Substance Abuse and Rehabilitation

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ORIGINAL RESEARCH

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

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Background: Datura metel is reported to induce hallucinations and mental disorders.

Objective: This study investigates the neurotoxic effects of *Datura metel* stramonium hydroethanolic root extract on the hippocampus and cerebral cortex of adult rats using biochemical, histological and immunohistochemical techniques.

Methodology: Twenty five adult rats were assigned to 5 groups (n = 5 each). Group A - negative control, group B (lead positive control). Groups C, D, and E exposed to 150 mg/kg, 300 mg/kg and 600 mg/kg body weight of extracts for 14 days once daily, respectively. Histology, biochemistry and immunohistochemical techniques were used to study cell injury in the brain tissue.

Results: Biochemical alterations were observed in superoxide dismutase (SOD), Malondialdehyde (MDA), Glutathionine S-transferase (GST) and Catalase among the experimental groups. Catalase was statistically significant at $P<0.05$. Histology reveals neurons damage, depletion and vacuolation. NFP and NSE were over expressed in the experimental groups.

Discussion: Oral administration of *Datura metel* root extracts at high concentration alters the antioxidant enzymes activity and body weight. Extracts cause cortex and hippocampus neurotoxicity through heightened oxidative stress.

Conclusion: *Datura metel* root extract is a neurotoxic agent and causes depopulation of hippocampus and cerebral cortex neurons. The use of this plant should be highly regulated to reduce neuropathies associated with consumption.

Keywords: *Datura metel*, oxidative stress, neuronal damage

Introduction

The abuse of herbal plants poses several burdens across all nations, race, age, and socioeconomic status.[1] Drug abuse in adolescence is a known health problem across the globe, particularly among handicaps,[2,3,] an etiologic factor of neurodegenerative disorders in adulthood.[4] Approximately 50% of drug users worldwide live in Africa,[5] as reported by the UN Office of Drugs and Crime.[6] Though the exact figure may be difficult to predict. This is because certain regions in Africa cannot be reached due to poor road network, and the inadequate record keeping of drug use and abuse throughout the continent.[6] Recently addicts are forced to import novel psychoactive drugs (NPS) from plants, due to tight laws prohibiting the usage of heroin, amphetamines, and cannabis.[6] The most common NPS among Nigerians youths is *Datura metel* plant extracts (roots, seeds, leaves, and stem bark). Tropane alkaloids present in *Datura metel* have been shown to induce hallucinations and metabolic disorders in rats[7] and disorders of hippocampus development in rats.[8] There have been reports of unintentional

Received: 2 October 2024
Accepted: 21 January 2025
Published: 25 January 2025

Datura metel poisoning in some households.[1,9,10] A case study of a family that made a revitalizing drink using a product bought from an apothecary (made from dried leaves and stems of *Datura metel)* recorded drug poisoning within five minutes of its consumption. Despite the potential benefits of herbal remedies, this inadvertent poisoning highlights the need for close monitoring of the plant distribution[11–13.] Human and animal studies have shown high levels of toxicity and poisoning of *Datura metel*[14,15,] with some tribes of Nigeria presenting with hallucinations, restlessness, and heavy falls after consuming the seeds of *Datura metel*.[16] In addition, agitation, confusion, and mydriasis were recorded in children who consume the seeds.[17] According to Maharazu et al,[18,19] the fundamental role played by the cerebellum in motor coordination and learning has long been known. However, since the Pharmacokinetics and pharmacodynamics of medicinal plants in humans is not yet fully understood, the mechanism on how *Datura metel* causes brain disorders was studied using standard techniques.

Materials and Methods

The current study used tissue homogenization assays (biochemical), histology, and immunohistochemistry markers to study the effects of *Datura metel* root extracts on the hippocampus and cerebral cortex of adult rats.

