water intake (L)
Distilled water	10	52.25 ± 8.14	110.20 ± 4.42	49.03 ± 4.37
<i>D. stramonium</i>	10	44.25 ± 6.48	79.26 ± 4.28****	60.88 ± 4.13
<i>D. stramonium</i>	50	43.92 ± 6.16	88.76 ± 4.34**	62.10 ± 4.14
<i>D. stramonium</i>	250	19.42 ± 2.43**	64.68 ± 3.99****	38.04 ± 4.39

Analyzed values showcased as mean ± Standard Error Mean (n = 8).

* P < 0.05,.

** P < 0.01,.

*** P < 0.001,.

**** P < 0.0001 vs. distilled water (One way ANOVA by Dunnet's multiple comparison test).

Bodyweight changes, food and water intakes were calculated for 90 days.

Table 2Assessment of Hydroethanol Leaf Extract of *Datura stramonium* Activities on weight of internal structures in Male and Female rats.

MALE								
Treatment	Dose (mg·kg ⁻¹)	Heart (g)	Liver (g)	Pancreas (g)	Spleen (g)	Brain (g)	Kidney (g)	Testes (g)
Distilled water	10	0.69 ± 0.01	4.51 ± 0.48	0.47 ± 0.05	0.53 ± 0.02	1.36 ± 0.17	1.12 ± 0.16	1.64 ± 0.19
<i>D. stramonium</i>	10	0.62 ± 0.05	4.32 ± 0.55	0.36 ± 0.04	0.36 ± 0.02	1.11 ± 0.13	0.85 ± 0.04	1.30 ± 0.08
<i>D. stramonium</i>	50	0.66 ± 0.04	5.04 ± 0.07	0.38 ± 0.05	0.45 ± 0.02	1.21 ± 0.16	0.98 ± 0.06	1.43 ± 0.07
<i>D. stramonium</i>	250	0.65 ± 0.01	4.55 ± 0.22	0.67 ± 0.12	0.43 ± 0.18	1.21 ± 0.03	1.45 ± 0.04	1.82 ± 0.12
FEMALE								
Treatment	Dose (mg·kg ⁻¹)	Heart (g)	Liver (g)	Pancreas (g)	Spleen (g)	Brain (g)	Kidney (g)	
Distilled water	10	0.46 ± 0.01	4.19 ± 0.14	0.46 ± 0.03	0.56 ± 0.01	1.13 ± 0.03	0.96 ± 0.03	
<i>D. stramonium</i>	10	0.59 ± 0.03*	5.65 ± 0.26**	0.49 ± 0.02	0.58 ± 0.02	1.26 ± 0.05	1.17 ± 0.05*	
<i>D. stramonium</i>	50	0.62 ± 0.04**	5.34 ± 0.37*	0.33 ± 0.05*	0.60 ± 0.03	0.85 ± 0.05**	1.02 ± 0.06	
<i>D. stramonium</i>	250	0.58 ± 0.03*	5.43 ± 0.28*	0.24 ± 0.01***	0.64 ± 0.03	1.08 ± 0.06	0.98 ± 0.05	

Analyzed values showcased as mean ± Standard Error Mean (n = 8).

* P < 0.05,.

** P < 0.01,.

*** P < 0.001 vs. distilled water (One way ANOVA by Dunnet's multiple comparison test).

po) increased ($P < 0.05-0.0001$) the WBC, monocytes and lymphocytes compared to the control. However, the extract at 20 and 250 mg·kg⁻¹/d, po It has elicited a significant decrease in neutrophils ($P < 0.05$) and lymphocytes ($P < 0.01$), respectively, relative to the vehicle. As regards PCV, RBC and Platelets, the extract (10–250 mg·kg⁻¹/d, po) produced a significant increase ($P < 0.05-0.001$) in the levels of these parameters against the control. Meanwhile, there was no significant change ($P > 0.05$) in Hb by the extract compared to the vehicle (Table 3).

3.5. DSE increased cholesterol, LDL and decreased HDL, ALP, ALT and urea in male and female rats

On cardiovascular function indicators, the extract (10–250 mg·kg⁻¹/d, po) produced a significant increase ($P < 0.01-0.001$) in cholesterol and LDL and a decrease in HDL compared to the control in male rats (Table 4). Meanwhile, in female rats, the extract (10–250 mg·kg⁻¹/d, po) elicited an increase ($P < 0.01-0.001$) in cholesterol, TG and LDL relative to the control. However, HDL was not affected ($P > 0.05$) by the extract (Table 4). On liver function test indices, *D. stramonium* (10–250 mg·kg⁻¹/d, po) produced a significant decrease ($P < 0.0001$) in ALP and ALT, while AST, TB and CB levels were not affected ($P > 0.05$) by the extract relative to the control in male rats (Table 4). In female rats, AST, ALP, TB and CB levels were not significantly ($P > 0.05$) affected by the extract compared to the vehicle.

Meanwhile, the extract (10–250 mg·kg⁻¹/d, po) produced a decrease ($P < 0.01$) in ALT against the control (Table 8). On kidney function parameters in male rats, *D. stramonium* (50 mg·kg⁻¹/d, po) elicited a decrease in the concentration of urea in the blood against the distilled water, while creatinine was not affected ($P > 0.05$) by the extract (Table 4). In female rats, there was a decrease ($P < 0.05, 0.001$) in urea concentration against the control by the extract (10 & 250 mg·kg⁻¹/d, po), but creatinine level was not significantly altered ($P > 0.05$) by the extract (Table 8). On electrolytes, most of the parameters were not significantly altered ($P > 0.05$) by the extract in both male and female rats (Table 4). However, the extract (250 mg·kg⁻¹/d, po) produced a significant decrease ($P < 0.01$) in sodium ions compared to the vehicle in male rats (Table 4).

3.6. DSE showed no effect on sperm parameters

Against the control, *D. stramonium* (250 mg·kg⁻¹/d, po) produced a non-significant decrease ($P > 0.05$) in sperm motility and sperm count (Table 5).

3.7. DSE Elicited no changes on urine parameters

In both male and female animals, *D. stramonium* produced no significant changes in the pH, specific gravity and glucose. There were

Table 3
Assessment of Hydroethanol Leaf Extract of *Datura stramonium* Activities on Hematological Parameters in Male and Female rats.

