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Effects of Hydroethanolic Leaf Extract of Datura Metel on the Hippocampus and Cerebellar Cortex of Adult Sprague Dawle Rats

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Abstract

Background: Datura metel is a plant of medical importance, often abused due to its psychostimulatory properties. Its use to relieve flu symptoms has led to an upsurge in consumption. ***Aim:*** To evaluate the impact of Datura metel leaf extracts on the histology and histochemistry of the brain. ***Materials and Methods:*** Five groups, A, B, C, D, and E, each having five adult rats, were formed out of 25 rats in total. Group C, D, and E were treated with hydroethanolic leaf extracts of 150mg/kg, 300mg/kg, and 600mg/kg body weight respectively. Whereas, group A served as the negative control and group B as the positive control. The methodology included biochemical analysis on antioxidants enzymes and lipid peroxidation. Also, haematoxylin and eosin stain was used to examine the general tissue morphology. Additionally, neurospecific enolase (NSE) and neurofilaments proteins (NFP) were used to evaluate the neurohistochemistry. ***Results:*** Exposure to hydroethanolic extracts of Datura metel significantly increased SOD and GST (p= 0.042 and 0.003). However, there was no significant increase in catalase and MDA after 14 days of exposure. Qualitatively, neuronal degenerations in adult rats confirmed the neurotoxicity of Datura metel. ***Conclusion:*** The hippocampus, and cerebellar cortex showed neurodegenerative signs including diminished neuronal cells, dendritic arborisation, vacuolation, increased neurofilament proteins and neuro-enolase enzymes. Therefore, people should be well guided on using the plant, especially given the negative effect on the brain as shown in experimental animals used in this study.

Keywords: ***Leaf extract, Datura metel, Hippocampus, Cerebellar cortex.***

Introduction

Datura metel is a plant from the Solanaceae family and seen almost everywhere. Datura is a family that has five species [1]. The most predominant species are D. arborea, D. fastuosa, D. innoxia, D. metel, and D. stramonium [1]. While Datura metel's toxicity was recently associated to unintentional ingestion, its utilization for psychedelic impacts and to ease influenza side effects has prompted an upsurge in utilization lately [2]. Because of Datura's hallucinogenic impacts, both conscious and inadvertent poisonings have been observed in many places in the world, whether consumed crude or through decoctions made from home preparations [3].

Mortality from Datura is uncommon, yet unfriendly impacts after its consumption are more frequent [4]. Datura metel contains atropine, scopolamine, hyoscine and hyoscamine, which are liable for its harmful properties. The poisons in Datura are obtainable in the leaves and even other parts of the plant. At the point when Datura plant is ingested, even minute quantities might cause gentle cerebral damage, fantasies and anxiety [5]. The poison is capable of causing erythema of the skin, dry mouth, hyperthermia, mydriasis, tachycardia, hypotension, hypertension, gastrointestinal dysmotility, muscle shortcoming, ataxia, mental trips, tumult, irrationality, spasms, disarray, unconsciousness, cardiovascular breakdown and others [6].

Datura metel is a common stimulant with a variety of purposes. A few restorative purposes of the plant incorporate its relaxing properties, excitement of the focal sensory system, respiratory decongestion, and treatment of dental and skin diseases, alopecia and toothache [6]. It has been helpful for treating cough, chest pain, asthma, epilepsy, and so on. In certain places, it is utilized in cocktails to increase inebriation, and smoked like tobacco on account of its psychoactive properties. It has likewise been used to

treat ineptitude, loose bowels, killing of parasites and as antipyretic for the control of fever [7].

Toxicity cases have been accounted for after eating the berries of Datura. Previous studies showed that death can occur from cardiovascular breakdown in the course of ingesting seeds of the plant. This is owing to the fact that the seed contain more tropane alkaloids [8]. The high rate of distribution, toxicity and its likelihood of been part of food stuffs are linked to the various episodes of injury in people [8]. It has anticholinergic properties, with scopolamine being a strong cholinergic impeding drug. Surplus consumption of the plant can bring about extreme cholinergic extermination [7]. A particular specie of the plant (Datura stramonium) has been reported to cause extremely durable transient cognitive decline and is said to impede learning since it equally contains gamma-l-glutamyl-l-aspartate [9]. Also, as a result of its psychoactive properties, it is one of the significant medications embroiled in the enhancement of neurological problems [9].

It was found that Datura metel leaf elicits neurotoxicity in the cerebral cortex, as seen in electron photomicrographs, which showed impaired and disintegrate mitochondria [9]. The utilization of any piece of the Datura plant might prompt an extreme anticholinergic impact that might cause harm. The whole plant is poisonous to some extent, however, the seeds are viewed as the most harmful; neither drying out nor bubbling obliterates the poisonous properties [10]. The consumption of huge portions of D. stramonium upsets the focal sensory system and inspires side effects like disarray, pipedreams and amnesia, and abnormal behaviors [11]. Also, the signs and side effects of Datura incorporate dryness of the lips and epidermis, pupil dilation, urinary retention, debilitated vision, and quick heartbeat [12].

Studies on the neurotoxicity ability of Datura metel pointed out neuronal degeneration in the hippocampus and medial prefrontal cortex (mPFC) with cell hypoplasia and loss of dendritic arborizations [13]. Similar studies revealed a marked increase in oxidative pressure and diminution of antioxidant status of all treated (experimental) mice. This characteristic neurotoxicity and expanded oxidative pressure in mice were demonstrated behavioural assessment of the animals, which revealed memory debilitation and mental deficiencies, nervousness and burdensome behaviours in mice following oral administration of Datura Metel [13]. Lipid peroxidation resulting from cell excitotoxicity, cascades of influence from a rapid increase in free radicals either through cyclooxygenase or nitric oxide synthases [14]. Additionally, free radicals' accumulation in the hippocampus and prefrontal cortex prompts memory decline by means of modification in long-term potentiation [14]. Administration of datura metel elicits a rise of MDA in mice. MDA as a biomarker of lipid peroxidation has high reactivity and toxicity, which results from oxidative stress. High MDA levels observed in animals after ingestion of Datura metel implies lipid peroxidation [14].