Collection and Processing of the Plant

The plant was collected in its entirety in the Amassoma forest in Bayelsa State, Nigeria, in August 2023. The plant was correctly identified and verified by Dr. Godwin Alade of the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University Wilberforce Island, Nigeria, and was assigned a voucher number NDUP/24/02. The fresh *Datura metel* roots was cleaned with water, allowed to air dry, and then ground to fine particles before being subjected to standard Soxhlet extraction. The extraction (hydroethanolic) of the roots was carried out using the methods of Adekomi et al.[7,20] The muggy mass of the extract was collected and kept refrigerated. The dehydrated extract was concentrated to obtain the desired doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg body weight and administered orally.

Dosage Calculation

The dosages were determined using the formula.

Dose = [Weight of Rat/1000] x Dosage. This amount of the extract was weighed and dissolved in 2.4 mL of distilled water and given to each rat in group 3, 4 and 5 according to the OECD guidelines (2001).[21]

Experimental Design

The rats were kept in clean cages at room temperature, with an adequate light source, and were allowed to acclimatize for 14 days and were assigned into five (5) groups. The administration of drug and extract was orally using gastric tube for 14 days once daily.

Group A served as control group and allowed to standard feed and water (negative control).

Group B were given 0.5 mL of 100mg/kg body weight of lead (a known neurotoxicant) (positive control).

Group C rats received 0.5 mL of 150 mg/kg body weight of *Datura metel* root extract (low dose).

Group D received 0.5 mL of 300 mg/kg body weight of *Datura metel* root extract (medium dose).

Group E received 0.5 mL of 600 mg/kg body weight of *Datura metel* root (high dose).

Ethical Approval

The study was approved by the Ethical Committee on Animal Use of the Faculty of Basic Medical Sciences, College of Health Sciences Niger Delta University Wilberforce Island Bayelsa State Nigeria with approval number FBMS/REC/23/A/0053. The voucher number NDUP/24/02 was deposited at the Herbarium of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The OCED (2001) guidelines for animal care was adopted for use in animal care.

Animal Sacrifice

The Sacrifice was carried out on the 15thday. The 2013 guidelines for the euthanasia of animals established by the American Veterinary Medical Association were adopted. Eight milliliters (8 mL) of 100mg/kg body weight of sodium

pentobarbital (PB) was administered intraperitoneally (IP). The rats were allowed to undergo sedation and loss of consciousness before the brain was harvested. The organ (brain) was washed with saline to remove excess blood and fixed in 10% formal saline solution for 72 hrs for histological and immunohistochemical analysis. Brain tissues for oxidative stress study were immediately homogenized and stored for biochemical examination at $4^{°c}$.

Determination of Biochemical Parameters

The brain tissue was rinsed in normal saline and homogenized in 10 mm potassium phosphate buffer (pH 7.4) that contained 30mM potassium chloride. The mixture was centrifuged for 10 min at 1000rpm. The antioxidant activities of Catalase, Superoxide dismutase (SOD), Malondialdehyde (MDA), and Glutathionine S-transferase (GST) were determined. Malondialdehyde (MDA) was determined using the methods of Lucky and Oboma and Adam and Seregi[11,22]. 30% TCA (0.5 mL) was added after 0.4 mL of the homogenate and 1.6 mL of KCl buffer was mixed. After adding Thiobarbituric acid, the mixture was placed in an 80°C water bath for 45 min. Following cooling, the mixture was centrifuged at 3000 g and measured using a calorimeter.

The method described by Sighn[23] was adopted for catalase estimation.19mL of distilled water was combined with 1 mL of homogenate,4mL of hydrogen peroxide and 5 mL of phosphate buffer (pH 7.0). Using a gently swirling motion, the reaction mixture was quickly mixed with 1 mL of the diluted sample at room temperature. At 60 s intervals, 1 mL of the reaction mixture was aspirated and blasted into 2 mL of the dichromate/acetic acid reagent. The Misra and Fridovich method[24] was utilized to assess the activity of superoxide dismutase (SOD). 1 mL of the sample was mixed with 9 mL of distilled water to create 1:10 dilution. To equilibrate in the spectrophotometric, 0.2 mL of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2). Followed by 0.3 mL of freshly prepared 0.3 mm adrenaline to the mixture, swiftly mixed by inversion. The method previously described was utilized to evaluate the activity of glutathionine S-transferase (GST).[25]