MALE											
Treatment	Dose (mg·kg ⁻¹)	WBC (× 10 ³ /μL)	NEU (%)	BAS (%)	EOS (%)	MON (%)	LYM (%)	PLT (× 10 ⁶ /μL)	PCV (%)	RBC (× 10 ⁶ /μL)	Hb (g·dL ⁻¹)
Distilled water	10	9.86 ± 1.73	30.87 ± 4.05	2.20 ± 1.36	3.93 ± 0.63	7.33 ± 1.45	64.20 ± 4.04	480.30 ± 59.18	30.23 ± 1.90	6.76 ± 0.21	8.23 ± 1.45
<i>D. stramonium</i>	10	9.74 ± 1.45	20.67 ± 1.48*	2.28 ± 1.41	2.93 ± 0.63	9.66 ± 1.76	73.22 ± 3.66	644.30 ± 56.29	61.45 ± 1.49***	8.85 ± 0.52*	15.18 ± 1.66*
<i>D. stramonium</i>	50	10.69 ± 1.29	39.03 ± 2.03	3.10 ± 1.15	3.83 ± 1.72	5.53 ± 1.44	58.77 ± 4.92	672.30 ± 56.29	49.53 ± 1.56***	8.93 ± 0.57*	7.43 ± 1.48
<i>D. stramonium</i>	250	13.14 ± 1.60	68.53 ± 1.47*	3.33 ± 1.18	4.13 ± 1.41	6.63 ± 1.38	66.59 ± 10.52	640.30 ± 115.10	51.55 ± 1.45***	7.67 ± 0.50	13.42 ± 1.46
FEMALE											
Treatment	Dose (mg·kg ⁻¹)	WBC (× 10 ³ /μL)	NEU (%)	BAS (%)	EOS (%)	MON (%)	LYM (%)	PLT (× 10 ⁶ /μL)	PCV (%)	RBC (× 10 ⁶ /μL)	Hb (g·dL ⁻¹)
Distilled water	10	7.67 ± 1.68	60.80 ± 7.39	0.43 ± 0.14	1.53 ± 0.99	8.63 ± 1.53	29.80 ± 1.28	539.30 ± 59.18	34.40 ± 1.40	6.98 ± 0.20	8.91 ± 1.29
<i>D. stramonium</i>	10	14.05 ± 1.63*	29.77 ± 2.29*	3.93 ± 1.62	2.93 ± 1.50	7.16 ± 1.50	65.40 ± 1.50***	677.30 ± 1.45*	59.52 ± 1.45***	8.35 ± 0.12***	14.45 ± 1.41
<i>D. stramonium</i>	50	13.46 ± 1.37	45.90 ± 7.82	1.66 ± 0.76	1.83 ± 0.69	15.93 ± 1.38*	46.50 ± 1.44***	745.30 ± 12.68**	35.73 ± 1.55	7.57 ± 0.08**	9.83 ± 1.33
<i>D. stramonium</i>	250	19.09 ± 1.49**	56.17 ± 5.09	0.46 ± 0.14	2.08 ± 0.50	22.50 ± 2.06**	20.77 ± 1.41**	835.00 ± 11.53***	49.87 ± 1.70***	7.18 ± 0.05	12.30 ± 1.51

Analyzed values showcased as mean ± Standard Error Mean (n = 8).

* P < 0.05.

** P < 0.01.

*** P < 0.001.

**** P < 0.0001 vs. distilled water (One way ANOVA by Dunnett's multiple comparison test). WBC: White blood cells, NEU: Neutrophils, BAS: Basophils, MON: Monocytes, EOS: Eosinophils, LYM: Lymphocytes, PLT: Platelets, PCV: Packed cell volume, RBC: Red blood cells, Hb: Hemoglobin.

no traces of protein, blood, ketone, nitrite, bilirubin and urobilin in the extracted treated groups in both male and female animals (Table 6).

3.8. Hormonal assay DSE Elicited no changes on sex hormones

In both male and female rats, *D. stramonium* (10–250 mg·kg⁻¹/d, po) produced a non-significant change (P > 0.05) on all the hormones evaluated in this study relative to the control (Table 7).

3.9. Histopathology

Liver, kidney, brain and testes exhibited mild to moderate cytoarchitectural distortions in male and female animals (Figs. 1–4).

4. Discussion

Previous studies have documented the traditional and modern uses of *D. stramonium*. However, extensive toxicological evaluation of *D. stramonium* is imperative to predict its effects or possible damage following long-term exposure correctly. Therefore, a sub-chronic (90 days) toxicological evaluation of the hydroethanolic leaf extract of *D. stramonium* was investigated in this study. In the oral acute toxicity study, oral administration of up to 2 g·kg⁻¹/d, po of *D. stramonium* did not elicit any semblance of toxicity or mortality within 24 h to 14 days in mice. The intraperitoneal LD₅₀ was estimated to be 900 mg·kg⁻¹/d, ip. It has been reported that an acute oral LD₅₀ estimate of more than 2000 mg·kg⁻¹/d, po, of the extract is considered safe and nontoxic (Ugwah-Oguejiofor et al., 2019; Alelign et al., 2020; VK et al., 2021). Absence of abnormal changes in the general behavior of animals, such as difficulty in breathing, tachycardia, hypokinesia, dullness, inactivity, lacrimation and death upon acute oral LD₅₀ estimate of 2–5 g·kg⁻¹/d, po of the extract is considered harmless and safe (Nugroho et al., 2020; Degu et al., 2021). Accordingly, *D. stramonium* can be said to be relatively safe.

