

Original Research Article

Behavioural alterations and cerebellar toxicity induced by *Datura metel* exposure in mice

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Abstract

Purpose: To investigate the neurotoxic effects of methanolic crude extract of leaves of *Datura metel* L. Solanaceae on the cerebellum and motor coordination.

Methods: A total of 24 mice (22.0 ± 2.0 g) were randomized into 4 groups (control, 200, 400, and 800 mg/kg ($n = 6$) doses of methanolic extract of *Datura metel* leaves and orally administered once daily for 28 days). Motor and neuromotor activity were evaluated after daily treatment for 28 days using the rota rod, bar test, catalepsy and the animals were sacrificed by cervical dislocation for histological analysis of the cerebellum.

Results: Treatment with *Datura metel* significantly increased motor activity with a significant reduction in muscle tone in both the rota rod and bar test models compared to control group ($p < 0.05$). No effect was demonstrated in the catalepsy model. There were cytoarchitectural degradative changes in the molecular, Purkinje and granular layer, with significant reduction in cerebellar volume at 800 mg/kg.

Conclusion: This study shows that *Datura metel* has a cerebellotoxic effect with attendant manifestation in movement activity and coordination. Further studies on *Datura metel* would be required to determine the biochemical components responsible for these effects, with focus on possible psychoneuroendocrine mechanisms.

Keywords: *Datura metel*, Neurotoxicity, Motor coordination, Rota rod, Cerebellum

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INTRODUCTION

Datura metel L. Solanaceae is a perennial plant widely distributed in Asian and African regions [1]. The genus *Datura* comprises 4 species,

however, *D. metel*, *D. innoxia*, and *D. stramonium* are the most prominent [2]. In Nigeria, it is called *Apikan* in Yoruba, *Zakami* in Hausa, and *Myaramuo* in Igbo [3]. The leaf is simple, alternate, dark green, broadly ovate and

lobed [1]. The leaves are used to induce psychoactive effects, while the seeds are used to treat piles [4]. The plant parts which include seeds, leaves and roots are used to treat insanity, fever with catarrh, diarrhea, skin diseases, and cerebral complications [5]. The leaves are prepared and consumed in different concoctions, and in some cases smoked. Consumption results in anticholinergic effects leading to hallucinations and other central nervous excitatory conditions [6]. As a result, abuse results from recreating these psychostimulatory experiences. The plant is readily available in rural communities and serves as a cheap source of substance abuse compared to orthodox drugs. This creates potential risks like stimulants, addiction, dependence as well as brain impairment [6].

Neurotoxic substances compromise the structural and functional integrity of nerves through several mechanisms [7]. Various hypothesized mechanisms include induction of oxidative pathways, upsetting the acetylcholine balance in the central nervous system, and leading to impairment of neuronal metabolic function and activities. The Cerebellum which orchestrates motor control, motor precision, movement timing, and motor learning contains several neurons [8], whose intricate structure and vital functions make it a remarkable component of our nervous system. It plays a crucial role in transmitting sensory signals to the motor portion of the brain, contributing to muscle coordination, equilibrium, and posture [9].

Aristotle first described the cerebellum as a structure at the back of the brain, coining the term “parencephalis” (meaning “little brain” in Latin) due to its distinct form and size [10]. The cerebellum which consists of grey matter (cerebellar cortex) and white matter (medulla) reveals distinct layers: molecular layer (outer) which contains granule cell axons, Purkinje cell dendrites, stellate cells, and basket cells, purkinje layer (middle) which houses Purkinje cell bodies and granular layer (inner) which contains granule cell bodies, Golgi cells, and Purkinje cell axons. These cell types play essential roles in cerebellar function and connectivity [11].

With increasing search for natural, affordable and accessible sources of substances of abuse, the use of *Datura metel* as a hallucinogen should be investigated, because it has the potential to cause neurologic damage with effect on motor activities and coordination, which is a prominent role of the cerebellum. It is important thus to evaluate the effect of *Datura metel* extract (DME)

on motor activities as well as its histological effects on the cerebellum.

EXPERIMENTAL

Animals

Albino Swiss mice weighing 22.0 ± 2.0 g used in the study were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka and were housed in plastic cages at room temperature with 12:12 h light-dark cycle with unrestricted access to food and water, and were allowed to acclimatize for 2 weeks. Ethical approval was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (approval no. REC/FBMS/DELSU/21/83) and conducted in accordance with the Guideline for the Care and Use of Laboratory Animals [12].