Histological Methods

After, The brain tissue was fixed in 10% formal saline for 72 hours sectioned, and stained with hematoxylin and Eosin technique using the methods of [26–28]

Hematoxylin and Eosin Method

This procedure involved dewaxing, where the tissue slides were placed in hot oven at 65°C for 1 min and placed in 2 changes of xylene. Slides were drained and hydrated by passing through decreasing concentration of alcohol baths (100%, 90%, 80%, 70%) and then water. Then slides were stained in Ehrlich hematoxylin bath for 15 minutes. Washed in running tap water until sections were clean for 2 minutes. Then the tissue section (slides) were dipped in 1% acid alcohol (1% HCl in 70% alcohol) for a few seconds for differentiation and rinsed in running tap water, blued in Scott tap water for 2 min. Counter-staining was done using 1% Eosin for 1 min. Then washed in running tap water for 1 min. Dehydrated by increasing concentration of alcohol. Tissue slides were cleared in two changes of xylene and mounted using DPX. Slides were examined under a high-resolution microscope (Olympus BX60MF, Japan), and photomicrographs were taken at a magnification of x400.

Immunohistochemistry

Immunohistochemical staining was performed for neuronal filament proteins (NFP) and neuron-specific enolase (NSE) using the method described by Lucky and Oboma.[11] The identification of the antigen in the formalin fixed paraffin embedded (FFPE) tissues was carried out using the above stated antibody. The antigen and antibody complex was achieved using an enzyme coupled (HRP) secondary antibody with specific lock to the primary antibody. The complex formed was then visualized by the enzymatic activation of a chromogen resulting in a visible colored reaction product at the antigenic site. Each and each step involves precise time and optimal temperature and the results are interpreted using a light microscope.

Heat retrieval under steam pressure for 15 min using EDTA (pH 8.0), for optimal retrieval of the epitopes and solution was allowed to cool at the room temperature. Endogenous peroxidase was blocked using 3% hydrogen peroxide

for 15 mins. The slides were transferred to the primary antibody at room temperature followed by the secondary antibody. Colour was developed using DAB chromogen for 10 mins and counter stain with hematoxylin for 3 min. Brownish colouration was indications of over-expression and reported as positive result when compared with the control.

Statistical Analysis

All statistical analysis was performed using SPSS soft ware. Unless otherwise specified, results were expressed as mean standard deviation (SD). Regression analysis was used to compare group data. A p value <0.05 was considered statistically significant.

Results and Discussion

Effect of Datura metel Hydroethanolic Root Extracts on Body Weight

There was reduction in body weight in all experiment groups though not statistically significant at <p.005 (before and after treatment with extract) see Figure 1. Alkaloids have striking pharmacological effects on body weight[29–31]. The impact observed in weight (Figure 1) may also be linked to the presence of alkaloids in *Datura metel*. The observed reduction in the body weight of animals might be due to changes in the biochemical parameters observed after extracts was administered. Biochemical analysis for markers of oxidative stress is presented as the Mean ±SD. Regression analysis showed the degree of impact of the extract on dependent variables such as SOD, Catalase, MDA, and GST.

Effect of Hydroethanolic Root Extracts of Datura metel on SOD

Superoxide dismutase (SOD) transforms radicals into safe molecules, shielding cells from the damaging effects of oxidative stress. Elevated SOD levels protect against cell injuries and deficiency causes cell injury. Assessment of the effect of hydroethanolic root extracts on SOD revealed that the negative control and lead groups had a Mean ±SD of 8.29 ±0.18 and 5.59±0.59. By contrast, the experimental groups administered with varying doses (low, medium and high) of root extracts showed reduced Mean ±SD values of 7.64±0.57, 7.12±0.54, and 7.01±0.38 compared to the negative group. Regression analysis of the root extract confirmed that a 0.14 increases in SOD accounted for a unit increase in the root extract dosage. However, the root extract had a 7% impact on the tissues ($R^2 = 0.73$; $p = 0.605$), see Table 1. This result disagrees with a study by,[32] which found that at 24 and 48 hours, superoxide dismutase activity in cells treated with Datura extract increased in a statistically non-significant manner compared to control cells.