Bodyweight, food, and water intake changes are preliminary signs of toxicity, disease development and signs of improvement upon treatment (Silva et al., 2012; Abebe et al., 2021). In male rats, the extract non-significantly decreased the body weight gain of the animal. However, food and water intakes were increased at the lower doses of the extract. Meanwhile, in female animals, *D. stramonium* decreased the body weight of the animals at 250 mg·kg⁻¹/d, po and food intake at all doses of the extract. In one of the previous toxicity studies conducted on *D. stramonium*, (Uddin et al.) submitted that *D. stramonium* leaves decreased the body weight of rats after 14–28 days of administration (Uddin et al., 2017). This observation agrees with our data, especially on female rats, which significantly reduced body weight gain. However, the lack of significant changes in the body weight of male rats seems to agree with the submissions of Abdelouahab et al. and Gidado et al. (Uddin et al., 2017; Abdelouahab et al., 2011; Gidado et al., 2007). who reported no changes in the body weight gain of male rats after 120 days of administration of *D. stramonium* extract. According to the previously reported findings, the reduction in body weight and food intake in female animals suggests toxicity, which links the loss of appetite and body weight to drug-induced toxicity (El Fakir et al., 2021). The decreased body weight and food intake in female animals could be attributed to the physiologic differences that affect drug activity (Whitley and Lindsey, 2009). Decreased intestinal enzymatic activity and glomerular filtration rate in females could be responsible for the *D. stramonium* toxicities on female rats' body weight and appetite (Anderson, 2008). The increase in water consumption in male animals does not indicate toxicity, which agrees with the findings of (Ahmad et al., 2020), which reported an increase in water consumption due to regular physiological changes. Elevation in food consumption in male animals indicates no sign of toxicity but rather the tendency of the extract to augment appetite, as previously reported by (Kognou et al., 2018). Therefore, the above findings suggest

Table 4
Assessment of Hydroethanol Leaf Extract of *Datura stramonium* Activities on Biochemical Parameters in Male and Female rats.

MALE												
TM	Dose (mg·kg ⁻¹)	CHOL (mmol·L ⁻¹)	TRIG (mmol·L ⁻¹)	HDL (mmol·L ⁻¹)	LDL (mmol·L ⁻¹)	ALB (μmol·L ⁻¹)	TP (g·dL ⁻¹)	TB (μmol·L ⁻¹)	CB (μmol·L ⁻¹)	ALP (u/L)	AST (u/L)	ALT (u/L)
Distilled water	10	65.33 ± 5.78	43.33 ± 2.33	29.67 ± 2.90	26.33 ± 5.45	5.16 ± 1.30	5.96 ± 0.02	0.08 ± 0.03	0.03 ± 0.00	62.50 ± 6.67	9.33 ± 1.45	30.25 ± 2.65
D. stramonium	10	87.67 ± 6.38*	49.67 ± 2.90	12.33 ± 1.45**	56.33 ± 5.81*	5.96 ± 1.39	6.84 ± 1.64	0.11 ± 0.03	0.03 ± 0.01	23.50 ± 3.17****	14.33 ± 1.45	8.25 ± 1.37****
D. stramonium	50	124.30 ± 4.33***	56.33 ± 3.18	20.33 ± 1.45	84.33 ± 4.91***	7.23 ± 1.39	9.00 ± 1.47	0.10 ± 0.04	0.02 ± 0.01	20.40 ± 2.72****	14.67 ± 2.60	9.75 ± 1.75****
D. stramonium	250	103.70 ± 5.20**	53.67 ± 7.44	14.33 ± 3.52**	76.67 ± 7.12***	6.80 ± 1.68	7.3 ± 1.56	0.11 ± 0.04	0.02 ± 0.01	18.75 ± 3.19****	12.33 ± 2.33	7.50 ± 1.55****
FEMALE												
TM	Dose (mg·kg ⁻¹)	CHOL (mmol·L ⁻¹)	TRIG (mmol·L ⁻¹)	HDL (mmol·L ⁻¹)	LDL (mmol·L ⁻¹)	ALB (μmol·L ⁻¹)	TP (g·dL ⁻¹)	TB (μmol·L ⁻¹)	CB (μmol·L ⁻¹)	ALP (U·L ⁻¹)	AST (U·L ⁻¹)	ALT (U·L ⁻¹)
Distilled water	10	46.67 ± 5.23	14.67 ± 2.33	20.25 ± 4.64	26.33 ± 4.09	5.73 ± 1.53	7.43 ± 1.03	0.05 ± 0.01	0.05 ± 0.01	8.92 ± 1.68	8.25 ± 1.49	27.67 ± 4.05
D. stramonium	10	91.67 ± 4.05**	46.67 ± 2.96***	21.50 ± 2.78	59.67 ± 8.11**	5.70 ± 0.92	8.76 ± 1.59	0.04 ± 0.01	0.06 ± 0.01	11.83 ± 1.33	14.00 ± 2.55	11.67 ± 1.85**
D. stramonium	50	111.30 ± 7.44***	54.70 ± 3.18****	20.50 ± 3.08	84.33 ± 5.36***	4.78 ± 0.70	7.53 ± 1.56	0.05 ± 0.01	0.05 ± 0.01	13.83 ± 1.33	13.75 ± 1.88	10.33 ± 1.66**
D. stramonium	250	102.70 ± 9.38**	51.33 ± 4.09***	17.00 ± 3.36	74.67 ± 4.09***	3.12 ± 1.09	5.53 ± 1.50	0.03 ± 0.01	0.04 ± 0.02	14.13 ± 1.43	11.50 ± 1.32	10.67 ± 2.02**
MALE												
Na ⁺ (mmol·L ⁻¹)	K ⁺ (mmol·L ⁻¹)	Cl ⁺ (mmol·L ⁻¹)	H ⁺ (g·L ⁻¹)	HCO ₃ ⁻ (mEq/L)	Urea (mg·dL ⁻¹)	Creatinine (μmol·L ⁻¹)						
141.80 ± 2.20	3.68 ± 0.41	98.67 ± 1.76	7.10 ± 1.44	19.33 ± 1.45	51.33 ± 2.02	1.65 ± 0.08						
136.70 ± 1.48	3.06 ± 0.29	96.33 ± 1.45	6.93 ± 1.02	18.68 ± 1.60	46.67 ± 1.76	2.00 ± 0.25						
141.30 ± 1.45	3.70 ± 0.11	101.30 ± 1.45	8.16 ± 1.20	22.72 ± 1.61	39.00 ± 1.15**	1.83 ± 0.26						
129.30 ± 1.50**	3.90 ± 0.14	98.67 ± 1.76	7.63 ± 1.54	20.52 ± 1.62	46.00 ± 1.52	1.73 ± 0.14						
FEMALE												
Na ⁺ (mmol·L ⁻¹)	K ⁺ (mmol·L ⁻¹)	Cl ⁺ (mmol·L ⁻¹)	H ⁺ (g·L ⁻¹)	HCO ₃ ⁻ (mEq/L)	Urea (mg·dL ⁻¹)	Creatinine (μmol·L ⁻¹)						
142.30 ± 1.45	5.61 ± 0.35	96.90 ± 0.63	6.80 ± 0.47	19.83 ± 0.73	57.52 ± 1.62	1.80 ± 0.05						
132.50 ± 1.32	4.63 ± 1.62	101.70 ± 2.02	7.50 ± 1.05	21.33 ± 1.45	48.58 ± 1.68*	2.20 ± 0.83						
153.80 ± 22.10	4.33 ± 1.45	102.30 ± 0.66	8.63 ± 1.59	18.85 ± 1.08	52.62 ± 1.71	2.60 ± 1.17						
136.20 ± 1.74	4.43 ± 1.51	98.83 ± 1.64	7.73 ± 0.61	20.58 ± 1.68	41.52 ± 1.44***	2.06 ± 0.78						

Analyzed values showcased as mean ± Standard Error Mean (n = 8).

