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Original Research Article

Behavioural, Biochemical, and Cerebral Histomorphological Effects of Aqueous Extract of *Datura stramonium L.* Leaves in Rats

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ABSTRACT

Datura stramonium L, commonly called devil's trumpet is a popular medicinal plant known for its psychoactive effect. This study aimed to investigate the behavioural, biochemical, and histomorphological changes in rats administered aqueous extract of *Datura stramonium* leaves. Eighty Wistar rats were divided into four groups of 20 rats per group. Group I was fed with standard rodent chow, while Groups II – IV were administered aqueous extract of *Datura stramonium* leaves at 50, 100, and 200 mg/kg, respectively once daily for 28 days orally. After the treatment period, the rats were subjected to behavioural tests, including conditioned place preference, open field, Y-Maze, elevated plus maze, tail suspension, and forced swim tests. After the tests, blood samples were collected for biochemical analysis, including evaluation for malondialdehyde and antioxidant enzymes (catalase and glutathione peroxidase) levels. The hippocampus and cerebral cortex were processed for histology, and brain dopamine and acetylcholine levels were determined. Results from the study showed that the sub-acute administration of the aqueous extract of *Datura stramonium* leaves significantly decreased feed consumption, and caused body weight loss in rats. Behavioural tests revealed that the administration of *Datura stramonium* leaf extract resulted in neurobehavioural symptoms like addictive tendencies, anxiety, depression, and memory impairment. In addition, the extract significantly increased brain dopamine and decreased brain acetylcholine levels. Histological examination of the brain showed degenerating pyramidal neurons in the cerebral cortex and hippocampus of rats administered *Datura stramonium* leaf extract. These findings suggest potential neurotoxic effect of the prolonged use of *Datura stramonium* leaf extract.

Keywords: Datura stramonium, Addictive tendency, Oxidative stress, Depressive-like behaviour, Anxiety-like behaviour, Neurotransmitters.

Introduction

Datura stramonium L, also known as Jimson weed, thorn apple, devil's trumpet, and various other names, is an annual poisonous plant belonging to the Solanaceae family.[1] The plant is commonly cultivated in temperate, tropical, and subtropical regions of the world, and is a widely recognized medicinal herb.[2] The seeds of *Datura stramonium* L. are commonly smoked for their hallucinogenic properties, but excessive consumption leads to toxic effect.[3] There has been a multidisciplinary endeavour cutting across diverse fields, including drug discovery and pharmacology, plant breeding and bioengineering, evolutionary biology, analytical chemistry, and ethnobotany.[1]

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The increasing prevalence of new psychoactive substances is a growing global public health concern. Over the last decade, there has been a significant surge in the quantity of newly developed psychoactive substances. Africa is only beginning to acknowledge the growing public health threat posed by these substances to its large population of young adults and adolescents.[4] The search for new psychoactive substances is driven by the desire to develop drugs that can circumvent chemical detection processes and legal restrictions on the use and possession of traditional drugs of abuse.[5] Healthcare providers must interpret and handle complex symptoms and indications of psychoactive substances, requiring continuous surveillance to detect potential threats to communities. This study aimed to address the dearth of research on the effects *of Datura stramonium* leaf on feed consumption and body weight, anxiety and depressive-like symptoms, memory impairment, addictive tendencies, lipid peroxidation, antioxidant status, brain neurotransmitters (dopamine and acetylcholine), and cerebral cortex histomorphology changes in rats.

Materials and Methods

Plant collection and identification

Datura stramonium leaves were procured on 5th August, 2021 at Stadium Area, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The plant was identified by a plant taxonomist, Dr. J. Ogunkunle, of the Department of Biology, LAUTECH Ogbomoso, Oyo State, Nigeria. Herbarium specimen with voucher number

LHO588 was prepared and deposited in the herbarium unit of the department.

Preparation and extraction of Datura stramonium leaves
Datura stramonium leaves were washed, and air-dried for thirty days at room temperature.[6] The dried leaves were ground into powder, and the powdered sample (70 g) was macerated in 700 mL of distilled water at room temperature for 24 hours. The aqueous extract was filtered, and the filtrate was evaporated in a rotary evaporator at 40°C.[7] The concentrated extract was subsequently kept in the desiccator to ensure complete dryness. The desiccated extract was then reconstituted in distilled water to achieve the desired concentration needed for the experiment.

Phytochemical screening of Datura stramonium leaf extract
The extract was subjected to basic qualitative and quantitative phytochemical screening, including gas chromatography-mass spectrometric (GC-MS) analysis for the presence or absence of phytochemical constituents.

GC-MS analysis
GC-MS analysis was performed according to the protocol previously described by Bouhaddouda *et al*. (2025).[8] The analysis was performed on a gas chromatograph coupled with a mass spectrometer (PerkinElmer Clarus 600 D) provided with an autosampler. A RESTEK Rtx®-5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) composed of 5% diphenyl and 95% dimethyl polysiloxane was employed, and sample injections were performed in split mode. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The temperatures of the injector and transfer line were both maintained at 250°C. The initial temperature was maintained at 60°C for 1 minute, followed by a ramp of 3°C per minute until reaching 200°C, which was then held isothermally for 13 minutes. An injection volume of 1 µL was used. Both the standard solutions and *Datura stramonium* leaf extract samples were diluted in absolute ethanol at a concentration of 1 g/L. The MS analysis was conducted using electron impact ionization (EI) mode with an ionization energy of 70 eV, employing scan mode acquisition over an m/z range of 40 to 600 atomic mass units (amu). Compound identification was achieved by matching the retention times of the samples with those of standard solutions, followed by comparison of the mass spectra with the NIST® (National Institute of Standards and Technology) commercial database. The relative percentage of each compound was determined using the internal standardization techniques.

Quantitative phytochemical analysis
A complementary quantitative method was used to quantify the phytoconstituents of *Datura stramonium* leaf extracts. The phytoconstituents were determined spectrophotometrically using UV-Visible spectrophotometer (Thermo Fisher Scientific, China). Using the calibration curve, the concentration of each phytoconstituent in the extract was calculated and expressed as mg of standard equivalent per 100 g of *Datura stramonium* leaf extract.[9]

Experimental animals
Eighty (80) male adult Wistar rats weighing between 150 and 170 grams were sourced from the Empire animal farms, Ara, Osun State, Nigeria. The animals were kept in well-ventilated cages, and acclimatized to the laboratory conditions for one week. The rats were fed with standard rodent pellets (Top Feeds, Premier Feed Mills Limited) and had access to drinking water *ad libitum*.

Ethical consideration
All procedures were conducted in compliance with approved institutional protocols, and international guidelines for animal care and use (European Council Directive EU2010/63 and NIH Publication No. 85-23). The study was approved by the Ladoke Akintola University of Technology Ethical Research Committee, Ogbomoso, Oyo State, Nigeria, with the ethical approval reference number NHREC/TR/UNIMED-HREC-Ondo St/22/06/21.

Acute toxicity test
The acute oral toxicity was conducted in rats according to the guidelines of the Organization for Economic Cooperation and Development (OECD).[10] The weight of each animal was measured every week.