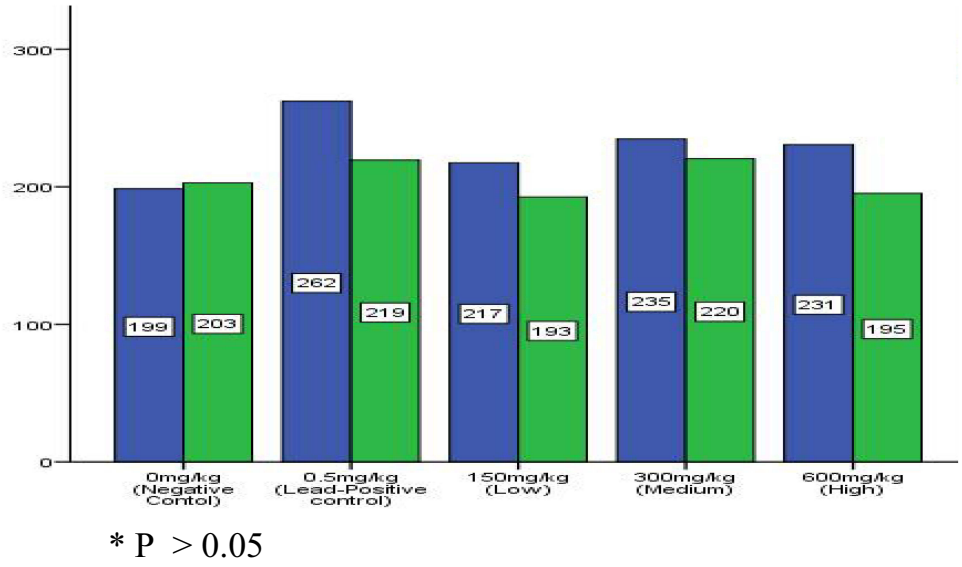

Figure 1 Bar chart showing mean weights of animals before and after extract's administration.
Notes: Weight of animals before extract administration (g) Weight of animals after extract administration (g).

Effect of Hydroethanolic Root Extract of Datura metel on Catalase

Catalase enzymes play a central role in opposing cellular oxidative stress. This action is pivotal for protecting cells against the oxidative damage caused by chronic psychological stress.[33] Elevated catalase activity is usually associated with enhanced protection against cell injury. The normal group showed mean ±SD = 5.56 ± 0.59, lead group = 2.35 ± 0.38, low dose group = 3.17 ± 0.08, medium group 3.17 ± 0.08, and high-dose group = 3.81 ± 0.09. Regression analysis revealed that as the dosage of the root increases, catalase increases by 0.33 units, alongside a size effect of 68%, see Table 1. After Datura metel root extract was administered, a significant dose-dependent drop in the level of catalase was recorded, indicating cell damage. The reduction in catalase levels observed in this study might be due to heightened stress, which results in oxidative stress by contributing to cellular toxicity.

Effect of Hydroethanolic Root Extract of Datura metel on Malondialdehyde (MDA)

Malondialdehyde (MDA) is a biomarker of lipid peroxidation, which is caused by ongoing oxidative stress and toxicity. Lipid peroxidation induced by cellular excitotoxicity causes cyclooxygenase or nitric oxide synthase to produce more free radicals.[34] Elevated MDA levels in tissue indicate oxidative stress and potential cell damage. MDA level was increased in experimental groups exposed to Datura metel extract, MDA level of 13.88±0.08 was observed for the negative control (normal), 28.29 ± 0.24 for lead (neurotoxicant), while the root extract groups for low, medium, and high had 18.89 ± 0.12, 19.45 ± 0.32, and 21.52 ± 0.84 respectively. Analysis showed that a unit increase in the dosage of the root resulted in a 0.31 increase in MDA, with a 19% impact, see Table 1. Similarly, oral administration of *Datura metel* extract increased the levels of MDA and nitric oxide (NO) in mice in a dose-dependent manner as observed in the study by Igben et al.[35] Lipid peroxidation in a significant dose-dependent rise in MDA concentration in the brain of Datura metel treated mice compared to the normal groups was indicated by the observed increase in MDA levels in the rats administered different dosages of Datura metel. Furthermore, Mu et al,[34] reported that elevated MDA levels are risk factors for a number of different brain disorders.