Plant collection

Leaves of *Datura metel* (DM) plant were harvested fresh from its growing habitat at Obinomba, Ukwuani Local Government Area (LGA), Delta State, and authenticated at the Department of Botany, Delta State University, Abraka (voucher no. UPH/P/281). The leaves were air-dried for 3 weeks, pulverized using an electric blender, and 50 g was extracted with 1 L of 70 % v/v methanol using cold maceration at $28 - 30^\circ\text{C}$ for 72 h. The resultant mixture was filtered using Whitman filter paper (No. 1), and the filtrate concentrated to dryness in vacuo at 40°C using a rotary evaporator to yield a dark green hygroscopic powder (9 g; 18 % w/w). The *Datura metel* extract (DME) was air-dried, and stored in universal bottles at $4 - 8^\circ\text{C}$ for further use.

Design

A total of 24 mice were randomized into 4 groups ($n = 6$). Control group received deionized water. Group 1 received 200 mg/kg/bw, Group 2 received 400 mg/kg/bw, and Group 3 received 800 mg/kg/bw of DME administered orally using oro-gastric tube between 9 - 10 am once daily for 28 days. All parameters were evaluated 24 h after the last administration on day 28.

Behavioural test

Assessment of motor coordination using rota rod test

In the rota rod test, each mouse was placed on the rotating bar of a rota rod set at 5 revolutions per minute (rpm). The duration spent by a mouse

on the rota rod before it falls, referred to as the latency of fall (LOF) was used as an index of motor function. Deionized water was used as the control. Determinations were done in triplicates and averages were taken [13].

Assessment of motor coordination using catalepsy

Catalepsy was evaluated using the bar test immediately after the rota rod test. Forepaws of each mouse were located in a half-rearing position on a horizontal metal bar, which was set at 9 cm above the base in a parallel form. Descent latency time (time taken for the mouse to remove one paw from the bar) was recorded. Determinations were done in triplicates and average taken. Deionized water was used as the control. The descent latency cutoff time was 180 sec for the bar test [14].

Open field test

The open field test (OFT) was used to determine spontaneous motor activity (SMA) immediately after the catalepsy test. Each mouse was placed at the centre of the activity cage and allowed to move freely. Deionized water was used as the control. The number of square lines crossed with all paws and duration of ambulation of each mouse was recorded [15].

Histological examination and estimation of neuronal density

The mice were euthanized by cervical dislocation 48 h following the completion of the neuro-behavioural study, and the brains were harvested. Brain samples were fixed in 10 % phosphate-buffered formaldehyde and embedded in paraffin blocks. Transverse sections (5 – 6 μ m thick) of the brains were obtained with the aid of a microtome (Leica, Germany), stained with Hematoxylin and Eosin and fixed on glass slides. Thereafter, images were acquired using an Optronics Digital Camera connected to a computer interface (MagnaFire) and an Olympus BX-51 Binocular research microscope. General structure of axons, pyramidal cells, and peri-glomerular and granule cells were characterized using inter-reader variability. Diameter, area and length of neurons were measured using ImageJ at X400 or X250 at different microscopic fields for all groups.

Statistical analysis

Data was analyzed using GraphPad InStat® Biostatistics software (GraphPad Software, Inc. San Diego, California, USA). Results were

analyzed using one-way analysis of variance (ANOVA) and compared using the Student's Newman–Keuls post-hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of plant extract on motor activity

There was no significant difference in latency of fall among animals receiving 200, 400, and 800 mg/kg compared to control group ($p > 0.05$; Figure 1 A) in the catalepsy model. However, the rota rod test showed a significant decrease in the latency of falls in all treatment groups compared to control group ($p < 0.05$; Figure 1 B). Furthermore, locomotor activity significantly increased in all treatment groups compared to control group in the open-field test ($p < 0.05$; Figure 1 C).

Effect plant extract on cell parameters

There was a significant increase in cell diameter, length, width, and intercellular distance due to cell death following treatment with DME compared to control ($p < 0.05$; Figure 2 and Figure 3).