Experimental design
A total of eighty (80) male adult Wistar rats (150 - 170 g) were randomly divided into four groups (I -IV) consisting of twenty animals per group. Ten animals in each group were used for the Conditioned Place Preference Test (CPP) while the other ten were used for the other behavioural tests.[7] The animals were treated as follows;
Group I (control group) was fed the standard rodent feed only for an additional 28 days after the initial 7 days of acclimatization while Groups II, III, and IV were fed the standard rodent feed alongside *Datura stramonium* aqueous leaf extract at doses of 50, 100, and 200 mg/kg, respectively.[11] Details of the groupings and extract administration are presented in Table 1.

Table 1: Animal grouping and extract administration

Group	Treatment
Group I (Control)	Normal rodent chow with 10 mL/kg bw of distilled water orally for 28 days.
Group II (Low dose *D. stramonium* leaf extract)	Normal rodent chow with 50 mg/kg bw of aqueous extract of *Datura stramonium* leaf extract and 10 mL/kg bw distilled water orally for 28 days.
Group III (Moderate dose *D. stramonium* leaf extract)	Normal rodent chow with 100 mg/kg bw of aqueous extract of *Datura stramonium* leaf extract and 10 mL/kg bw distilled water orally for 28 days.
Group IV (High dose *D. stramonium* leaf extract)	Normal rodent chow with 200 mg/kg bw of aqueous extract of *Datura stramonium* leaf extract and 10 mL/kg bw distilled water orally for 28 days.

The body weights of the rats were measured weekly for four weeks. After the treatment period, the animals were subjected to behavioural tests, including Open Field, Conditioned Place Preference, Elevated Plus Maze, Y-Maze, Tail Suspension, and Force Swim tests.
Animals were sacrificed by cervical dislocation 24 hours after the last behavioural test, and blood samples were collected by cardiac puncture for use in the evaluation of antioxidant enzymes [catalase activity, glutathione peroxidase (GPX), and reduced glutathione (GSH)] and malondialdehyde (MDA). The hippocampus and cerebral cortex were immediately dissected and weighed.

Five brain homogenates in each group were made in ice-cold phosphate-buffered saline with the aid of a Teflon-glass homogenizer. The homogenate was centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant obtained was used for the estimation of dopamine and acetylcholine levels.

Behavioural tests
Conditioned place preference test: The conditioned place preference (CPP) test assessed the rewarding and aversive effects the extract, with rats exposed to increasing oral doses of aqueous extract of *Datura*

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stramonium leaves and paired with their preferred or non-preferred chamber.[12]

Open field test
The open field test assessed the emotional behaviour of the rats by measuring horizontal locomotion, rearing, and grooming after exposure to increasing oral doses of aqueous extract of *Datura stramonium* leaves.[13]
Forced swim test: The forced swim test measured behavioural despair in rats, measuring three distinct behaviours: floating, head shaking, and body bobbing.[14,15] The test is beneficial in identifying active behaviours in rats.

Tail suspension test
The Tail Suspension Test (TST) was used to assess the antidepressant effect of the extract in mice by measuring the ability of the rat to maintain a suspended position for six minutes.[16,17]
Y-maze test: The Y-maze test was used to assess spatial working memory capacity.[18] The rats were tested on their spontaneous alternation behaviour (SAB) by placing them in a whitewashed Y-maze with three separate forks. The percentage of arm changes in a given period was calculated by subtracting the actual arm changes from the total arm entries. The number of successive arm entries in each arm was then counted.

Elevated plus-maze test
The elevated plus-maze was designed to aid in guiding and directing the rats. The percentage of open arm entries was calculated by dividing the number of open arm entries by the total number of arm entries and multiplying by 100.[19]

Biochemical analysis
The plasma concentration of marker of lipid peroxidation (malondialdehyde) and antioxidant enzymes were determined as follows;
Malondialdehyde (MDA) concentration was determined by reacting the blood sample with 2-thiobarbituric acid, heated at 98^{o}C for 10 minutes, cooled to room temperature, then centrifuged at 10,000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 532 nm.[20]
Catalase activity was determined by reacting the plasma sample with saturated solution of ammonium sulfate and hydrogen peroxide. Catalase activity was estimated by measuring the hydrogen peroxide substrate remaining after catalase action. The amount of hydrogen peroxide remaining was measured spectrophotometrically by determining the absorbance of the reaction mixture at 540 nm.[20,21]
Glutathione peroxidase activity was measured using the Fortress Glutathione Peroxidase kit, which quantitatively determine total glutathione peroxidase (GPx) activity. The kit specifically measures the ability of GPx to catalyze the oxidation of reduced glutathione (GSH) by cumene hydroperoxide.[22] Glutathione activity was determined using spectrophotometric method based on Ellman's reagent.[23]
The level of acetylcholine in the brain was assessed using acetylcholine assay kit. The enzyme-driven reaction detects acetylcholine via acetylcholinesterase enzyme and choline oxidase. Samples were compared to a known concentration of acetylcholine standard in a 96-well microtiter plate.[24] The concentration of dopamine in the brain was determined using dopamine assay kit, which is based on the ELISA principle. Absorbance was measured using a micro-plate reader set at 450 nm.[25]

Statistical analysis
Statistical analysis was performed using Chris Rorden ezANOVA statistical software (version 0.98). Data were presented as Mean ± Standard Error of Mean (S.E.M.), n = 10. Data were analysed by one-way analysis of variance (ANOVA) followed by Turkey's post-hoc multiple comparison test. Statistical significance difference between means was established at $p < 0.05$.

Results and Discussion

Acute toxicity of aqueous extract of Datura l. stramonium leaves
The acute toxicity test was used to determine the median lethal dose (LD_{50}) of *Datura stramonium* aqueous leaf extract. LD_{50} represents the dose that causes death in 50% of the test population during short-term exposure. Acute oral administration of the aqueous extract of *Datura stramonium* leaves resulted in no sign of toxicity, and no death was recorded even up to the maximum dose of 2000 mg/kg. In addition, there was no significant weight change in all treated groups. Therefore, the LD_{50} of the extract was estimated at >2000 mg/kg bw.