Effects of Hydroethanolic Root Extract of Datura metel on Glutathionine-S-Transferase (GST)

A reduction in GST makes cells more vulnerable to damage, which hinders their defence against oxidative stress. Regression analysis revealed a non-significant decrease. All experimental animals given *Datura Metel* showed a statistically significant dose-dependent increase in oxidative stress and a decrease in antioxidant state.

Effects of Hydroethanolic Root Extract of Datura metel on Histomorphology of the Brain

Microscopic Assessment of the Hippocampus

Figure 2 shows the transverse section of the hippocampus tissue showing the CA3 neuronal population, dentate gyrus, and pyramidal cells at x100 and x400 magnification, respectively, and a scale Bar of 50um. The administration of *Datura metel* resulted in a significant dose-dependent increase in the inter-cellular distance of the pyramidal cells, with a corresponding significant dose-dependent decrease in the area, length, width, and perimeter of the hippocampus pyramidal cells compared with the control group. Tissue morphology showed that the neuronal population decreased

Table 1 Effect of Hydroethanolic Root Extract of Datura Metel on Biochemical Markers

Parameter	Normal	Lead	Low ($\bar{X} \pm SD$)	Medium ($\bar{X} \pm SD$)	High ($\bar{X} \pm SD$)	B	R^2	P-value
SOD (U/mg)	8.29±.18	5.59±.59	7.64±.57	7.12±.54	7.01±.38	0.137	0.073	0.605
Catalase (U/mg)	5.56±.59	2.35±.38	3.17±.08	3.17±.08	3.81±.09	0.333	0.679	0.044*
MDA (μMol/mg)	13.88±.08	28.29±.24	18.89±.12	19.45±.32	21.52±.84	0.310	0.194	0.382
GST (μMol/mg)	2.05±.156	2.03±.09	2.06±.11	2.03±.16	2.02±.17	0.023	0.029	0.748

Note: *statistical significant P >0.05.