* P < 0.05.

** P < 0.01.

*** P < 0.001.

**** P < 0.0001 vs. distilled water (One way ANOVA by Dunnett's multiple comparison test). TM: Treatment, DW: Distilled water, DS: *Datura stramonium*, CHOL: Cholesterol, TRIG: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, ALB: Albumin, TP: Total protein, TB: Total bilirubin, CB: Conjugated bilirubin, ALP: Alkaline phosphatase, AST: Aspartate transaminase, ALT: Alanine transaminase, Na⁺: Sodium ion, K⁺: Potassium, Cl⁺: Chloride ion, H⁺: Hydrogen ion, HCO₃⁻: Bicarbonate ion.

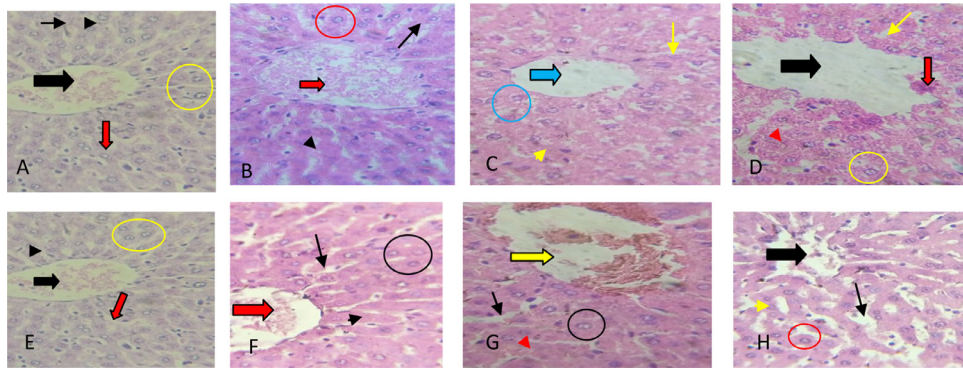


Fig. 1. Photomicrograph of male (A-D) and female (E-H) rat liver tissue.

- A. Control group (10 mL·kg⁻¹/d, po) with liver tissue showing normal appearance and histomorphology of the central vein (black thick arrow), hepatocytes (yellow circle), sinusoids with kupfer cells (red thin arrow) and hepatic plate (black arrow head)
- B. Treated group with DSE 10 mg·kg⁻¹/d, po. showed no severe morphological changes, the hepatic plate (black arrow head), hepatocytes (red circle), slight congestion of the central vein (red thick arrow) and sinusoids (black thin arrow)
- C. Treated group with DSE 50mg·kg⁻¹/d, po. extract showed mild morphological changes and irregularity of the hepatic plate (yellow arrow head), constricted sinusoid (yellow thin arrow), microveside hepatocyte (blue circle) with a clear central vein (blue thick arrow)
- D. Treated with DSE 250 mg·kg⁻¹/d, po. extract showed severe necrotic degeneration of the tissue (red thin arrow), enlarged central vein (black thick arrow), and hepatic plate irregularity (red arrow head) with loss of function. H/E ×400; DSE: *Datura stramonium* extract.
- E. Control group (10 mL·kg⁻¹/d, po) with liver tissue histology showed normal histomorphology of the central vein (black thick arrow), hepatocytes (yellow circle), sinusoids housing the kupfer cells (red thin arrow) and the hepatic plate (black arrow head) were well defined
- F. Treated group with DSE 10 mg·kg⁻¹/d, po. extract showed slight morphological changes, irregularity of the hepatic plate (black arrow head), slight congestion of the central vein (red thick arrow), sinusoids with reduced kupfer cells (black thin arrow) and hepatocytes (black circle)
- G. Treated with DSE 50 mg·kg⁻¹/d, po. extract showed mild morphological changes, congested central vein (yellow thick arrow), microvesides hepatocytes (blue circle), constricted sinusoids with reduced kupfer cells (black thin arrow) and hepatic plate (red arrow head)
- H. Treated with DSE 250 mg·kg⁻¹/d, po. extract shows degradation and hydropic degeneration of the hepatocytes (red circle), congested central vein (black thick arrow), and dilated sinusoids (black thin arrow) with loss of function. H/E ×400; DSE: *Datura stramonium* extract.

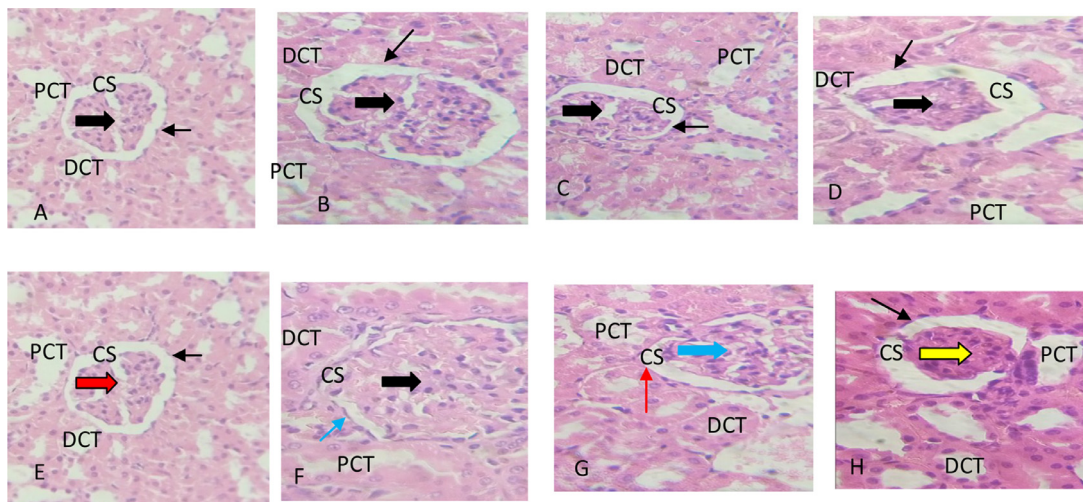


Fig. 2. Photomicrograph of male (A-D) and female (E-H) rat renal tissue.