Datura metel may incite oxidative harm in the brain by catalyzing the peroxidation of biomolecules in the cerebrum, bringing about cell proliferation, cell injury and cell death [15]. Antioxidants metabolise oxygen species created from the peroxidation of lipid into less hurtful elements; for instance, SOD which is essential for switching superoxide radicals to hydrogen peroxide (H2O2), captures oxidative pressure by reacting with other neuroprotective antioxidants [16]. Previous studies have shown that Datura metel consumption affects biomolecules as well as the histomorphology of the brain, therefore, the necessity to investigate the toxicity of leaf.

The study aimed to evaluate the impact of Datura metel leaf extracts on neuro-histology and neurochemistry. The specific objectives of the study are to assess the effect of Datura metel hydroethanolic leaf extracts on oxidative stress markers and body weight using mean difference and regression analysis. Also to evaluate the consequence of Datura metel hydroethanolic leaf extract on the hippocampus and cerebella cortex of sprague dawley rats via haematoxylin and eosin staining and immunohistochemical staining techniques.

Materials And Methods

Experimental Design

Twenty five (25) healthy adult rats were used as experimental subjects. Before the extracts were administered, the animals were given two weeks to acclimatize with the environment. Five groups, A, B, C, D, and E, each including five adult rats were formed out of the 25 rats. Group C, D, and E rats were treated with Datura metel hydroethanolic leaf extracts of 150mg/kg, 300mg/kg, and 600mg/kg body weight respectively. Whereas, group A rats served as the negative control and group B rats as the positive control administered with lead.

Extract Preparation

Fresh leaves were dried at 40°C in a hot air oven. The dried seeds were pulverized with a metal pestle and mortar. An electronic weighing balance was used to weigh the minced leaf material, which was then immersed in 50% ethanol for 72 hours. After that, it was shaken intermittently for 72 hours, drained, and sieved using filter paper. The material was concentrated using a rotary evaporator, which lowers the boiling point of ethanol by using vacuum. The rotary evaporator comprised of a condenser, water bath, chiller, concentrating flask and solvent receiving flask. The rotation of the concentrating flask at 30oC promotes heat circulation which enables the evaporation of the extract into the condenser as vapour. Then, the vapour was transformed back into liquid and added to the receiving flask [17].

Animal Treatment

Rats in group A received their regular diet and water, group B received lead injections, and groups C, D, and E received Datura metel leaf extracts of 150 mg/kg, 300 mg/kg, and 600 mg/kg body weight, in that order. The extract was administered orally for duration of fourteen (14) days.

Organ Resection and Processing

Following the animal's anaesthesia, the brain tissue was removed, weighed, and then quickly placed in a 10% formal saline fixative. Tissues were processed using standard techniques as adopted [18]. Brain tissues were cleared in xylene, infiltrated with molten paraffin wax, and then dehydrated from low-grade to absolute alcohol. Tissue-tek embedding mould was used to embed the tissues. Also, the rotary microtome was used to make thin sections [19].

Staining and Microscopy

Sections were deparaffinised in two (2) changes of xylene and hydrated through descending grades of alcohol. Sections were stained with Harris haematoxylin for 5 minutes, and rinsed in water. Sections were stained with eosin for 2 minutes, rinsed in water and dehydrated. Tissue slides were finally mounted using dibutylphyltalatepolysterene xylene (DPX). Tissue slides were then viewed under the microscope (OlympusBX60MF, Japan) at ×10 and ×40 objective lenses [18,19].

Estimation of Antioxidants and Lipid Peroxidation

For Malondialdehyde (MDA), a portion of the sample, equal to 0.4 millilitres, was combined with 1.6 millilitres of Tris-KCl buffer and 0.5 millilitres of 30% TCA. Next, 0.5ml of 0.75% TBA was added, and the mixture was heated to 80°C for 45 minutes in a water bath. After being cooled in ice, this was centrifuged at 3000g. The absorbance was calculated at 532nm using distilled water as blank [20]. Lipid peroxidation was calculated using a molar extinction coefficient of 1.56 x 105 M-1Cm-1 in units/mg protein.

Catalase was estimated using the principle; dichromate in acetic is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H2O2), with the formation of perchromic acid as an unstable intermediate. Hydrogen peroxide concentration is directly proportional to the concentration of chromic acetate produced from the reaction. The chromic acetate produced is measured calorimetrically at 570 nm [21]. $Cr2O7-2+ 7H2O2 \rightarrow 2CrO8-3 + 5H2O + 4H+$.

Also, for superoxide dismutase (SOD) estimation, 1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2ml of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds [22].

The activity of glutathione-S-transferase was measured using the method of Habig *et al* [23] according to the protocol table below.

Table1: Protocol Table for Glutathione-S-Transferase

Reagent	Blank	Test
Reduced glutathione (0.1M)	30µl	30µl
CDNB (20mM)	150µl	150µl
0.1M Phosphate buffer, pH 6.5	2.82ml	2.79ml
Homogenate	-	30µl

Statistics

Data was presented in mean±sd and charts for descriptive statistics. Inferential statistics was carried out using regression analysis to ascertain the effect of the different doses of the leaf extracts on the biochemical parameters. The significance level was set at $\alpha<0.05$. For statistical analysis, we used the statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 23).