Phytochemical constituents of Datura stramonium aqueous leaf extract
Phytochemical analysis of the aqueous extract of *Datura stramonium* leaves revealed the presence of tannins, flavonoids, alkaloids, saponins, steroids, cardiac glycosides, reducing sugars, and phenolic compounds at varying concentrations (Figure 1). Alkaloids found in *Datura stramonium* leaves can cause damage to neurons through changes in calcium homeostasis, free radical generation, mitochondria malfunction, protease activation, gene expression changes, and inflammation.[26] In addition, reducing sugar found in *Datura stramonium* leaves, has been hypothesized to contribute to oxidative stress, leading to alterations in mitochondrial dynamics and neuronal death.[27]

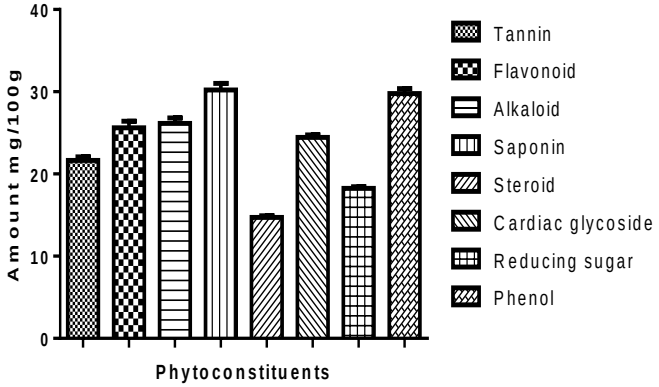

Figure 1: Quantitative phytochemical constituents of the aqueous extract of *Datura stramonium* leaves

Furthermore, the GC-MS analysis of the extract identified 3Thirty-two (32) secondary metabolites (Table 2). Among these metabolites were tropane alkaloids such as 2,5-Methano-2*H*-furo[3,2b] pyrrole, hexahydro-4-methyl; 6*β*,7*β*-epoxy-1α,5α-tropan-3α-ol; and 1-benzene acetic acid, alpha-(hydroxymethyl-8-methylazabicyclo oct-3-yl ester. These tropane alkaloids are commonly known as 3,6-epoxytropane, scopolamine, and atropine, respectively. These alkaloids are known for their anticholinergic properties, a predictor of addiction-like behaviour, memory impairment, anxiety, and dementia. The corresponding retention times and peak area percentages for these compounds were as follows: 7.064 and 6.78 for 3,6-Epoxytropane, 13.478 and 6.03 for Scopolamine, and 14.606 and 7.21 for Atropine, as outlined in Table 2.

Effect of aqueous extract of Datura stramonium leaf on feed consumption and body weight of rats
There was a significant ($p < 0.05$) reduction in feed consumption of the rat treated with aqueous extract of *datura stramonium* compared to the control at the end of the first week. A similar observation was made in the second, third, and fourth weeks (Figure 2). As a result, a significant weight loss was observed in the rats at the end of the fourth week following the administration of the extract compared to the control group (Figure 3). These observations were contrary to the findings from a previous study which showed that the ethanol extract of *Datura stramonium* leaves had no significant effect on body weight or feed

consumption.[28] The reduced feed intake and weight loss observed in this study may be attributed to the presence of atropine in the leaves of *Datura stramonium*. Atropine inhibits muscarinic receptors, impacting the central and peripheral nervous systems which results in decreased antral contractility and delayed stomach emptying.[29] On the other hand, acetylcholine plays a crucial role in encouraging food consumption, and a reduction in acetylcholine at post-synaptic muscarinic receptors may disrupt regular food intake patterns.[30]

In addition, the presence of tannins in *Datura stramonium* leaves may also have contributed to the reduced feed consumption and weight loss in rats administered *Datura stramonium* leaf extract. Tannins have antinutrient properties, hence, it alters the digestibility of protein and dry matter in some animals.[31]

Table 2: Phytochemical composition of aqueous extract of *Datura stramonium leaves*

No.	Retention Time (min)	Compound Name	Synonym	Molecular Formula	Area%
1	3.167	Tetraethyl silicate	Chloro(trimethyl)silane	$C_{11}H_{29}ClO_4Si_2$	6.85
2	3.310	1-(2 Adamantylidene) semicarbazide	2-Adamantanone semicarbazone	$C_{11}H_{17}N_3O$	0.68
3	3.402	Triethoxysilanol	SCHEMBL57766	$C_6H_{16}O_4Si$	1.10
4	5.668	1-Dodecene	Adacene 12	$C_{12}H_{24}$	5.42
5	6.131	7-Azabicyclo[4.1.0]heptane		$C_6H_{11}N$	0.80
6	7.064	2,5-Methano-2H-furo[3,2-b] pyrrole, hexahydro-4-methyl	Deoxyoscine; Deoxyscopoline; 3,6-Epoxytropane	$C_8H_{13}NO$	6.78
7	7.241	Hexanoic acid, 1-methylethyl ester	Isopropyl heptanoate	$C_{10}H_{20}O_2$	0.55
8	8.008	9-Octadecene, (E)-	(E)-octadec-9-ene	$C_{18}H_{36}$	10.20
9	9.954	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)	(2E)-3-Ethyl-2,4-pentadien-1-ol	$C_7H_{12}O$	1.51
10	10.097	9-Eicosene, (E)-	Icos-9-ene; 9-Icosene	$C_{20}H_{40}$	9.24
11	10.286	6-Amino-1-methylpurine	1H-Purin-6-amine	$C_6H_7N_5$	0.70
12	10.297	Phenol, 2-amino-4-(1H-1,2,3,4-tetrazol-1-yl)-	-	$C_7H_7N_{5O}$	0.72
13	11.962	Acetic acid, trifluoro-, dodecyl ester	Trifluoroacetic acid	$C_{14}H_{25}F_3O_2$	5.94
14	12.374	7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	-	$C_9H_{16}O_2$	0.63
15	12.443	Cyclooctene, 3-ethenyl-	3-Ethenylcyclooctene	$C_{10}H_{16}$	0.61
16	12.695	cis,cis,cis-7,10,13-Hexadecatrienal	USMAGXMGZXKLHT-PDBXOOCHSA-N	$C_{16}H_{26}O$	0.95
17	13.032	7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	-	$C_9H_{16}O_2$	1.34
18	13.112	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	-	$C_{16}H_{28}O_2$	1.15
19	13.478	6beta,7beta-epoxy-1alphaH,5alphaH tropan-3alpha-ol	Scopolamine; (-)-Hyoscine	$C_{17}H_{21}NO_4$	6.03
20	13.656	Octanoic acid, ethyl ester	Ethyl caprylate	$C_{10}H_{20}O_2$	5.53
21	14.423	9,12-Octadecadienoic acid (Z,Z)-	Methyl octadec-9,12-dienoate	$C_{19}H_{34}O_2$	3.32
22	14.463	Ethyl 6,9,12-hexadecatrienoate	MZCUMMBTDLDIGZ-QNEBEIHSSA-N	$C_{18}H_{30}O_2$	2.18
23	14.508	9,17-Octadecadienal, (Z)-	SCHEMBL3966133	$C_{18}H_{32}O$	3.30
24	14.606	1 benzene acetic acid, alpha-(hydroxymethyl-8-methylAzabicyclooct-3-yl Ester endo-(±)-	Atropine; Hyoscyamine; Tropyl tropate	$C_{17}H_{23}NO_3$	7.21
25	14.755	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid; Industrene 120	$C_{18}H_{30}O_2$	1.70
26	14.846	1,5-Cyclodecadiene, (E,Z)-	Cyclodeca-1,5-diene	$C_{10}H_{16}$	1.31
27	15.006	Oleic Acid	9-octadecenoic acid; Elaidoic acid	$C_{18}H_{34}O_2$	5.70
28	15.184	Cyclododecyne	DTXSID30150209	$C_{12}H_{20}$	2.95