Effect of DME on Golgi section of the cerebellum

Control group demonstrated well-outlined granular and molecular layers including a distinct neuronal nucleus (Figure 4 A). Group 1 (200 mg/kg/bw DM) showed a delineated Purkinje layer indicative of cell shrinkage (Figure 4 B). Group 2 (400 mg/kg/bw) showed a less distinctive Purkinje layer with larger cell sizes, and irregular outline, possibly due to degeneration from apoptotic process (Figure 4 C). Group 3 (800 mg/kg/bw) showed fewer cells in the Purkinje layer, smaller cell sizes, greater intercellular distance with embedded vacuolations (Figure 4 D).

DISCUSSION

This study showed a dose-dependent distortion in the histo-architecture of cerebellar cortex with degenerative changes across all three cell layers (Molecular, Purkinje and Granular layer). This finding is in agreement with earlier studies [16]. There were cellular changes in the Purkinje layer, with the cells appearing less distinctive, and irregular, with clumped cell bodies and higher intercellular distance following treatment with *Datura metel* extract (DME).

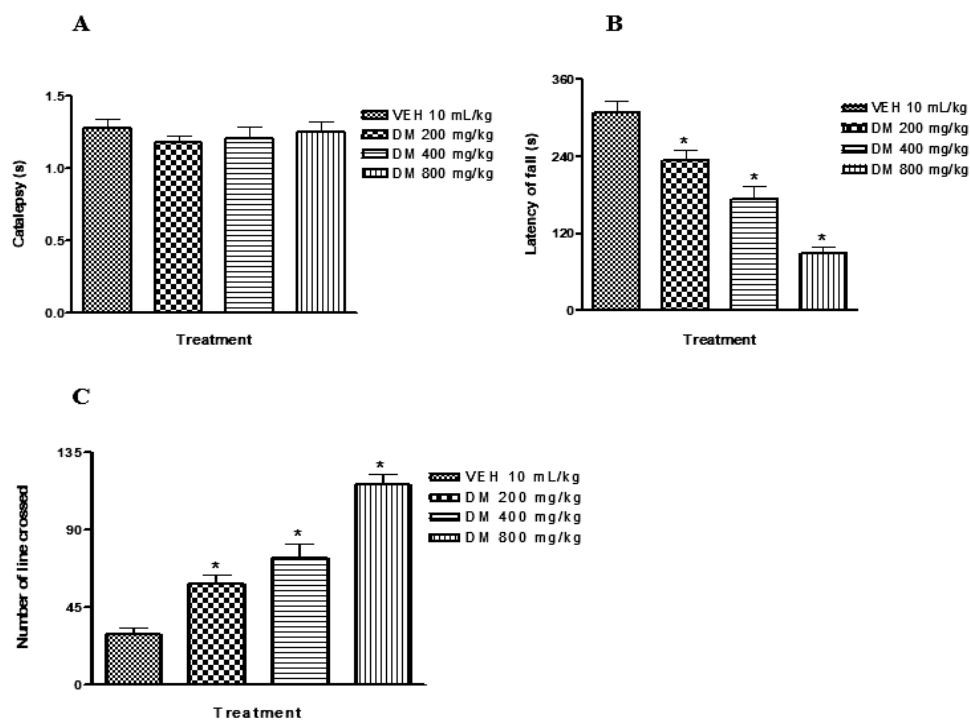


Figure 1: Effect of DM on catalepsy (A), motor coordination (B), and locomotor activity (C) of mice in treated groups compared to control. Values presented in mean $\pm$ SEM ($n = 6$). * $P < 0.05$ vs control. VEH (control), DM (*Datura metel*)