Results

Effect of Hydroethanolic Leaves Extract of *Datura metel* on Biochemical Parameters

Estimation of superoxide dismutase was performed using descriptive statistical analysis and regression analysis across the study groups. Study groups administered with the leaf extract which comprised the low, medium and high dose revealed the following mean±sd; 8.93±0.12, 9.39±0.66, 10.24±0.54. However, mean±sd for the normal group and the positive control (lead) = 8.29±0.18, 5.60±0.59. B =.657, $R^2 = 0.685$, p = 0.042. This means that there is a statistically significant difference in SOD level between the study groups administered with the leaf extract. The analysis also portrays that a unit increase in the dose of the hydroethanolic leaf extract of *Datura metel* is likely to cause 0.66 increase in the SOD level. In addition, the effect of the extracts (leaves) on SOD is 68.5%. The analysis revealed a statistically significant effect on SOD.

Furthermore, for catalase, the respective Mean±SD values for the study groups of the leaves extract are; normal = 5.56±0.59, lead =2.35±0.38, low = 5.58±0.57, medium = 6.21±0.07, high = 6.21±0.10. Consequently, B = 0.315 and the coefficient of determination (R^2) of 0.459 means that 45% variance in the dependent variable (catalase) can be explained by the leaves extract. However, the mean difference for MDA was not statistically significant. The assessment of the normal and lead groups demonstrates accordingly; Mean±SD 13.88±0.08 for the normal group and 28.29±0.24 for lead group. Also, the different groups of the leaves extract - low, medium, and high subsequently displayed 12.44±0.04, 12.13±0.75, and 11.62±0.29 in that order. B = -0.41, R^2 = 0.50, by implication, a unit increase in the leaves extract will result to (-0.4) decrease in MDA level. The mean difference in the concentration of MDA among the animals exposed to the leaves extracts was not statistically significant.

Subsequently, analysis on GST across the groups explains that the negative control and lead groups recorded 2.05±0.16 and 2.03±0.09. Whereas, the respective groups of the leaf extract have; low dose = 1.95±0.04, medium = 2.15±0.07, high dose = 2.28±0.05. The coefficients of regression were also assessed to determine the influence of the factor on the criterion variable (leaves extract). Leaf extract has a significant positive impact on GST. Regression analysis revealed that B = 0.16, $R^2 = 0.91$, p value = 0.003. The model explains 91% variance in GST. Moreover, as dosage increases by 1 unit, GST increases by 0.2. See table 2 for details.

Table 2: Effect of Hydroethanolic Leaves Extract of *Datura Metel* on Biochemical Parameters

Parameter	Normal	Lead	150mg/kg (x̄±SD)	300mg/kg (x̄±SD)	600mg/kg (x̄±SD)	B	R^2	P-Value	Remark
SOD (U/mg)	8.29±.18	5.59±.59	8.93±.12	9.39±.66	10.24±.54	.657	.685	0.042*	Sig
Catalase (U/mg)	5.56±.59	2.35±.38	5.58±.57	6.21±.07	6.21±.09	.315	.459	0.139	N/S
MDA (µMol/mg)	13.88±.08	28.29±.24	12.44±.04	12.13±.75	11.62±.29	-.410	.504	0.114	N/S
GST (µMol/mg)	2.05±.16	2.03±.09	1.95±.01	2.15±.07	2.28±.05	.163	.917	0.003*	Sig

Figure 1: Box plot showing weights of animals before extract's administration.

Figure 2: Box plot showing weights of animals after extract's administration. Discriptive statistics reveals a decline in animals' body weight in the experimental groups.

Assessment of the Hippocampus

In comparison to the normal control group, figure 2 shows a decline in the population of the neurons in the low dose group and a severe degeneration at the high dose group. At the concentration of 600mg/kg body weight, the leaf extracts demonstrates significant neuronal damage. Haematoxylin and eosin staining technique was to demonstrate the pyramidal cells, the CA3 neuronal population, and the dentate gyrus. Figure 3 shows the transverse section of the hippocampal tissue at x100 and x400 magnification respectively.

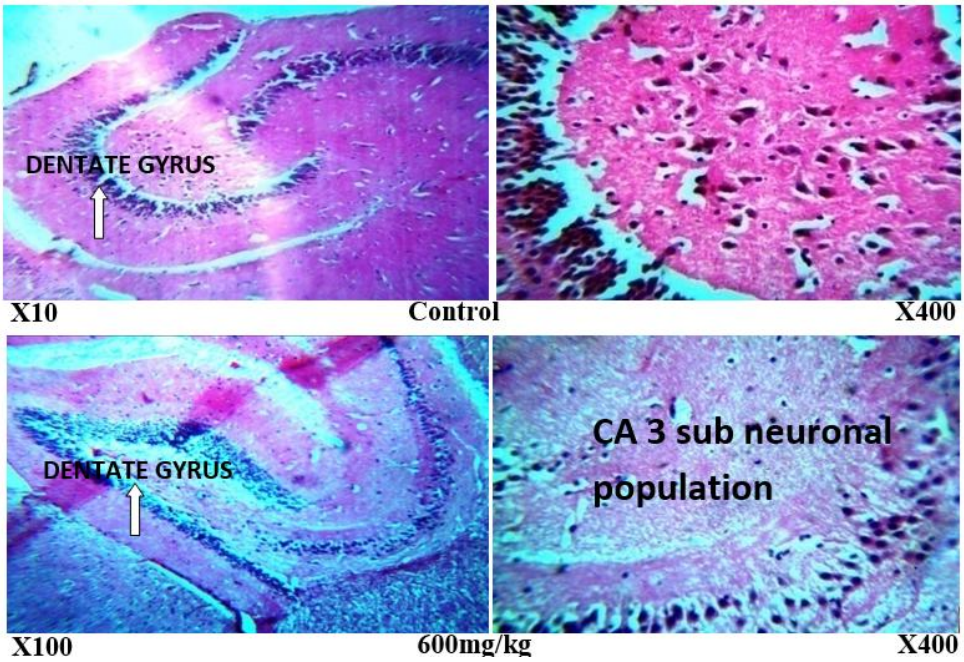

Figure 3: Hippocampus showing cornu ammonis and dentate gyrus. Control rats showing normal histological architecture. At higher concentration, the leaf extracts exhibited serious depopulation of the neurons. The damage to the neurons is expressed by a decline in the number of the neurons when compared with the control group.