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29	15.395	10-Undecyn-1-ol	ω-Undecenyl alcohol; 11-Hydroxy-1-undecene	$C_{11}H_{22}O$	0.51
30	17.301	4-Nitrophenyl laurate	p-nitrophenyl dodecanoate SCHEMBL1916980	$C_{18}H_{27}NO_4$	0.61
31	17.684	Dodecanoic acid, 1,2,3-propanetriyl ester	Laurin, Glycerin trilaurate	$C_{39}H_{74}O_6$	0.60
32	18.846	Mono(2-ethylhexyl) phthalate	1,2-Benzenedicarboxylic acid;	$C_{16}H_{22}O_4$	2.82

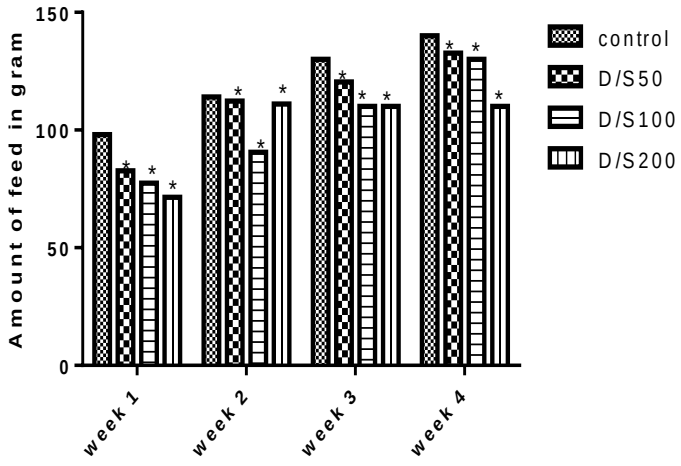

Figure 2: Effect of aqueous extract of *Datura stramonium* leaves on feed consumption in rats. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to Control

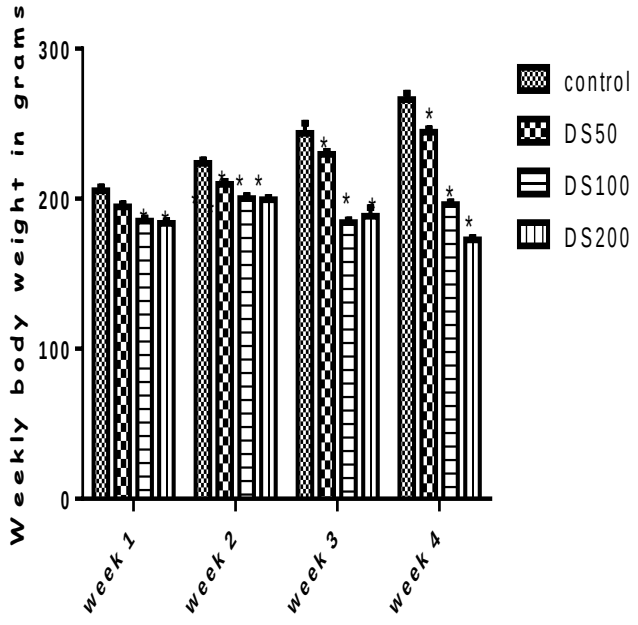

Figure 3: Effect of aqueous extract of *Datura stramonium* leaves on body weight of rats. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

Behavioural effect of aqueous extract of Datura stramonium leaves in rats

In the open field test, there was a significant ($p < 0.05$) increase in line-crossing, rearing, and self-grooming behaviours of the rats administered aqueous extract of *D. stramonium* at all the doses tested except for the 50 mg/kg dose where no significant effect was observed in the self-grooming behaviour compared to that of the control group (Figures 4 - 6). The increase in locomotor activity in the animals administered *Datura stramonium* aqueous leaf extract especially at 100 and 200 mg/kg may be attributed to increase in dopamine concentration in the brain. Dopamine is a neurotransmitter that promotes movement and locomotor activity, with the nucleus accumbens playing the most crucial role in the dopaminergic pathway, it receives stimulating input from the prefrontal cortex, hippocampal formation, and amygdala, which are crucial for its function.[32] Recent scientific evidence supports the hypothesis that an increase in the dopaminergic activity in the nigrostriatal system is responsible for the observed increase in locomotor activity in rats.[33] Dopamine is crucial to the effects of psychostimulants, such as cocaine, amphetamine, and drugs prescribed for attention deficit hyperactivity disorder.[34] Dopamine agonists, such as apomorphine and amphetamine, have been studied for their potential to stimulate locomotor activity.[35] However, systemic treatment with antidopaminergic drugs has been demonstrated to reduce locomotor activity.[36]

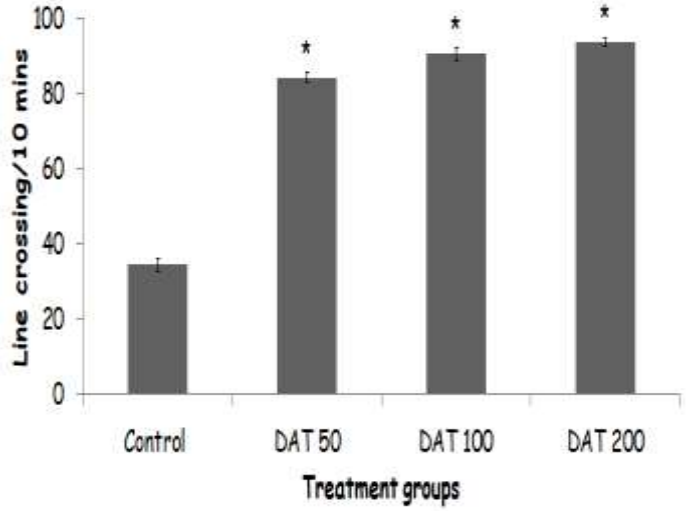

Figure 4: Effect of aqueous extract of *Datura stramonium* leaves on line crossing behaviour of rats in the Open Field Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

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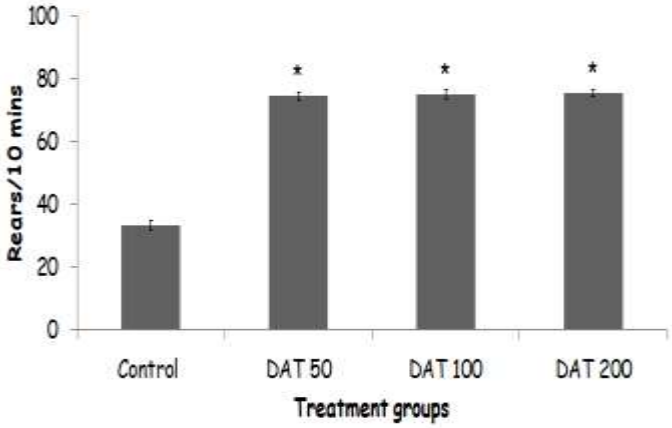

Figure 5: Effect of aqueous extract of *Datura stramonium* leaves on rearing behaviour of rats in the Open Field Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