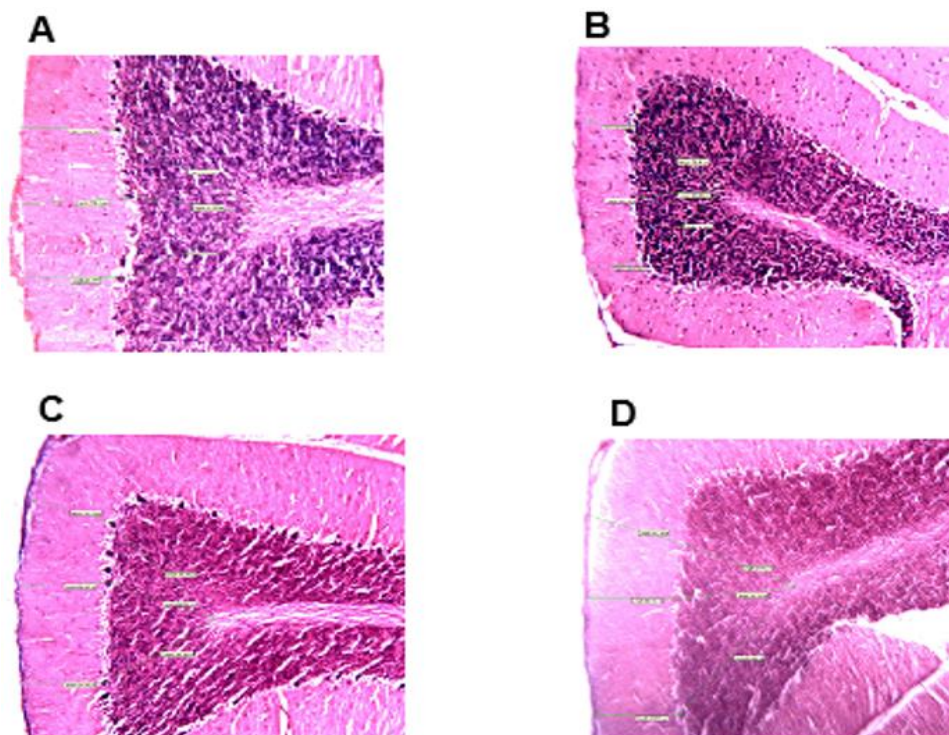


Figure 2: Effect of DM on cell parameters. **A:** Coronal section showing thickness of molecular and granular layers of cerebellum (control group). **B:** Coronal section showing thickness of the molecular and granular layers of cerebellum (DM treated 200 mg/kg). **C:** Coronal section showing thickness of the molecular and granular layers of cerebellum (DM treated 400 mg/kg). **D:** Coronal section showing thickness of the molecular and granular layers of cerebellum (DM treated 800 mg/kg). H and E stain $\times 250$. MCL - molecular cell layer, PCL - Purkinje cell layer, GCL - Granule cell layer