- A. Control (10 mL·kg⁻¹/d, po) group showing normal histoarchitecture of renal tissue. The glomerulus (black thick arrow), capsular space (CS), epithelia cells (black thin arrow) and the proximal convulated tubules (PCT) and distal convulated tubules) DCT) appeared normal
- B. Treated group with DSE 10 mg·kg⁻¹/d, po. extract showed no significant morphological changes, the glomerulus (black thick arrow), epithelial cells (black thin arrow), capsular space (CS), and the proximal and distal convulated tubules (PCT and DCT) were well organized without any loss of function
- C. Treated group with DSE 50 mg·kg⁻¹/d, po. extract showed distorted glomerulus (black thick arrow), constricted distal convulated tubules (DCT), capsular space (CS) with dilated proximal tubules (PCT)
- D. Treated group with DSE 250 mg·kg⁻¹/d, po. extract showed collapsed glomerulus (black thick arrow), dilated capsular space (CS) and dilated proximal and distal tubules (PCT and DCT) with loss of function. H/E ×400; DSE: *Datura stramonium* extract.
- E. Control (10 mL·kg⁻¹/d, po) group showing normal histoarchitecture of renal tissue. The glomerulus (red thick arrow), capsular space (CS), epithelia cells (black thin arrow) and the proximal convulated tubules(PCT) and distal convulated tubules) DCT) appeared normal
- F. Treated group with DSE 10mg·kg⁻¹/d, po. extract showed no significant morphological changes, the glomerulus (black thick arrow), epithelial cells (black thin arrow), constricted capsular space (CS), the proximal and distal convulated tubules (PCT and DCT) were well organized without any loss of function
- G. Treated group with DSE 50 mg·kg⁻¹/d, po. extract showed distortion of the glomerulus (blue thick arrow), dilated distal and proximal convulated tubules (DCT and PCT) and irregular capsular space(CS)
- H. Treated group with DSE 250 mg·kg⁻¹/d, po. extract showed collapsed and extrusion of the glomerulus (yellow thick arrow), dilated capsular space (CS) and severe dilated proximal and distal tubules (PCT and DCT) with loss of function. H/E ×400; DSE: *Datura stramonium* extract.

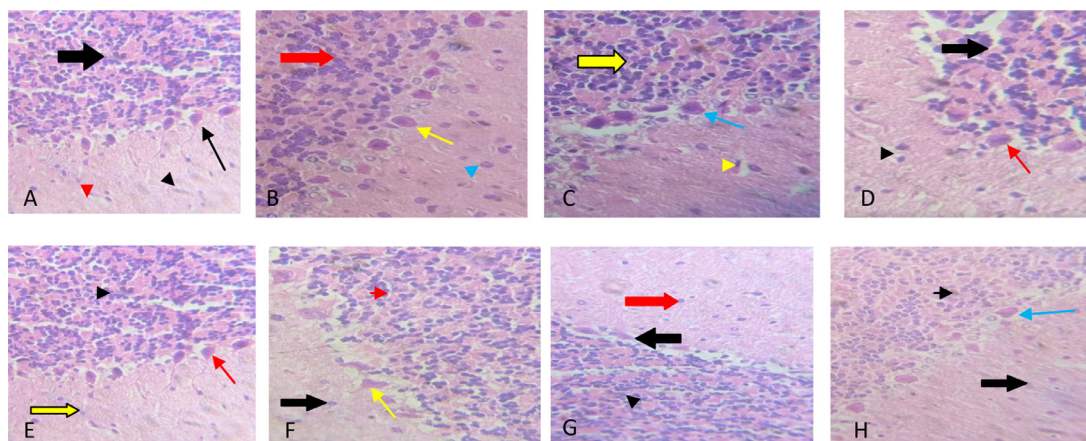


Fig. 3. Photomicrograph of male (A-D) and female (E-H) rat brain histology.

A. Control (10 mL.kg⁻¹/d, po) group showing well differentiated layers of brain tissue, the molecular layer (red arrow head), granular layer (black thick arrow) and the purkinje cells at the purkinje layer (black thin arrow) were well organized

B. Treated group with DSE 10 mg.kg⁻¹/d, po.. of extract showed no significant morphological changes the molecular layer (blue arrow head), the granular layer (red thick arrow) and the purkinje cells (yellow thin arrow) were well organized without any lesion

C. Treated group with DSE 50 mg.kg⁻¹/d, po. extract showed slight purkinje layer cells (blue thin arrow) distortion, vacuolation of the neuroglia cells at the molecular layer (yellow arrow head) and granular layer (yellow thick arrow).

D. Treated group with DSE 250mg.kg⁻¹/d, po. showed degenerating neurons and neuroglia cells on the molecular layer (black arrow head), hyperchromatic granular layer (black thick arrow) and purkinje cells (red thin arrow) distortion with loss of functions. H/E ×400; DSE: *Datura stramonium* extract.

E. Control (10 mL.kg⁻¹/d, po) group showing well differentiated layers of brain tissue, the molecular layer (red arrow head), granular layer (black thick arrow) and the purkinje cells at the purkinje layer (black thin arrow) were well organized

F. Treated group with DSE 10 mg.kg⁻¹/d, po. of extract showed no significant morphological changes at the molecular layer (blue arrow head), the granular layer (red thick arrow) with slight distortion at the purkinje cell layer (yellow thin arrow) without any visible lesion

G. Treated group with DSE 50 mg.kg⁻¹/d, po. extract showed slight constriction of the purkinje layer (black thin arrow) distortion of the neuroglia cells at the molecular layer (red thick arrow) and granular layer (black arrow head).