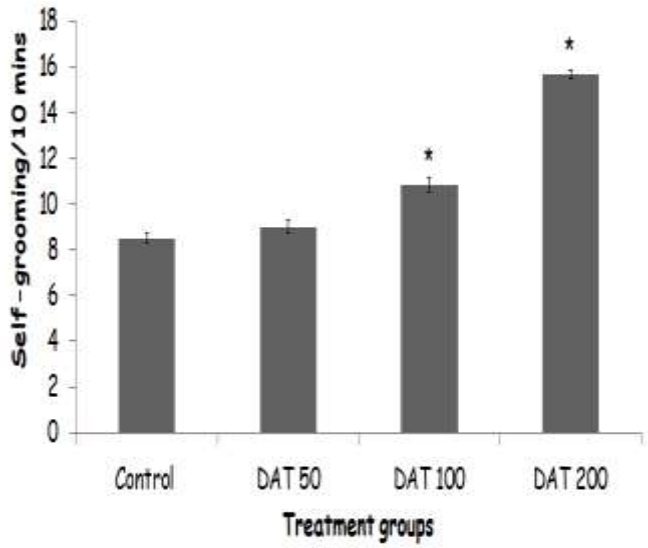

Figure 6: Effect of aqueous extract of *Datura stramonium* leaves on self-grooming behaviour of rats in the Open Field Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

In the force swim and tail suspension tests, administration of aqueous leaf extract of *Datura stramonium* resulted in a significant ($p < 0.05$) increase in the immobility time compared to the control (Figures 7 and 8). This suggests that the organism's ability to cope with stress may be diminished.[37] The reduction in the brain acetylcholine level coupled with the induced oxidative stress by the aqueous extract of *Datura stramonium* leaves may be responsible for this observation.[38]

A significant ($p < 0.05$) decrease in the percentage alternation in the Y-maze, as well as a decrease in the time spent in the open arm of the elevated plus maze were observed in rats treated with aqueous extract of *Datura stramonium* leaves at 50, 100, and 200 mg/kg BW compared to the control group (Figures 9 and 10), whereas, the time spent in the close arm of the elevated plus maze significantly increased in rats treated with the extract compared to the control (Figure 11).

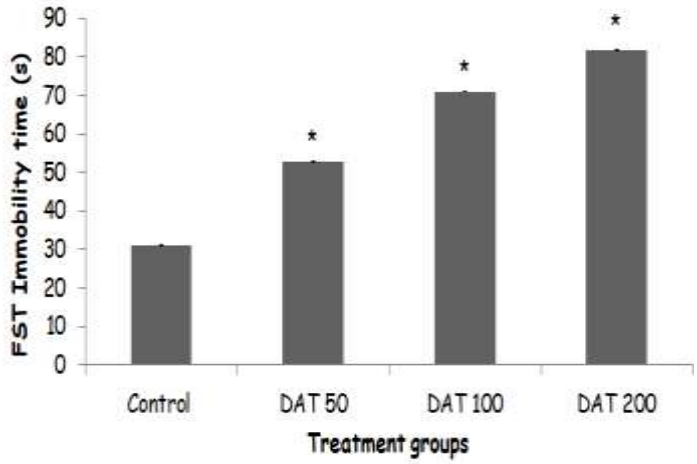

Figure 7: Effect of aqueous extract of *Datura stramonium* leaves on immobility time of rats in the Force Swim Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

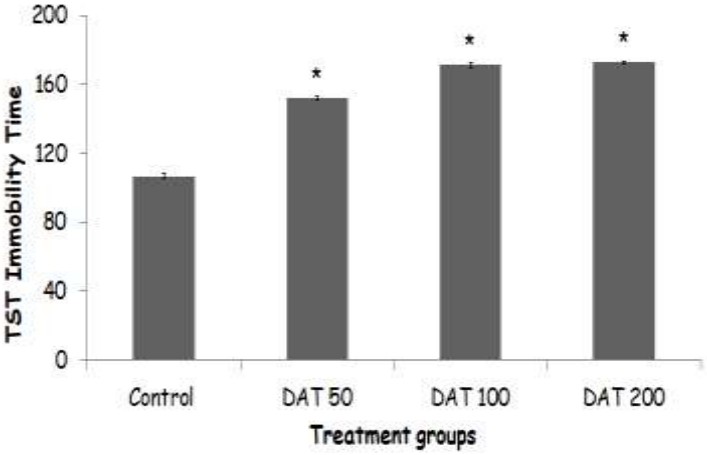

Figure 8: Effect of aqueous extract of *Datura stramonium* leaves on the immobility time of rats in the Tail Suspension Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

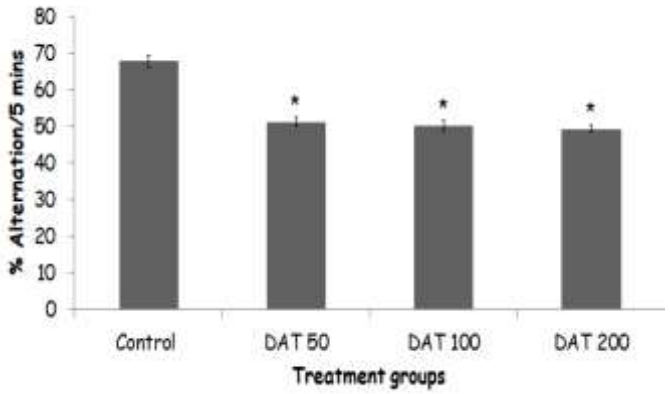

Figure 9: Effect of aqueous extract of *Datura stramonium* leaves on Percentage Alternation in the Y-Maze Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

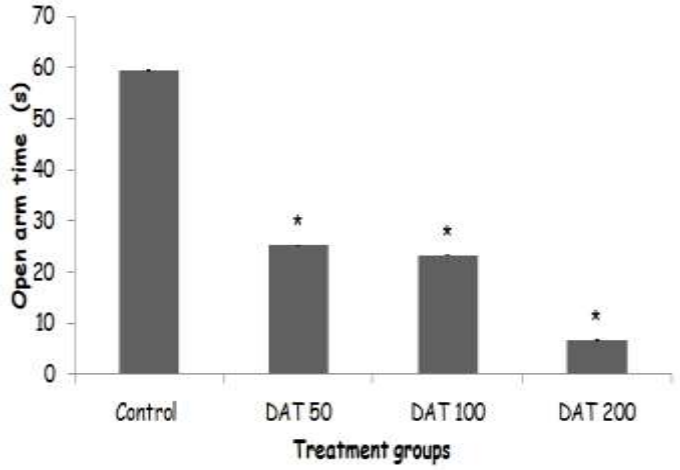

Figure 10: Effect of aqueous extract of *Datura stramonium* leaves on the time spent on the open arm of the Elevated Plus Maze. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