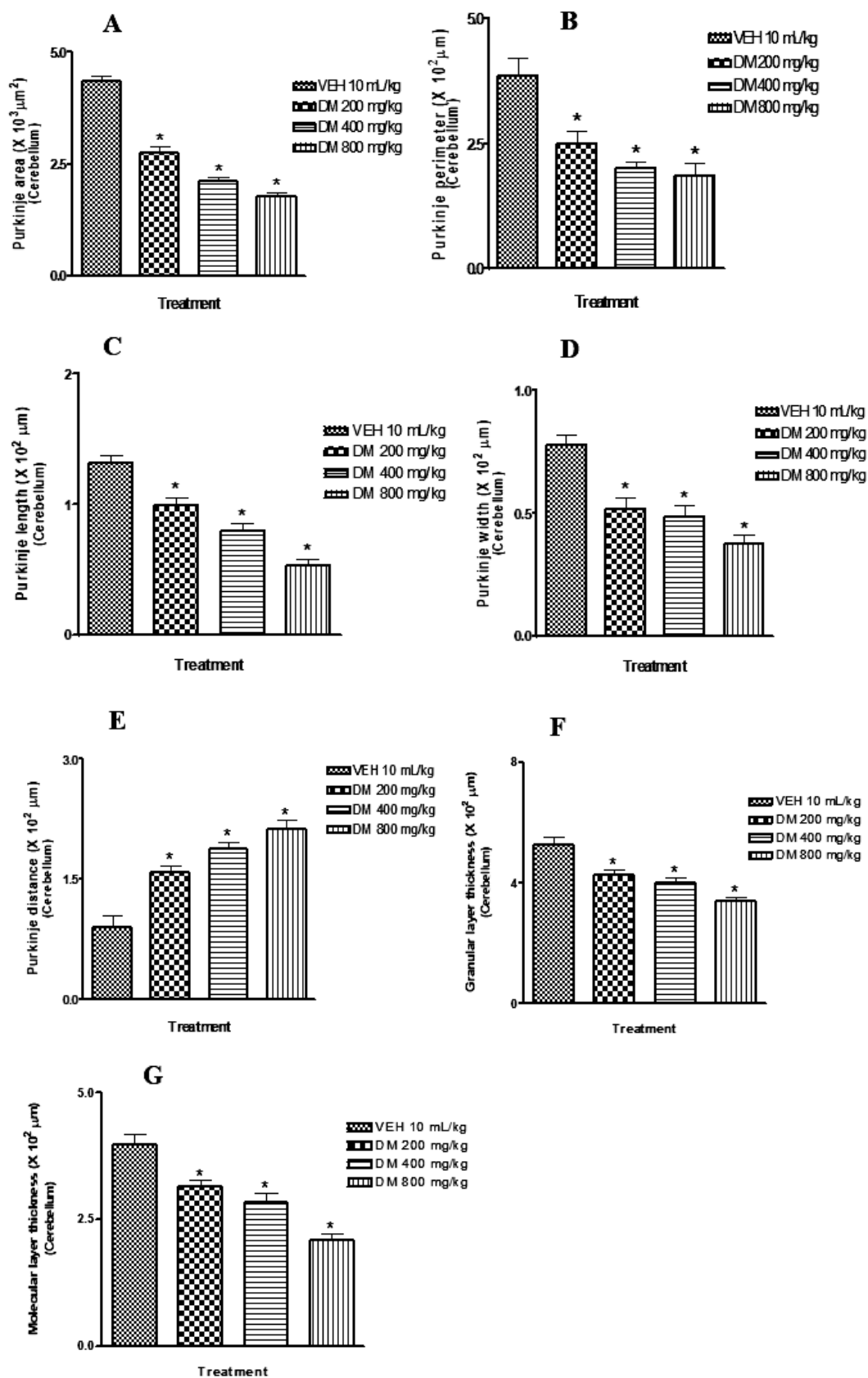


Figure 3: A. Purkinje cell area, B. Purkinje cell perimeter, C. Purkinje cell length, D. Purkinje cell width, E. Purkinje intercellular distance, F. Granular layer thickness, G. Molecular layer thickness

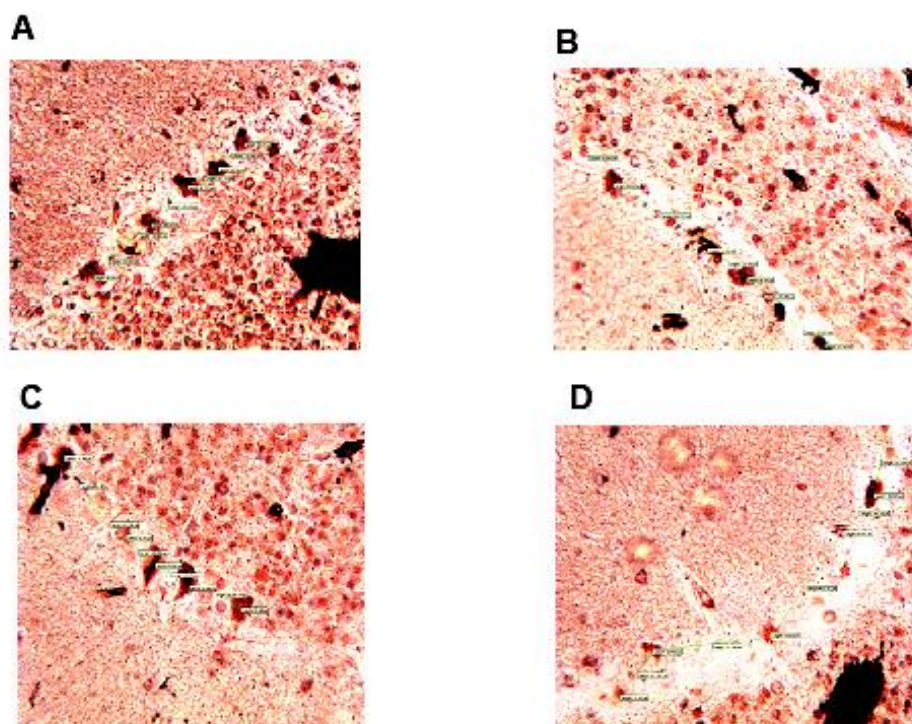


Figure 4: **A** (control group) showed three layers, with well outlined neurons, granular and molecular layers with distinct nucleus of the neurons clearly distinctive. **B** (DM treated 200 mg/kg) showed clearly delineated Purkinje layer with clumped cell bodies with increased inter-cellular distance, indicative of cell shrinkage. **C** (DM treated 400 mg/kg) showed less distinctive Purkinje layer with larger cell sizes, sparsely distributed molecular and granular layer cells. **D** (DM treated 800 mg/kg) showed vacuolation at the granular layer, with fewer cells in the Purkinje layer, and spare cells at the granular layer (Golgi Stain x 400)