H. Treated group with DSE 250mg.kg⁻¹/d, po. showed reduced degenerating neurons and neuroglia cells on the molecular layer (black thick arrow), hypochromatic granular layer (black arrow head) and purkinje cells (blue thin arrow) distortion with loss of functions. H/E ×400; DSE: *Datura stramonium* extract.

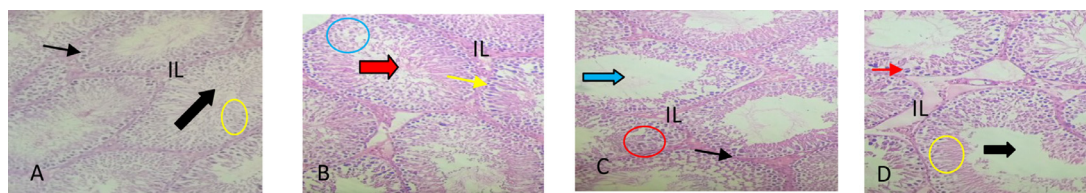


Fig. 4. Photomicrograph of testicular tissue histology.

A. Control (10 mL.kg⁻¹/d, po) testicular tissue showing normal testicular morphology, the seminiferous tubules (black thick arrow) housing the spermatozoa, sertoli cells (yellow circle), spermatogonia cells (black thin arrow) and the leydis cells at the interstitial layer (IL) were well defined

B. Treated group with DSE 10 mL.kg⁻¹/d, po extract showed no significant morphological changes. The seminiferous tubule (red thick arrow) housing the spermatozoa, sertoli cells (blue circle), spermatogonia cells (yellow thin arrow) were well defined with slight distortion at the interstitial layer housing the leydis cells (IL)

C. Treated with DSE 50 mg.kg⁻¹/d, po. extract showed severe distortion and dilation of the seminiferous tubules (blue thick arrow) with drastic reduction of the spermatozoa, sertoli cells (red circle), interstitial layer housing the leydig cells (IL)

D. Treated with DSE 250 mg.kg⁻¹/d, po. extract showed severe degradation of the tissue, dilated seminiferous tubules (black thick arrow) with loss of spermatozoa cells, distorted interstitial layer housing the leydig cells(IL), reduction of spermatogonia cells (Red thin arrow) and sertoli cells (yellow circle) with loss of function. H/E ×400; DSE: *Datura stramonium* extract.

Table 5
Semen Evaluation of Hydroethanol Leaf Extract of *Datura stramonium* in Male rats.

Treatment	Distilled water	<i>Datura stramonium</i>	<i>Datura stramonium</i>	<i>Datura stramonium</i>
Dose	10 mL.kg ⁻¹	10 mg.kg ⁻¹	50 mg.kg ⁻¹	250 mg.kg ⁻¹
Sperm motility (%)	91.50 ± 2.84	96.40 ± 3.98	95.33 ± 4.64	80.83 ± 3.63
Sperm count (× 10 ⁶)	30.63 ± 1.76	34.50 ± 4.10	27.27 ± 3.31	20.68 ± 1.49
% Abnormality (× 10 ⁶)	93.90 ± 3.35	91.92 ± 0.79	89.97 ± 4.64	86.68 ± 2.41

Analyzed values showcased as mean ± Standard Error Mean (n = 8). *P< 0.05 vs. distilled water (One way ANOVA by Dunnet’s multiple comparison test).

Table 6
Urine Evaluation of Hydroethanol Leaf Extract of *Datura stramonium* in Male and Female rats.

MALE										
Treatment	Dose (mg·kg ⁻¹)	pH	Specific gravity (g·cm ⁻³)	Glucose (mmol·L ⁻¹)	Protein (g·L ⁻¹)	Blood (mL)	Ketone (mmol·L ⁻¹)	Nitrites (mg·L ⁻¹)	Bilirubin (mg·dL ⁻¹)	Urobilinurubin (mg·dL ⁻¹)
Distilled water	10	8.66 ± 0.33	1.02 ± 0.00	4.33 ± 0.66	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>D. stramonium</i>	10	7.66 ± 0.16	1.03 ± 0.01	4.83 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>D. stramonium</i>	50	8.16 ± 0.33	1.01 ± 0.00	5.33 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>D. stramonium</i>	250	8.33 ± 0.16	1.01 ± 0.00	5.50 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

FEMALE										
Treatment	Dose (mg·kg ⁻¹)	pH	Specific gravity (g·cm ⁻³)	Glucose (mmol·L ⁻¹)	Protein (g·L ⁻¹)	Blood (mL)	Ketone (mmol·L ⁻¹)	Nitrites (mg·L ⁻¹)	Bilirubin (mg·dL ⁻¹)	Urobilinurubin (mg·dL ⁻¹)
Distilled water	10	7.83 ± 0.33	1.01 ± 0.01	4.83 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>D. stramonium</i>	10	7.66 ± 0.44	1.00 ± 0.00	5.00 ± 1.32	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>D. stramonium</i>	50	7.33 ± 0.44	1.00 ± 0.00	5.83 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>D. stramonium</i>	250	7.50 ± 0.57	1.01 ± 0.00	5.00 ± 0.28	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Analyzed values showcased as mean ± Standard Error Mean (n = 8). * P < 0.05 vs. distilled water (One way ANOVA by Dunnett's multiple comparison test). **0.00 ± 0.00-No traces.**

that long-term administration of *D. stramonium* may be more toxic to female than male subjects.

Alterations in the internal organs' weight and histopathology are indicators for assessing toxicities and the general health status of animals in toxicological studies (Porwal et al., 2017; Tiwari et al., 2020). Cellular constriction or swelling, which could result in a decrease or increase in the weight of organs respectively, may indicate potentially harmful tendencies of chemicals/drugs (Tabasum and Khare, 2019). (Muia et al., 2020) reported that a decrease in the mean body weight and animal food consumption might stem from drug-induced toxicity, which could negatively impact the weight of the internal organs. In male animals, evidence of any significant alteration in the weight of the internal organs by the extract was not observed, suggesting any toxicity in this regard. This outcome agrees with the observations of (Abdelouahab et al., 2011) and (Gidado et al., 2007), who, in their evaluations, submitted that *D. stramonium* did not elicit any changes in the internal organs of the male rats. However, in female rats, the extract increased the heart, liver and kidney weights, suggesting toxicity; it also decreased the weight of the pancreas and brain, indicating toxicity. The decrease in the brain's weight may be suggestive of the extract's neurotoxic effect, which agrees with the findings of (Ogunmoyole et al., 2019). The histopathological findings supported the internal organ toxicities observed in female rats in our study, which revealed some cytoarchitectural distortions in the liver, kidney and brain. Internal organ toxicity in female animals could be linked to reduced pharmacokinetic activities in female animals (Anderson, 2008). Based on the above outcomes, *D. stramonium* is relatively safe in male rats but toxic in female rats.