Assessment of the Cerebellar Cortex

Animals administered with 600mg/kg of Datura metel's leaf extracts have a sparse granular cell layer, mulecular layer, and purkinge layer in their cerebellar cortex. Also, only one active Purkinje neurone and five deaths per high power field are visible in the Purkinje layer. Additionally, the neurone exhibits vacuolation as shown in figure 5 below.

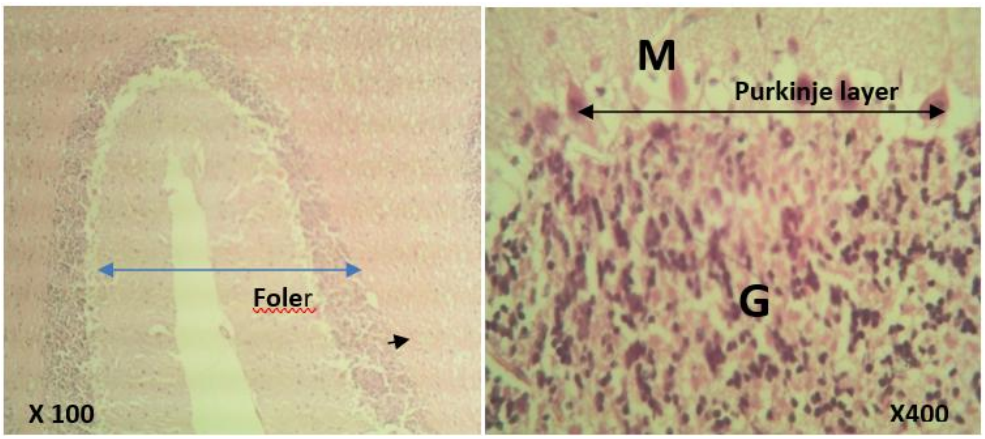

Figure 4: Cerebellar cortex of normal (control) rat showing the molecular layer (M), Purkinje cell layer (black arrow), and granular layer (G).

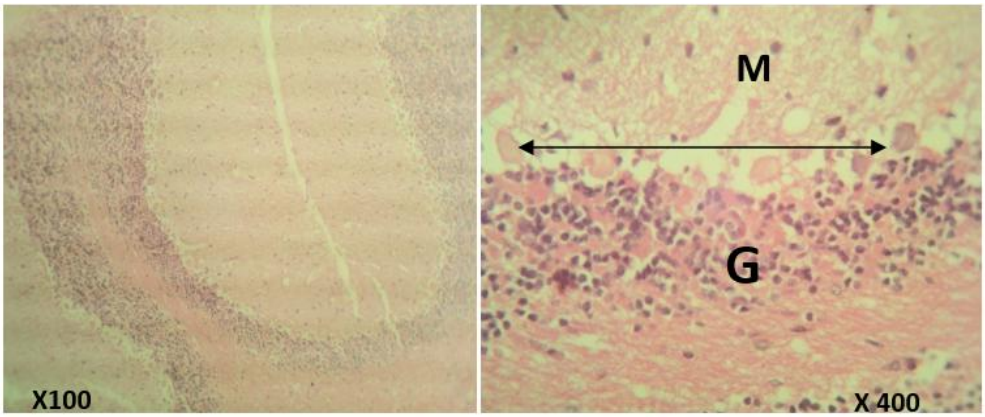

Figure 5: Histology of the cerebellar cortex of rats exposed to the leaf extracts of Datura metel. Histology shows scanty granular cell layer (G), molecular layer (M) and purkinge layer (black arrow). The purkinje layer show 1 active purkinje neurone and 5 deaths per high power field. The neurones shows vacuolation.

Staining Result for Neurofilament Proteins

Figure 6 below shows immunohistochemical staining for neurofilament proteins. The photomicrograph of subjects that received the normal feed and high doses of hydroethanolic leaf extract of Datura metel are shown below. The result of the study showed that control group has less expression of neurofilament proteins. Neurofilament protein expression is moderately expressed at the medium dose, but at the high dose, there is marked expressions.