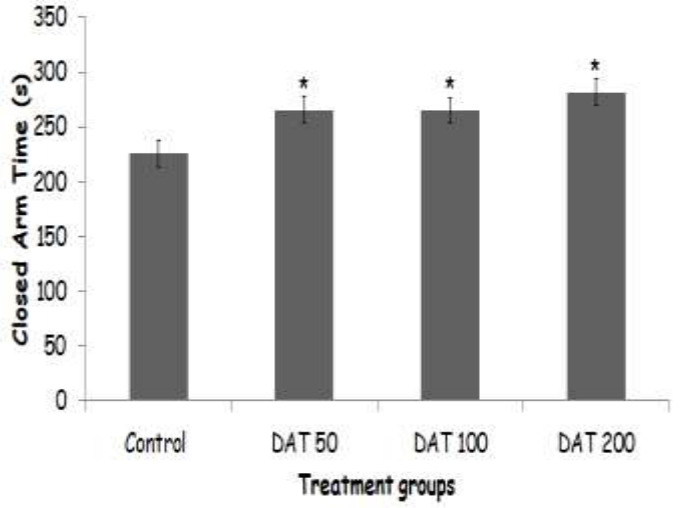

Figure 11: Effect of aqueous extract of *Datura stramonium* leaves on the time spent on the close arm of the Elevated Plus Maze. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

The decrease in the percentage alternation in the Y-maze test indicates impaired spatial working memory in the rats administered aqueous extract of *Datura stramonium* leaves. Memory formation is a complex process that involves several brain regions, including the hippocampus and prefrontal cortex.[39] *Datura stramonium* extract has been linked to short-term memory impairment in rats, as it can lower acetylcholine levels in the brain and increase oxidative stress.[40] Acetylcholine plays a crucial role in the function of the central cholinergic system, facilitating the consolidation and creation of memories. However, reduced acetylcholine levels may aid in the consolidation of memories retrieved from long-term storage. The muscarinic acetylcholine receptor (mAChR) is recognized for its pivotal role in cognitive processes related to learning and memory. It has been suggested as a therapeutic target for improving cognitive abilities in people with Alzheimer's disease (AD).[41] Acetylcholine may gradually modify hippocampal function in ways that facilitate either the encoding of new information or the retrieval of previously stored information. In addition to the effect of reduced acetylcholine level on memory, oxidative stress induced by the plant may be implicated in the genesis of memory impairment. Insufficient antioxidant level may hasten the interaction between free radicals and crucial molecules, including those necessary for learning and memory.[42]

Anxiety-related behaviours in rats were found to increase after being fed *Datura stramonium* as evidenced by increased rearing activity, a shorter exploration time, and increased time spent on the closed arm of the Elevated Plus Maze in contrast to a study where a dosage of 200 mg/kg bw of ethanol extract from *Datura stramonium* leaf had an anxiolytic effect.[43] Recent studies suggest that an increase in dopamine levels may be associated with anxiety-like behaviour, as dopamine plays a crucial role in managing anxiety in various parts of the brain.[44] Dopamine release via Ventral Tegmental Area-Interpeduncular Nucleus (VTA-IPN) pathways has been extensively studied using *in vivo* optogenetics to stimulate and inhibit anxiety-related behaviour.[45] Dopamine and noradrenaline have significant overlap in various domains, with co-release from noradrenergic terminals, shared innervation, non-specific receptor and transporter affinity, and common intracellular signaling pathways.[46] In addition, the neurotoxic effect of the extract on the frontal lobe and hippocampus is a likely risk factor for anxiety-like behaviour in rats, and the amygdala plays a role in this process, combined with a network that includes the prefrontal cortex and hippocampus.[47] Furthermore, the induced oxidative stress may be linked to the development of anxiety-like symptoms in the brain.[48] Studies have shown that male Balb/cJ mice exhibited more anxious behaviour after being separated from their mothers, possibly due to long-term increases in oxidative stress.[49] Oxidative stress can affect neurotransmission, neuronal function, and overall brain activity, potentially leading to anxious-like behaviour.[50]

For the conditioned place preference test, the extract exhibited a noteworthy reduction in the duration of time spent on the light side (drug-paired side) when compared to the control group during the pre-conditioning phase, whereas, during the post-conditioning phase, a significant ($p < 0.05$) increase in the duration of time spent on the light side (drug-paired side) was observed in the extract-treated group compared to the control group (Figure 12). This indicate an increase in conditioned place preference in animals fed with aqueous extract of *Datura stramonium* leaves, which may be related to its addictive tendency. This observation may be a result of the increased level of brain dopamine. This neurotransmitter displays unconditioned reactions to intense, novel, rewarding, or punishing stimuli, known as phasic activation or burst firing.[51]

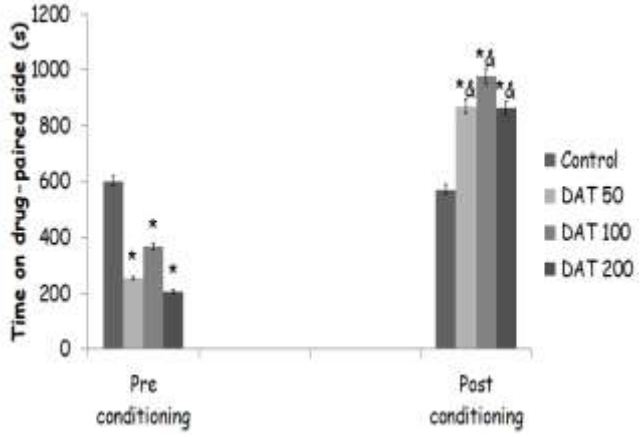

Figure 12: Effect of aqueous extract of *Datura stramonium* leaves on the preconditioning and Postconditioning time in the Conditioned Place Preference Test. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

Addictive drugs like amphetamine, cocaine, morphine, and heroin can induce conditioned place preference, and Dopamine D2 receptor antagonists like haloperidol and metoclopramide may help prevent or minimize these effects.[52] A study reported that dopamine levels in the nucleus accumbens of conditioned place preference (CPP) rats for cocaine were significantly higher after a vehicle injection compared to the vehicle-paired compartment.[53] Another study revealed that the

amphetamine-paired compartment has been demonstrated to increase dopamine levels in the prefrontal brain. Similarly, reducing norepinephrine levels in the prefrontal cortex can block the release of dopamine in the nucleus accumbens in response to amphetamine and morphine, preventing the development of CPP.[54] The nucleus accumbens is a key player in the pathophysiology of addiction, and drugs with addictive potential can increase its extracellular levels, potentially affecting the order of pleasure derived from eating.[55]

Effect of aqueous extract of Datura stramonium leaves on Lipid peroxidation and antioxidant status of rats

Administration of aqueous extract of *Datura stramonium* leaves resulted in an increased serum level of malondialdehyde, which was found to be significant ($p < 0.05$) at 100 mg/kg bw dose of the extract compared to the control group. On the other hand, the serum levels of catalase, glutathione peroxidase, and reduced glutathione activity were found to be significantly ($p < 0.05$) reduced in the extract-treated groups compared to the control (Table 3). These observations suggest increased oxidative stress in the rats administered *Datura stramonium* leaf extract, potentially causing neurotoxicity. Oxidative stress induced by the aqueous extract of *Datura stramonium* leaf may contribute to the development and progression of neuronal damage, as there is often an unfavorable ratio between the body's natural antioxidant levels and the production of free radicals.[56] This imbalance may lead to a chain reaction, including mitochondrial dysfunction and lipid peroxidation.[57]