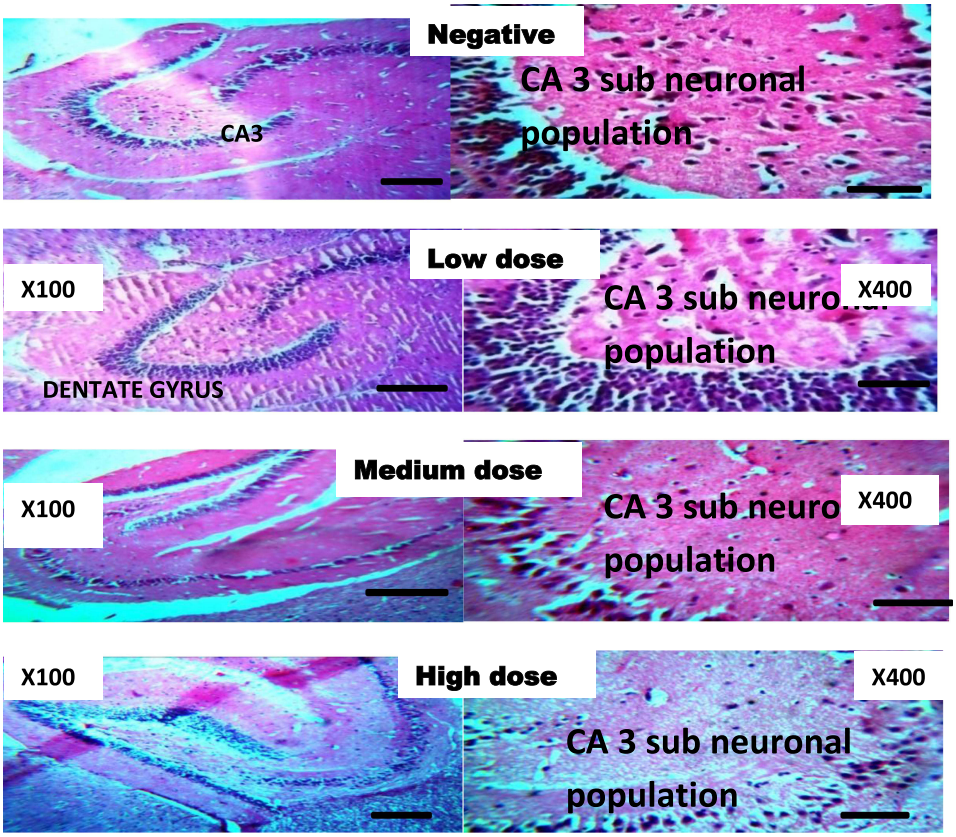

Figure 2 Photomicrographs of the Hippocampus tissue exposed to hydroethanolic root extracts of *Datura metel* and stained with hematoxylin and Eosin (x100 and x400). Scale bar= 50um.

from a low dose to severe degeneration in the high-dose group compared to that in the control group. The root extract caused severe neuronal damage at higher concentrations. The effects of the leaf extract on the morphology of hippocampus pyramidal cells have been documented by Igben et al.[35] Rats that received the highest dose of *Datura metel* showed a higher increase in the inter-cellular distance of the pyramidal cells with a corresponding decrease in the area, length, width, and perimeter of the hippocampus pyramidal cells compared to the control group.

The administration of *Datura metel* resulted in cellular hypoplasia, loss of dendritic arborization, and neuronal degeneration in the frontal cortex and hippocampus regions. Furthermore, we noted a noteworthy reduction in the number of neurons in a dose-dependent manner. Neuronal degeneration in the hippocampus pyramidal cells was observed in the hippocampus of rats given oral Datura metel. Furthermore, cells exposed to Datura metel exhibit lysis,[35] pyknosis, and a loss of cellularity. The current result also supports a prior study[8] that found hippocampus impairment in foetus exposed to ethanolic Datura metel leaf extract. This study demonstrates that Datura metel exposure results in neurotoxicity, which alters the hippocampus neuronal structure and function. In a behavioral context, Igben et al[35] investigated the effects of graded oral administration of Datura metel methanolic extracts on memory and cognition. They also studied the neurotoxicity of Datura metel in the medial prefrontal cortex and hippocampus of mice. According to the study, oral administration of Datura metel caused neuronal degeneration in the medial prefrontal cortex and the hippocampus, along with a loss of dendritic arborizations and an increase in cell quantity (hypoplasia) as shown in Figure 2.

Microscopic Examination of the Cerebral Cortex

The Purkinje layer of the normal group in Figure 3 shows active Purkinje cells per high-power field. The granular cell layer exhibited an even cell distribution. Conversely, photomicrograph of the cerebellar cortex from the low-dose (150 mg/kg) *Datura metel*. The histology shows a granular cell layer, molecular layer, and Purkinje layer. The