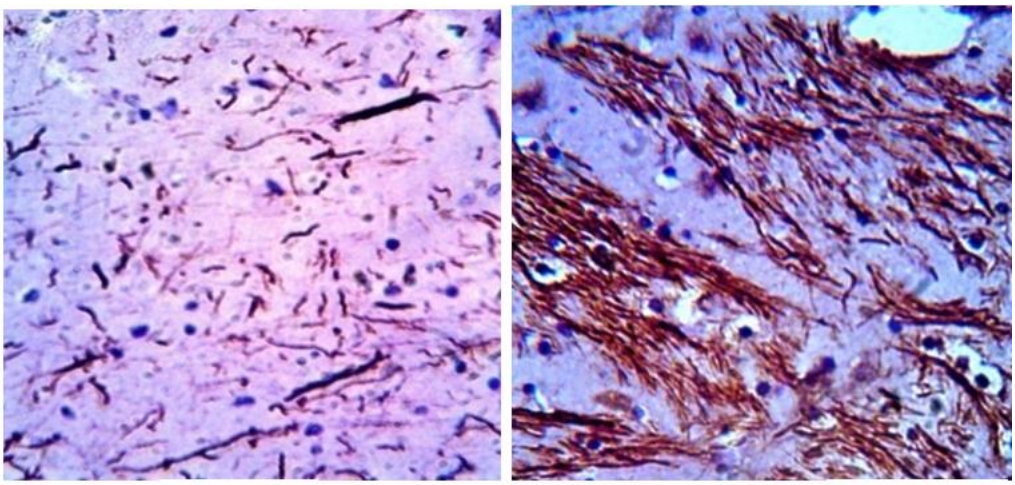

Figure 6: Photomicrographs showing NFP expression on brain tissue exposed to hydroethanolic leaf extract of Datura metel. The subjects exposed to 600mg/kg showed marked expressions of NFP.

Staining Result for Neuron-specific Enolase (NSE)

Neuron-specific enolase (NSE) was detected by immunohistochemical labelling. The photomicrographs below demonstrate how neuron-specific enolase (NSE) is expressed in the adult Sprague Dawley rats' brain. The dendritic processes are stained dark brown. Neuron-specific enolase was not expressed in the control animals. In contrast to the lower concentrations and control, only the high dose (600mg/kg) exhibited a mild expression of neuron-specific enolase.

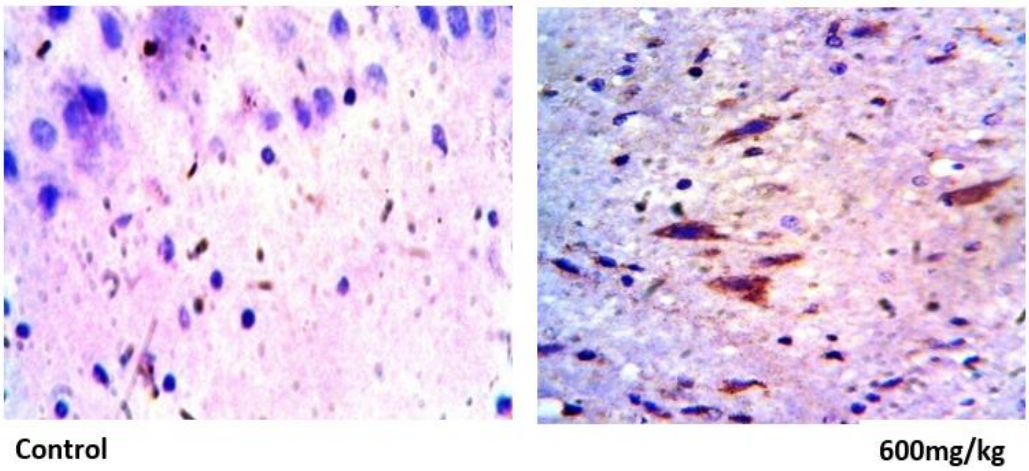

Figure 7: Photomicrographs displaying neuron-specific enolase (NSE) in rats' brain after exposure to Datura metel. Moderate expression is shown at high doses, while lost expression is seen in the control groups. The expression of the NSE varies with the severity of the injury. Positivity is shown by dark brown deposits.

Discussion

Impact of Leaf Extracts of *Datura Metel* on Superoxide Dismutase (SOD).

The metalloenzyme superoxide dismutase converts two superoxide radicals (O2-) into hydrogen peroxide (H_2O_2) and oxygen (O_2) by incorporating two hydrogen ions (H+). Pathologies like stroke, cancer, and neurological illnesses are caused by reactive oxygen species (ROS). Antioxidants are essential for maintaining good health by reducing the negative effects of reactive oxygen species [24].

The study groups for the leaf extract including low, medium, and high doses, revealed the mean±sd as 8.93±0.12, 9.39±0.66, and 10.24±0.54. Also, the lead (positive control) group and the normal group yielded: 8.29±0.18, 5.60±0.59. B = 0.657, R^2 = 0.685, p = 0.042. This indicates a statistically significant difference between the study groups that received the leaf extract and the control groups. The data also shows that an increase in dosage of *Datura metel's* hydroethanolic leaf extracts is likely to result in a 0.66 increase in the SOD level. Furthermore, the leaves' extracts have a 68.5% effect on SOD. Table 2 demonstrates that SOD increases alongside with an increase in the concentration (dosage) of the leaf extract. This occurred in the following order: low dose less than medium dose, medium dose less than high dose. This is in conformity with a study conducted by Alum *et al* [25]. Test results following analysis of antioxidant enzymes showed elevated SOD activity in rats given

Datura stramonium leaves extract. This also supports the phytochemical studies on datura which revealed the plant contains bioactive chemicals including health-promoting bioactive alkaloids, gallic acid, catechin, rutin, apigenin and others [26]. Additionally, compared to rats in the normal group, the introduction of Datura extract increased SOD activity [27]. The study findings indicate that the study groups that received *Datura metel* had higher levels of SOD; this higher level of SOD may be the consequence of oxidative stress that was forming in the tissue and thus, an adaptive and/or protective mechanism against it.

Impact of Leaf Extracts of *Datura Metel* on Catalase

The corresponding Mean±SD values for the controls and study groups administered with the leaf extracts were as follows: normal = 5.56±0.59, lead = 2.35±0.38, low dose = 5.58±0.57, medium dosage = 6.21±0.07, and high dose = 6.21±0.10. As a result, B = 0.315 and the coefficient of determination (R^2) = 0.459 indicate that the leaf extracts can account for 45% of the variation in the dependent variable, catalase. In recent years, medicines from natural sources has gained huge interest owing to their ability to effectively prevent and treat several health issues associated with oxidative stress in a harmless way [28]. Plants are considered as the first source of drug detection, and drugs of plants origin have played a crucial role in the medical world [29]. Most medicinal plants have been known to be useful in alleviating several disease conditions. This is for the reason that plants do possess a mixture of constituents each having its pharmacological impacts. The working system of each constituent differs, and some have mutual effects, while others differ in therapeutic outcome. Anti-inflammatory actions are some of the roles played by such constituents, thus the ability to repair injured cells or preventing the harmful effects of inflammatory product [30].