Determining hematological parameters is an important index to predict the toxicity of drugs (Christian et al., 2017; Bariweni et al., 2018). Studies have shown that ingesting toxic drugs/plants can alter blood biomarkers (Arome and Chinedu, 2013; Zahmati and Shokooh, 2016; Olayode et al., 2020). In male animals, *D. stramonium* non-significantly increased the WBC and its differentials. Meanwhile, the extract (10–250mg·kg⁻¹/d, po) significantly increased the PCV, RBC and Hb. In female animals, *D. stramonium* (10–250 mg·kg⁻¹/d, po) significantly increased the WBC, its differentials, PCV, RBC and platelets. In the 14–28 days toxicity studies conducted by (Uddin et al., 2017), a reduction in the hematological parameters by *D. stramonium* was reported, which does not agree with our observations in this study. However, an increase in hematological parameters upon administration of *D. stramonium* seed to West African Dwarf bucks for about ten days had been reported (Fatoba et al., 2013). This finding corroborates our observations on the hematological parameters after 90 days of oral ingestion of *D. stramonium* extract. The non-significant increase in WBC and its differentials in male rats suggested that the extract could initiate an inflammatory process. This is evident in the submission of (Olayode et al., 2020), who linked increased leukocytes with C-reactive protein (CRP), an inflammation biomarker, to inflammation. However, the increased WBC and its differentials in female animals suggest inflammatory reactions, indicating a possible toxicity sign. A decrease in PCV, RBC, Hb, MCV, MCH and MCHC suggests anemia (Nigatu et al., 2017; Rehan et al., 2018). Therefore, an increase in PCV, RBC and Hb by the extract in both studies suggests no toxicity but rather the extract's tendency to stimulate erythropoiesis possibly. Stimulation of bone marrow by thrombopoietin, an amino acid glycoprotein, can induce platelet release (Olayode et al., 2020). Increased circulating platelets have been linked to severe haemorrhages and inflammation mediated by toxic compounds (Arika et al., 2016). Therefore, an increase in female animal platelets may suggest an inflammatory response. Based on the above findings, *D. stramonium* can be toxic in female rats but relatively safe in male rats.

Liver and kidney function tests, lipid profile and electrolytes assay, are essential biochemical screenings for organ function (Adedapo et al., 2007; Arika et al., 2016; Nigatu et al., 2017). Regarding the lipid profile assay, the extract (10–250 mg·kg⁻¹/d, po) increased the cholesterol and LDL; and decreased the HDL in male animals. In female animals, *D. stramonium* increased cholesterol, triglycerides and LDL levels.

Table 7
Gonadal Hormone Assay of Hydroethanol Leaf Extract of *Datura stramonium* in Male and Female rats.

MALE					
Treatment	Dose (mg·kg ⁻¹)	FSH (U·mg ⁻¹)	LH (U·mg ⁻¹)	Progesterone (U·mg ⁻¹)	Testosterone (U·mg ⁻¹)
Distilled water	10	0.65 ± 0.27	0.78 ± 0.04	5.50 ± 1.34	0.87 ± 0.01
<i>D. stramonium</i>	10	0.68 ± 0.01	1.30 ± 0.11	6.48 ± 1.50	1.78 ± 0.29
<i>D. stramonium</i>	50	0.87 ± 0.06	1.82 ± 1.06	7.34 ± 1.45	1.74 ± 0.34
<i>D. stramonium</i>	250	1.66 ± 0.69	0.96 ± 0.07	5.40 ± 1.47	1.76 ± 0.32
FEMALE					
Treatment	Dose (mg·kg ⁻¹)	FSH (U·mg ⁻¹)	LH (U·mg ⁻¹)	Progesterone (U·mg ⁻¹)	Testosterone (U·mg ⁻¹)
Distilled water	10	0.70 ± 0.01	0.79 ± 0.01	4.62 ± 1.35	0.79 ± 0.01
<i>D. stramonium</i>	10	0.76 ± 0.07	1.63 ± 0.99	6.29 ± 1.75	0.34 ± 0.15
<i>D. stramonium</i>	50	0.80 ± 0.05	0.76 ± 0.01	5.35 ± 1.83	0.44 ± 0.14
<i>D. stramonium</i>	250	0.77 ± 0.01	0.81 ± 0.02	6.46 ± 1.50	0.37 ± 0.16

Analyzed values showcased as mean ± Standard Error Mean (n = 8). *P < 0.05 vs. distilled water (One way ANOVA by Dunnet's multiple comparison test).

The increase in total cholesterol, triglycerides and low-density lipoproteins (LDL) by the extract in this study indicates distortions in lipid metabolism. The body's inability to effectively regulate membrane lipids could emanate from temporary liver injury, oxidative stress and lipid peroxidation (Aberare et al., 2011). Significant elevation in serum LDL, cholesterol and triglycerides and a decrease in HDL undermine heart function and could lead to cardiovascular degeneration and thickening of the arterial walls (Owoade et al., 2018). Based on these findings, the increase in cholesterol, triglycerides and LDL levels in male and female studies suggests toxicity to the heart. These findings support the reports of (Ogunmoyole et al., 2019) on *D. stramonium* seed extract.

Regarding liver function, *D. stramonium* (10–250 mg·kg⁻¹/d, po) significantly decreased ALP and ALT, while AST, total bilirubin, total protein and conjugated bilirubin were not altered in male animals. In female animals, serum ALT level was significantly decreased by the extract (10–250 mg·kg⁻¹/d, po), while other parameters were unaffected. AST, ALP and ALT are important intrinsic biomarkers for evaluating liver damage, and an increase in the circulating liver enzymes indicates liver toxicity (Salasanti et al., 2014; Prasanth et al., 2015). The significant decrease in ALP and ALT in both male and female animals does not indicate toxicity, which is consistent with the submission of (Aljarba et al., 2021), who linked increased levels of SGOT, SGPT, ALP, and Bilirubin in the carcinogen control group to liver damage. Therefore, it is suggested that the extract can be safe on the liver.