Table 3: Effect of aqueous extract of *Datura stramonium* leaves on lipid peroxidation and anti-oxidant status in rats

Groups	MDA (µM)	Catalase (IU/L)	GPX (IU/L)	GSH (mM)
Control	1.76 ± 0.06	580.39 ± 0.46	46.95 ± 0.83	1.74 ± 0.26
DAT 50	1.95 ± 0.20	460.33 ± 0.44*	41.25 ± 0.40*	1.23 ± 0.09
DAT 100	3.22 ± 0.31*	385.70 ± 0.16*	26.13 ± 0.76*	1.72 ± 0.20
DAT 200	3.06 ± 0.48	475.04 ± 0.21	17.77 ± 0.28*	1.48 ± 0.04

Data are Mean ± SEM (n = 10). * P < 0.05 compared to control. MDA = Malondialdehyde. GSH = Reduced Glutathione, GPX = Glutathione peroxidase.

Effect of aqueous extract of Datura stramonium leaves on brain acetylcholine and dopamine levels

Administration of the aqueous extract of *Datura stramonium* leaves resulted in a significant decrease in the level of acetylcholine in the brain of Wistar rats at all tested doses (50, 100, and 200 mg.kg) (Figure 13), while dopamine level was found to increase significantly in the groups treated with 100 and 200 mg/kg of the extract compared to the control group (Figure 14). Cholinergic systems, including acetylcholine (ACh), play a crucial role in addiction development. The regulation of the nucleus accumbens circuit is mediated by ACh and dopamine, which work together to maintain appropriate ratios. Removal of cholinergic cells has been reported to accelerate addiction-related behaviours in mice.[58]

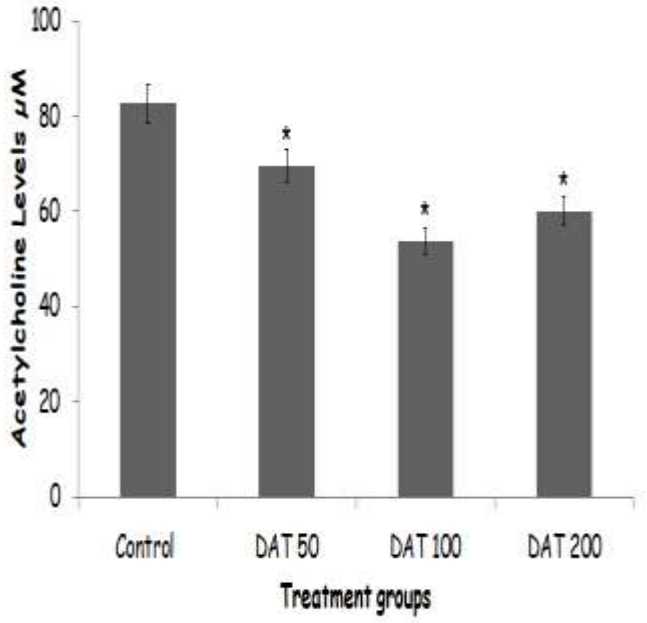

Figure 13: Effect of aqueous extract of *Datura stramonium* leaves on the brain level of acetylcholine in rats. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

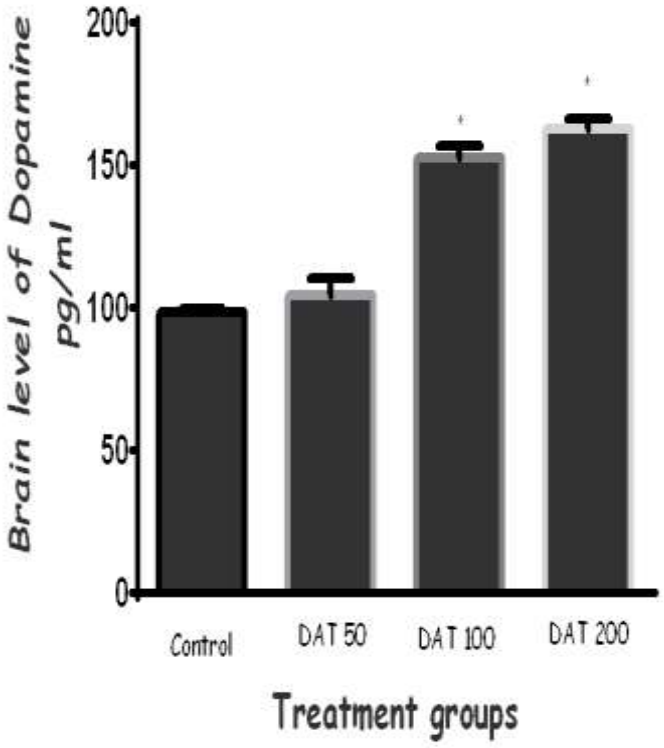

Figure 14: Effect of aqueous extract of *Datura stramonium* leaves on the brain level of dopamine in rats. Data represent the Mean ± Standard Error of mean (SEM), (n = 10). *P < 0.05 compared to control

The neurotoxic effect of aqueous extract of *Datura stramonium* on the cerebral cortex and hippocampus was supported by a previous study.[59] Glial function plays a significant role in neuronal damage, with acetylcholine having anti-inflammatory and neuroprotective characteristics in various neurodegenerative illnesses.[60] Acetylcholine's beneficial effect on protecting neurons from lipopolysaccharide-induced damage is attributed to its ability to lower the inflammatory response of microglia and enhance the generation of neurotrophic factors by microglia.[61] This implies that the anticholinergic property of

the leaf extract may be implicated in the neurotoxic effect. The role of vagus nerve stimulation leading to immune system regulation, which is connected to the understanding of the cholinergic anti-inflammatory pathway has been reported.[62] In addition, prolonged and increased dopamine activity caused by the extract may also lead to neurotoxicity.[60] Increased dopamine metabolism may lead to more reactive oxygen species (ROS) being produced and less energy being generated, exacerbated by mitochondrial dysfunction and the Inflammatory responses already existing in the brain may be triggered by oxidative stress caused by dopamine, leading to neurodegeneration.[63]

Effect of Datura stramonium aqueous leaf extract on cerebral cortex and hippocampal morphology

The photomicrographs shown in Figure 15 presents a visual examination of the hematoxylin and eosin-stained slides of the cerebral cortex in rats. Upon examination, it was observed that the cerebral cortex of rats in the control group displayed normal architecture of the cerebral cortex showing pyramidal cells with multipolar shapes, rounded vesicular nuclei, and granule cells that were identifiable as circular-shaped neurons with large open-face nuclei. Furthermore, there were small round-vesicular-shaped glial neurons dispersed throughout a pink-stained neutrophils. The observed features align with the expected histology of a healthy cerebral cortex. For the rats treated with different doses (50, 100, and 200 mg/kg body weight) of *Datura stramonium* aqueous leaf extract, it appeared that there are both normal pyramidal cells with deeply stained nuclei and degenerating pyramidal cells with pale edges, shrunken nuclei, and pale staining. In addition, there were observable signs of granule cells undergoing degeneration. These cells displayed pyknotic nuclei, which appeared pale when stained. Based on the presented information, it appears that these characteristics align with potential neuronal damage.