Impacts of Hydroethanolic Extracts of *Datura metel* on Malondialdehyde (MDA) and Glutathion-s-Transferase (GST).

The MDA level for lead in the group exposed to the leaf extract was found to be 13.88±0.08 for the negative control (normal). But the levels for the low, medium, and high leaf extract groups were 12.44±0.04, 12.13±0.75, and 11.62±0.29, respectively. Furthermore, R^2 = 0.50 and B = -0.4. Similarly, a unit increase in the leaves' dose will have a non-significant effect (p = 0.114) on MDA levels and a 50% impact. Additional, examination of GST for each group given the leaf extract revealed that the lead and negative control groups recorded 2.03±0.09 and 2.05±0.16, respectively. Conversely, the corresponding leaf extract groups have the following: high dosage = 2.28±0.05, medium dose = 2.15±0.07, low dose = 1.95±0.04. To determine the factor's influence on the criterion variable (leaves extract), the regression coefficients were also evaluated, thus, GST is significantly impacted by the seed extract. The results of the regression analysis has B = 0.16, R2 = 0.91, and p = 0.003. The GST variation is 91% as explained by the regression model. This suggests that GST rises by 0.2 for every unit increase in dosage. Details are shown in table 1. The result showed that taking *Datura metel's* leaf orally would cause a rise in GST. The results from a biochemical study for oxidative and antioxidant activity markers revealed dose-dependent, statistically significant increase in GST. Conversely, in a previous study, it was reported that the administration of the leaves extracts of a similar specie caused significant diminution in glutathione and malondialdehyde in rats induced with cyclophosphamide [31].

Impact of Datura Metel Leaf Extracts on Body Weight

The decrease in the animals' body weight in all the treatment groups as shown in figure 2 above can be linked to anorexia subsequent to the administration of the leaf extracts of *Datura metel*. It was previously reported that decrease in body weight can occur as a result of the harmful effects of the extract on the regular biological, biochemical, functional and metabolic processes, with resulting depletion of proteins in the treated animals [32]. The decrease in body weight of the extract-treated animals signified the possible alterations in the functions of organs regulated by the nervous system and metabolic activities [33].

Impact of *Datura Metel* leaf Extracts in the Hippocampus

Light micrographs of the hippocampus tissue from animals given hydroethanolic leaves extracts are shown in figure 3 above. Subsequent to haematoxylin and eosin staining, reduction in the number of neurons was observed. This decrease occurred in a dose-dependent manner. Impliedly, the decrease in low dose is more than the medium and high dose accordingly. Administration of the leave extract caused a remarkable injury at higher concentrations. In a similar study, the impact of *Datura metel* leaves extract in the pyramidal cells architecture was reported to impact considerably in a dose-dependent decrease in the area, length, width and perimeter of the pyramidal cell of the hippocampus as compared to control group [13].

Impact of *Datura Metel* Leaf Extracts in the Cerebellar Cortex

The animals exposed to the leaf extract from figure 4 above have insufficient (scant) purkinge layer, granular cell layer and molecular layer. More inactive neurons were recorded per high power field and accompanied with vacuolations. Also, as reported in previous literatures, there was atrophy in the axons, cell death and prominent vacuolization in animals treated with Datura extract [7]. These degenerative signs occurred in both male and female treated rats. This also contributed to signs like nausea, head ache, stimulation of the nervous system, dilated pupils etc [34,35]. The study revealed that the datura plant can induce neurotoxicity. The mitochondria and nuclear membranes were damaged in the experimental animals. The study also reaffirmed a dose-dependent toxicity [34,35]. Also exposing adult rats to the smoke of datura leaves provoked changes in histomorphology in some organs [33].

Expression of Neurofilament Proteins and Neuro-specic Enolase.

Animals in the control group (figure 6 above) showed no expression of Neurofilament proteins (NFP), whereas high dose (600mg/kg) showed marked expressions. However, the 150mg/kg and 300mg/kg *Datura metel* group had no expression of neuro-specific enolase. At the same time the 600mg/kg *Datura metel* confirmed a moderate demonstration of neuro-specific enolase as shown in figure 7 above. NSE could be a biomarker to estimate neuronal damage in brain leisions [36]. NSE has also been measured in cerebrospinal fluid of rats after injection of kainnic acid, a glutamic kainite receptor agonist that induces excitotoxic neurone death at high concentrations [37,39,40]. Measurement of NSE could be a sensitive index of neuronal damage. Interactions of NSE with many other nuclear, cytoplasmic, or membrane molecules in the CNS also raise the possibility that NSE is associated with neuronal damage [38,41,42].

Conclusions

This study has established some of the impacts of hydroethanolic leaf extracts of *Datura metel* in adult rats. Neuronal degenerations in adult rats confirmed the neurotoxicity of *Datura metel.* The hippocampus and cerebellar cortex showed neurodegenerative signs including diminished neuronal cells, increased neurofilament

proteins and neuro-enolase enzymes. Many people consume herbal medicines without prescription from a medical doctor or pharmacist. Taking into account some of the toxic effects of *Datura metel* leaf on the cellular components of the hippocampus and cerebellar cortex in rats, people should be well guided on the use of the plants, especially given the negative effects it has in the hippocampus and cerebellar cortex as shown in experimental animals used in this study.