This indicates cell shrinkage which may possibly be due to degeneration from apoptotic processes. Also, vacuolations due to cell absence were also observed which may be due to cell death and phagocytosis. Cells in the molecular layer were sparsely distributed compared to the granular cell layer which showed cellular vacuolation (evidence of cell death). The features reported in this current study, especially perinuclear vacuolation, and reduction in the dimensions of the Purkinje cells were similar to the finding of Ekanem *et al* [17] which revealed histoarchitectural distortion of the medial prefrontal cortex (mPFC) and Hippocampus of mice treated with 200 - 800 mg/kg/bw of DM. This suggests neurodegenerative induction properties of DM with attendant altered neuronal integrity, including chromatolysis, reduced density of Nissl substances, neuronal loss as well as axonal injuries. The mechanism of induction of the histological changes (perineuronal vacuolations, distortions and cellular degenerative changes) may be due to unopposed excitotoxicity following inhibition of acetylcholine as a result of the phytochemical constituents in DM (atropine, hyoscyamine, scopolamine and several alkaloids) [18]. These promote cellular degeneration through increased activities of

malondialdehyde (MDA) and superoxide dismutase (SOD) as well as decreased tissue level of glutathione (GSH).

The cerebellum is responsible for maintaining equilibrium, muscle tone and body posture. It also precisely coordinates skilled voluntary movements by controlling strength, duration and force of contraction [19]. An injury to the cerebellum will manifest as distortion in movement sequence which is assessed using various neurobehavioral model such as open field test, catalepsy and Rota rod [20]. Locomotion is a characteristic adaptive mechanism of survival, to help in the food search, avert unfriendly environments, and locate safe havens in cases of danger, and impairment of such a vital process is detrimental to health and survival [21]. Also, exploratory behaviours such as rearing, grooming, and ambulation) demand a significant degree of motor function, and as a result, are indices of motor control [22].

Locomotor activity was assessed using the open field test [15], and the results showed significantly increased locomotor activity, evidently demonstrated by increased distance covered, which signifies an augmented locomotor activity. The phytochemical

constituents of *Datura metel* which include scopolamine and atropine, have anticholinergic activities, with resultant sympathetic stimulation [23]. However, there was associated uncoordinated movement sequence, which may likely be due to cerebellar dysfunction [24]. A component of cerebellar activity was also assessed using the Rotarod and catalepsy model, and the results demonstrated significant reduction in fall time which further corroborates the uncoordinated motor activity (locomotion) in the open field test. These findings may be due to possible impaired muscle coordination [25].

The synergy of function and coordination between various components of the brain, such as the basal ganglia, cerebellum, motor cortex, and pre-frontal cortex helps in the initiation and coordination of muscle movement. Purkinje cells play an inhibitory coordinating role of signal transmission [26]. Thus, degeneration of Purkinje cells as evident in the photomicrograph sections, would greatly impair muscle tone and locomotion, with dysequilibrium between excitation and inhibition. This favours excitatory propensity which was observed in the open field test. Also, a reduction in cerebellum thickness, decrease in perimeter, length, width and area of the Purkinje cells, further contribute to reduced synergistic neural communication because cells need proximity to help initiate and maintain intercellular communication.

The cell death evidenced by increased intercellular distance impairs this basic signal transmission sequence. The Purkinje fiber plays a primary role in signal transduction and a reduction in thickness of the molecular and granular layer signify cerebellar atrophy and sparsely distributed Purkinje cells with wider intercellular distance, reduced length, perimeter, width and areas following treatment with DME. This finding is in tandem with similar study [17] which demonstrated histological modifications with atrophy of the cerebellar parallel fibers in the granular layer but no significant change in Purkinje cells. This contributes to the uncoordinated distorted movement.

CONCLUSION

This study shows that methanol extract from *Datura metel* leaves causes dose-dependent distortion in brain histoarchitecture and altered behavioural patterns in mice. Further studies on *Datura metel* would be required to determine the biochemical components responsible for these effects, with focus on possible psychoneuroendocrine mechanisms.

DECLARATIONS

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Ethical approval

As reported in the experimental section.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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