Meanwhile, this finding is at variance with the observation of (Abdelouahab et al., 2011) and (Ogunmoyole et al., 2019), who reported an increased ALP and AST following 14–28 days of *D. stramonium* administration. As observed in our study, the decrease in ALP and ALT levels could be linked to the regenerative ability of the liver and its capacity to handle or break down toxic compounds following long-term exposure. However, evidence of alterations was found in the histopathological evaluation of the liver at the supra-therapeutic dose, suggesting that the extract is unsafe at a high dose. Creatinine (unwanted product of muscle metabolism) and urea (unwanted product of protein metabolism) are biomarkers for evaluating kidney damage; an increase or decrease in serum levels of these parameters suggests the state of the kidney (Akindede et al., 2015; Abbas et al., 2018). In this study, serum urea levels decreased in male and female rats.

Meanwhile, there was no significant change in the creatinine level. This finding suggests that the extract did not cause any significant damage to the kidney. (Ogunmoyole et al., 2019) reported decreased serum urea levels in the aqueous extract of *D. stramonium* and increased serum urea levels in the methanol leaf extract. (Imo et al., 2019) submitted that there was no significant change in serum urea level after ten days of administration of *D. metel* extract. Our findings in this study are consistent with the observations of the above authors, except for the *D. stramonium* methanol extract study, where increased urea level was at-

tributed to an increased level of toxic alkaloids in methanol extraction. Serum electrolytes are bio-indicators for evaluating the viability of the kidneys and heart functions (Burton, 1997; Abbas et al., 2018). Elevation or depletion in the level of any of these ions may indicate kidney or heart toxicity (Imo et al., 2019; Amagon et al., 2020). In this study, *D. stramonium* did not alter the levels of most of the electrolytes in both male and female animals except sodium ion, which showed a significant decrease at 250 mg·kg⁻¹/d, po of the extract in male animals. Based on these findings, the decrease in the serum level of sodium ion in male rats at 250 mg·kg⁻¹/d po of the extract could indicate distortions in the kidney or heart function. The stability in the potassium, chloride, hydrogen and bicarbonate ions indicates the absence of nephrotoxicity and cardiotoxicity.

A semen function test (sperm count, motility and morphology) is a conventional procedure to evaluate testicular/sperm abnormalities or infertility (Muhammad et al., 2018). In this study, there was a non-significant decrease in the percentage of sperm count and motility at supra-therapeutic dose. Based on these findings, *D. stramonium* could be slightly toxic on the testes at high doses. This finding agrees with the histological examination of the testes, which showed distortions of the testicular cells at supra-therapeutic dose. This is consistent with the findings of Alwirfli (Alwirfli, 2021), who reported some abnormal histological alterations in the testes of Swiss albino mice.

Urinalysis and serum electrolytes evaluations could index kidney and liver functions (Tan et al., 2018). No significant changes were observed in the treated animals' urine parameters. Therefore, it could be submitted that the extract did not elicit any toxicity in the urine.

Histopathological examination of rat liver and kidney revealed some cytoarchitectural distortions at 250 mg·kg⁻¹/d, po of the extract in both male and female studies, suggesting that the extract could be toxic at supra-therapeutic dose. The brain of both sexes showed severe neuronal degeneration and necrosis at 250 mg·kg⁻¹/d, po of the extract, suggesting neurotoxicity at the high dose of the extract. The testes at 250 mg·kg⁻¹/d, po the extract showed severe tissue degradation, dilated seminiferous tubules with loss of spermatozoa cells, distorted interstitial layer housing the Leydig cells, reduction of spermatogonia cells and Sertoli cells with loss of function, suggesting testicular toxicity at a supra-therapeutic dose of the extract. Qualitative phytochemical screening of *D. stramonium* revealed the presence of saponins, flavonoids, phenols, reducing sugar, steroids, terpenoids, alkaloids, and tannins (Murtala et al., 2022). Quantitative phytochemical investigation of the extract showed a high level of alkaloids (Alwirfli, 2021; Murtala et al., 2022). Alwirfli (Alwirfli, 2021), (Ogunmoyole et al., 2019) and (Abdelouahab et al., 2011) established the toxic effects of *D. stramonium* on various organs by linking it with a high level of alkaloids in the plant. Therefore, the toxicities produced by *D. stramonium* on various organs in this study can be attributed to the high level of alkaloids.

5. Conclusion

The hydroethanolic leaf extract of *D. stramonium* is nontoxic on acute exposure and relatively safe at lower doses after sub-chronic oral administration. However, at very high doses, the plant may induce lipid metabolism, cardiotoxicity, nephrotoxicity and neurotoxicity distortion. The extract could suppress appetite and cause loss of body weight at a very high dose. In addition, the extract could also cause internal bleeding (haemorrhages) and inflammation at high doses. In male rats, the extract can attack testicular cells, which could affect the quality and quantity of sperm at high doses. Therefore, the plant should be used in moderate doses to avoid loss of appetite, loss of body weight, and brain, heart, testes and kidney toxicities. However, a further investigation that may involve 180 days of administration is recommended to understand its toxicity pattern following prolonged use fully.

Ethical Approval

Ethical approval was procured from the Health Research Ethics Committee (HREC) of CMUL, and the handling of animals was by the provisions of the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

Data Availability

Nil

Funding

Nil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Abdullahi A. Murtala: Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition. **Oyinloye E. Oladapo:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition. **Aderonke A. Aderionla:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition. **Wasiu E. Olooto:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition. **Oluwatosin O. Soyinka:** Methodology, Writing – review & editing, Project administration. **Royhan O. Folarin:** Methodology, Writing – review & editing, Project administration. **Farouk A. Oladoja:** Methodology, Writing – review & editing, Project administration. **Oluwatoyin O. Shonde:** Methodology, Writing – review & editing, Project administration. **Luqmon E. Osipitan:** Formal analysis, Data curation. **Emmanuel B. Adegbe:** Formal analysis, Data curation. **Julius A. Abolarinwa:** Formal analysis, Data curation.

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Supplementary Materials

Nil.

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