Declarations

Conflicts of interest

None

References

[1] Damilare A, Adekomi AA, Tijani OK. Some Effects of the Aqueous Leaf Extract of Datura Metel on the Frontal Cortex of Adult Wistar Rats (Rattusnorvegicus). *European Journal of Anatomy*. 2010;14 (2): 83-89.

[2] Adegoke SA, Alo LA. Datura Stramonium Poisoning in Children. *Nigerian Journal of Clinical Practise*. 2013; 16: 116-118.

[3] Meenakshi S, Inderpreet D, Kusum R, Anil K, Delta PK. Phytochemistry, Pharmacology, and Toxicology of Datura Species - A Review. Antioxidants. 2021: 10, 1291. https://doi.org/10.3390/antiox10081291.

[4] Krenzelok EP. Aspects of Datura Poisoning and Treatment. Clinical Toxicology (Phila). 2010; 48: 104-110.

[5] Ahmet KO, Ekrem G, Nihal K, Ali RN, Yalçın G. Datura Stramonium Poisoning in a child. *The Turkish Journal of Pediatrics*. 2015; 57: 82-84.

[6] Priyanka S, Anees AS, Jawa D, Vishal S. Pharmacological Properties of Datura Stramonium L. as a Potential Medicinal Tree: An Overview. *Asian Pacific Journal of Tropical Biomedicine.* 2012; 2: 1002-1008.

[7] Peter E, Ekanem R, Ekanem KG. Histological Patterns of Neurodegeneration of Frontal Cortex Neurons in *Datura stramonium* Treated Wistar Rats. *Journal of Behavioral and Brain Science*. 2016; 2. DOI: 10.4236/jbbs.2016.62009

[8] Das S, Puneet K, Basu SP. Phytoconstituents and Therapeutic Potentials of Datura Stramonium. *Linn. Journal of Drug Delivery & Therapeutics*. 2012; 2: 4-7.

[9] Davis CA. Commentary on the Associations Among 'Food Addiction', Binge Eating Disorder and Obesity: Overlapping Conditions with Idiosyncratic Clinical features. Appetite. 2016;115: 3-8. http://www.jddtonline.info/index.php/jddt/article/viewFile/141/79

[10] Maibam RD, Meenakshi B, Paul SB Sharma GD. Characterization of the Toxic Effects Induced by Datura Stramonium Leaveson Mice: A Behavioral, Biochemical and Ultrastructural Approach. Asian *Journal of Pharmaceutical and Clinical Research*. 2012; 5: 143-146. http://www.ajpcr.com/Vol5Suppl3/1188.pdf

[11] Setshogo MP, A Review of Some Medicinal and or Hallucinogenic Solanaceous Plants of Botswana: The Genus Datura L. *International Journal of Applied Research in Natural Products*. 2015; 1:15-23.

[12] Mukhtar Y, Tukur S, Bashir RA. An Overview on Datura stramonium L. (Jimson Weed): A Notable Psychoactive Drug Plant. *American Journal Natural Science.* 2019; 2: 1-9.

[13] Naik V. Babu KS, Latha J, Kolluru BA. Review on Phytochemical and Pharmacological Activity of Datura stramonium. (Medicinal Plant). *Research journal of Pharmacognosy and Phytochemistry*. 2018; 10: 77-8.

[14] Igben V, Onoriode W, Josiah I, Omogbiya AI, John Chukwuma O, Peter S. *et al*. Datura Metel Stramonium Exacerbates Behavioral Deficits, Medial Prefrontal Cortex, and Hippocampal Neurotoxicity in Mice via Redox Imbalance. *Laboratory Animal Research*. 2023; https://doi.org/10.1186/s42826-023-00162-

[15] Mu S, Yang W, Huang G. Antioxidant Activities and Mechanisms of Poly-saccharides. *Chemical Biology and Drug Design*. 2021;97(3):628-32.

[16] Ogunmoyole T, Adeyeye RI, Olatilu BO, Akande OA, Agunbiade OJ. Multiple Organ Toxicity of Datura stramonium Seed Extracts. *Toxicology Reports.* 2019; 6: 983-989.

[17] Atuadu V, Benneth BA, Oyem JC, Esom E, Mba C, Nebo K. *et al*. Adansonia Digitata L. Leaf extract Attenuates Lead-induced Cortical Histoarchitectural Changes and Oxidative Stress in the Prefrontal Cortex of Adult Male Wistar Rats. Drug Metabolism and Personalised therapy. 2020. https:// doi. org/ 10. 1515/ dmdi- 2020- 0116.

[18] Pepple IA, Nnaemeka JC, Okolie NJ, Godsman CE, Okara NP, Bumaa Joy. The Potential Protective Effects of Ethanolic Extracts of Plantain Root on Diclofenac-Induced Nephrotoxicity and Hepatotoxicity Through Biochemical Analysis. 2024; Volume 04 | Issue 01: https://gjrpublication.com/gjrms/

[19] God'sman CE, Amalachukwu I, Gift O, Ibiere P. The Effect of Rapid Tissue Processing on Fixation Time Using Microwave Techniques. *Journal of Applied Health Sciences and Medicine*. 2023; 3(1): 10-20.

[20] God'sman CE, Amalachukwu OI, Pepple IA. Xylene-Free Histoprocessing using an Alternative Method: Effect of Microwave Temperature". *Healthcare Studies.* 2;(1): 33 - 45

[21] Adam V, Seregi M. Receptor Dependent Stimulatory Effect of Noradrenaline on Na+/K+ ATPase in Rat Brain Homogenate. Role of Lipid Peroxidation. *Biochemical Pharmacology*. 1982; 31, 2231-2236. http://dx.doi.org/10.1016/0006-2952(82)90106-X

[22] Hadwan MH. New Method for Assessment of Serum Catalase Activity. *Indian Journal of Science and Technology;* 2016.