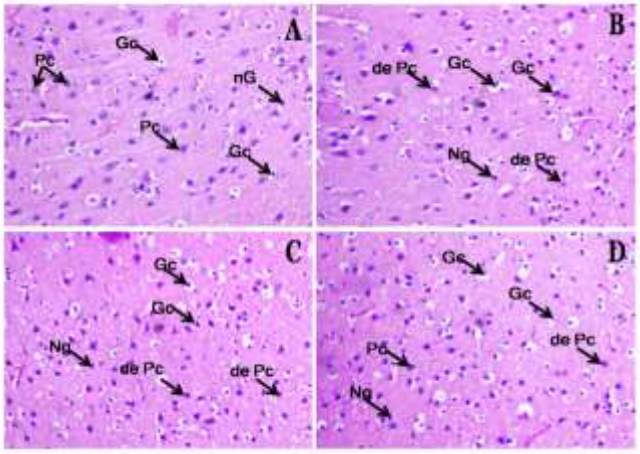

Figure 15: Representative photomicrographs of rat brain showing the effect of *Datura stramonium* leaf extract on the cerebral cortex (Haematoxylin and Eosin stain). (A) Control, (B) *Datura stramonium* leaf extract 50 mg/kg, (C) *Datura stramonium* leaf extract 100 mg/kg, (D) *Datura stramonium* leaf extract 200 mg/kg. Pc: Pyramidal cell, Gc: Granular cell, Ng: Neuroglia, dePc: degenerating Pyramidal cell. Magnification 100X

Figures 16 and 17 are representative photomicrographs of hematoxylin and eosin-stained slides of the dentate gyrus in the rat hippocampus. The present study focused on analyzing the dentate gyrus and Cornu ammonis 3 (CA3) regions of the hippocampus, which displayed the characteristic structure of the hippocampus. It has been observed that the CA3 region consist of relatively small number of pyramidal cells, which undergo a gradual transformation into the larger multipolar pyramidal cells found in the Cornu ammonis 4 (CA4) region. In the region referred to as CA4, there is a notable projection that extends into the concave area of the dentate gyrus. In the present investigation, there were small granular cells with vesicular nuclei in both the ascending and descending arms of the dentate gyrus. These cells appeared to be tightly packed together. The molecular layer contained astrocytes, microglia, neuronal processes, and nerve cells. These components were dispersed throughout this layer, which is located between the compact zones of the Cornu ammonis and dentate gyrus regions. The observed characteristics align with the expected histology of the dentate gyrus in the hippocampus of healthy rats. The histological examination of the hippocampus of rats administered *Datura stramonium* aqueous leaf extract at different doses (50, 100, and 200 mg/kg body weight) showed the loss of small pyramidal cell layer. Within the dentate gyrus, it was observed that there were a few small pyramidal neurons that appeared to be functioning normally. These neurons were found amidst a population of pyramidal cells that were undergoing degeneration, characterized by pale edges. In addition, it appeared that cells in the molecular layer were scarce, and a decrease in the density of the granular cells in the dentate gyrus was observed. These observations are indicative of neuronal damage in certain areas.

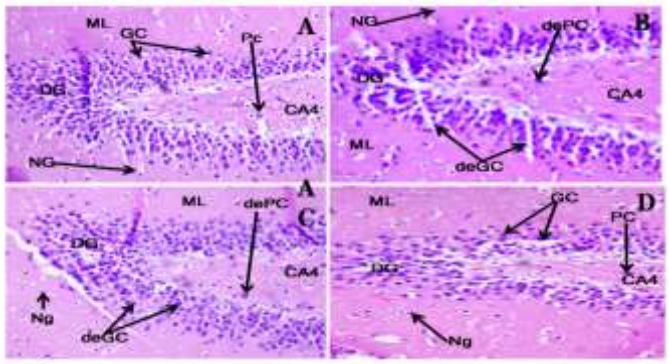

Figure 16: Representative photomicrographs of rat brain showing the effect of *Datura stramonium* leaf extract on the hippocampus (CA4) (Haematoxylin and Eosin stain). (A) Control, (B) *Datura stramonium* leaf extract 50 mg/kg, (C) *Datura stramonium* leaf extract 100 mg/kg, (D) *Datura stramonium* leaf extract 200 mg/kg. DG: Dentate gyrus, CA4: Cornu ammonis, Pc: Pyramidal cell, Gc: Granular cell, Ng: Neuroglia, ML: Molecular Layer, dePc: Degenerating Pyramidal cell, deGc: Degenerating Granular cell. Magnification 100X

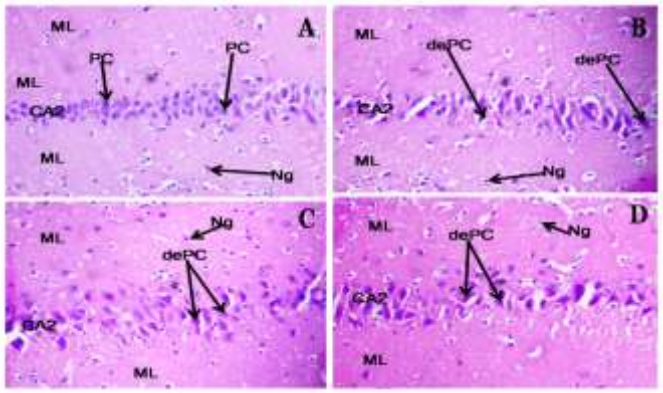

Figure 17: Representative photomicrographs of rat brain showing the effect of *Datura stramonium* leaf extract on the hippocampus (CA2) (Haematoxylin and Eosin stain). (A) Control, (B) *Datura stramonium* leaf extract 50 mg/kg, (C) *Datura stramonium* leaf extract 100 mg/kg, (D) *Datura stramonium* leaf extract 200 mg/kg. CA: Cornu ammonis, Pc: Pyramidal cell, Gc: Granular cell, Ng: Neuroglia, ML: Molecular Layer, dePc: Degenerating Pyramidal cell, deGc: Degenerating Granular cell. Magnification 100X

Conclusion

Datura stramonium L., a weed widely distributed around the world, has been shown to possess numerous medicinal properties. The plant has been used for centuries as medicine due to its psychoactive effect. However, the increasing popularity of the plant poses serious public health problems, as increasing use of the plant is associate with serious toxic effect, including addiction and overdose fatalities. The findings from the present study showed that the sub-acute administration of *Datura stramonium* aqueous leaf extract altered dopamine and acetylcholine levels, resulting in neurobehavioural symptoms like addictive tendencies, anxiety, depression, and memory impairment.

Conflict of Interest

The author`s declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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