[23] Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The Application and Mechanisms of Superoxide Dismutase in medicine, Food and Cosmetics. *Antioxidants.* 2023; 12(9): 1675.

[24] Habig WH, Michael J. Pabst W, Jakoby B. "Glutathione S-transferases: the first enzymatic step in mercapturic acid formation." *Journal of biological Chemistry*. 1974; 249 (22): 7130-7139.

[25] Saxena A, Lakshmi J, Bhattacharjya R, Singh PK, Mishra B, Tiwari A. The role of Antioxidant Enzymes in Diatoms and their Therapeutic role. InMarine Antioxidants 2023 Jan 1 (pp. 89-118). Academic Press.

[26] Alum EU, Inya JE, Ugwu OP, *et al*. Ethanolic Leaf Extract of Datura stramonium Attenuates Methotrexate-induced Biochemical Alterations in Wistar Albino rats. *RPS Pharmacy and Pharmacology Reports*. 2023 Jan 1;2(1): rqac011.

[27] Nasir B, Baig MW, Majid M, *et al*. Preclinical Anticancer studies on the Ethyl Acetate leaf Extracts of Datura Stramonium and Datura inoxia. BMC *Complementary Medicine and Therapies*. 2020 Dec; 20:1-23.

[28] Hacheked M, Emery TD, Sidiki A, Perfusion AA, Jacqueline ZM. Cardioprotective Effect of the Aqueous Extract of Seeds of Datura Metel (Solanaceae) on acute Cardiotoxicity Induced with Doxorubicin in wistar rats. *GSC Biological and Pharmaceutical Sciences*. 2020;13(3):008-18.

[29] Aruoma OI. Methodological Considerations for Characterizing Potential Antioxidant Actions of Bioactive Components in Plant Foods. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2003 Feb 1; 523:9-20.

[30] McRae J, Yang Q, Crawford R, Palombo E. Review of the Methods used for Isolating pharmaceutical Lead Compounds from Traditional Medicinal Plants. The Environmentalist. 2007 Mar; 27:165-74.

[31] Otimenyin SO. Antiinflammatory Medicinal Plants: a Remedy for Most Disease Conditions?. In Natural Products and Drug Discovery 2018 Jan 1 (pp. 411-431). Elsevier.

[32] Joshua PE, Asomadu RO, Eze CS, Nnamani IV, Kingsley CO, Nweje-Anyalowu CP. Effect of Datura Stramonium on Cyclophosphamide-induced Oxidative Stress in Albino Rats: Study on Kidney Markers. 2019: 926-932.

[33] Akinlolu AA, Shokunbi MT. Neurotoxic Effects of 25 mg/kg/bodyweight of Artemether on the Histology of the Trapezoid Nuclei and Behavioural Functions in Adult Male Wistar rats. Acta Histochemica. 2010 Mar 1;112(2):193-8.

[34] Adekomi DA, Musa AA, Tijani AA, Adeniyi TD, Usman B. Exposure to Smoke Extract of Datura Stramonium leaf: Some of its Effects on the Heart, Liver, Lungs, kidneys and Testes of Male Sprague Dawley Rats. *Journal of pharmacognosy and Phytotherapy*. 2011;3(1):6-12.

[35] Parrotta JA. Healing plants of peninsular India. CABI Publishing, Wallingford and New York. 2001 Sep 17.

[36] Devi MR, Bawari ME, Paul SB, Sharma GD. Characterization of the Toxic Effects induced by Datura stramonium L. leaves on Mice: a Behavioral, Biochemical and Ultrastructural Approach. *Asian Journal of Pharmaceutical and Clinical Research*. 2012;5(3):143-6.

[37] Sahu S, Nag DS, Swain A, Samaddar DP. Biochemical Changes in the Injured Brain. *World journal of biological Chemistry*. 2017 Feb 2;8(1):21.

[38] Scarna H, Delafosse B, Steinberg R, *et al*. Neuron-specific Enolase as a Marker of Neuronal Lesions during Various Comas in Man. *Neurochemistry International*. 1982 Jan 1;4(5):405-11.

[39] God'sman CE, Yibala O, Tatfeng YM. Neurological and Biochemical Impacts of datura metel hydroethanolic Seed Extracts on the Hippocampus and Cerebellar Cortex of Apparently Healthy Adult rats. *Annals of Innovation in Medicine*, 2024;*2*(4).

[40] Yibala IO, God'sman CE. Alteration in Oxidative Stress Enzymes and Proteins by Datura Metel Stramonium Hydroethanolic Root Extract Promotes Hippocampus and Cerebral Cortex Neurons Damage in Adult Sprague Dawley rats. *Substance Abuse and Rehabilitation*. 2025;16: 13-21

[41] Clement K. Okpora, God'sman Chidugam Eberechukwu, Teme Raphael (2022). A comparative Study on the use of Conventional Tissue Processing Method and Microwave Technique. Publication date; 2022. International Conference on Research and Development, University of Abomey-Calavi, Cotonou, Republic of Benin. 2022.Vol: 17 pg: 83-93.

[42] God'sman Chidugam Eberechukwu. Assessment Of Histological and Histochemical Effects of *Datura Metel* Ethanolic Extracts in the Brain of Adult Sprague Dawley Rats. International Conference and General Meeting of the Association of University Academic medical Laboratory Scientist (AUAMLS), Federal University Otuoke, Bayelsa State, Nigeria. November, 2024; AUAMLS-FUO-2024020, pg 38.