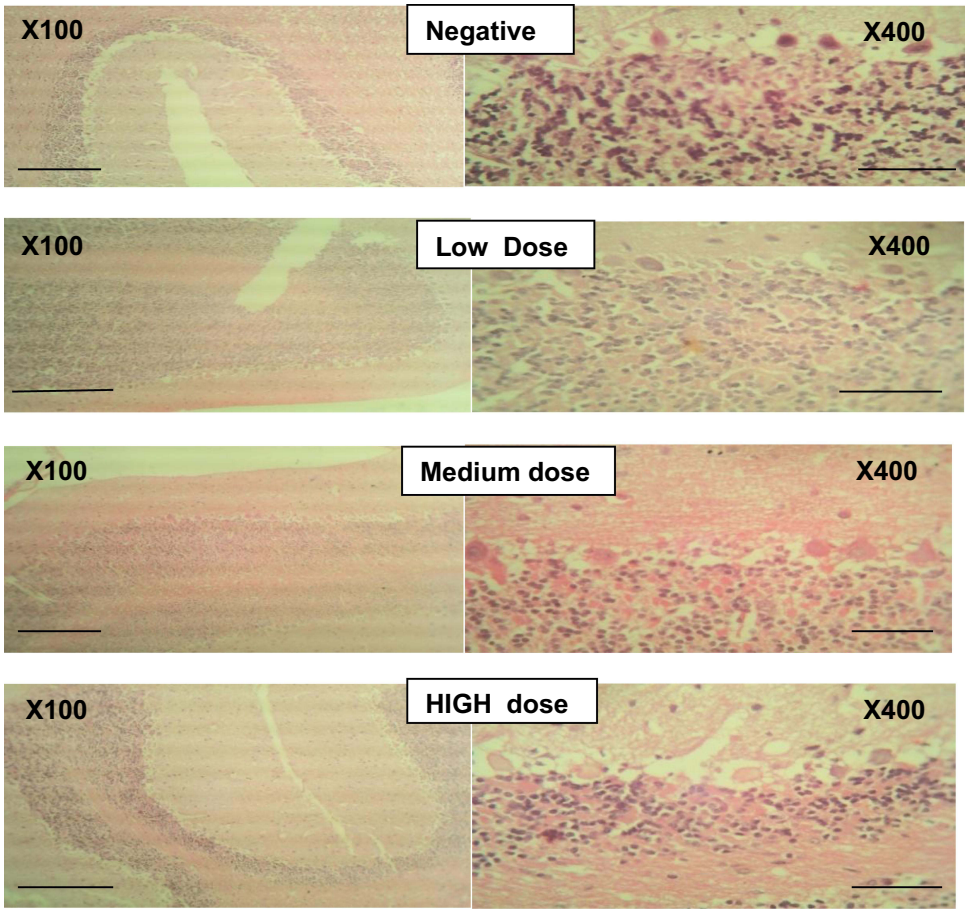

Figure 3 Photomicrographs of the cerebral cortex tissue exposed to hydroethanolic root extracts of *Datura metel* and stained with hematoxylin and Eosin (x100 and x400). Scale Bar 50um.

Purkinje layer displayed active Purkinje neurons per high power field. The granular cell layer showed an even distribution of cells, similar to that of the molecular layer. Plate 3 represents the cortex of rats exposed to (300) extract showed less active Purkinje neurons and neuronal cell death per high-power field with vacuolation. In addition, the granular material proliferated into a molecular layer. Furthermore, the rats were exposed to a high dose (600 mg/kg) of scanty granular cell layer, molecular layer, and Purkinje layer. The Purkinje layer shows damage to Purkinje neurons per high-power field with vacuolation.

Effects of Hydroethanolic Root Extract of Datura metel on Immunohistochemical Markers

Expression of Neurofilament Protein and Neuron Specific Enolase

Immunohistochemical staining for neurofilament proteins as shown in Figures 4 and 5. The photomicrograph represents the normal group, the low, medium, and high doses of hydroethanolic extracts of *Datura metel* root. The findings revealed mild expression of neurofilament proteins in the low group compared with the control group. The medium dose resulted in moderate expression of neurofilament proteins, whereas the high dose resulted in marked expression of NFP. Neurofilament has been found in the cell bodies and proximal axons of damaged neurons in some pathological situations.[36,37] One characteristic of neuronal pathological lesions is NFP buildup. The results of the study showed that, in comparison to the control group, the low group had milder expression of neurofilament proteins. Neurofilament proteins were moderately expressed at the medium dose, while NFP was significantly expressed at the high dose. The expression of neurofilament proteins is an indication of injury and increases from low to high doses as presented in Figure 4. The study findings suggest

https://doi.org/10.2147/SAR.S490392

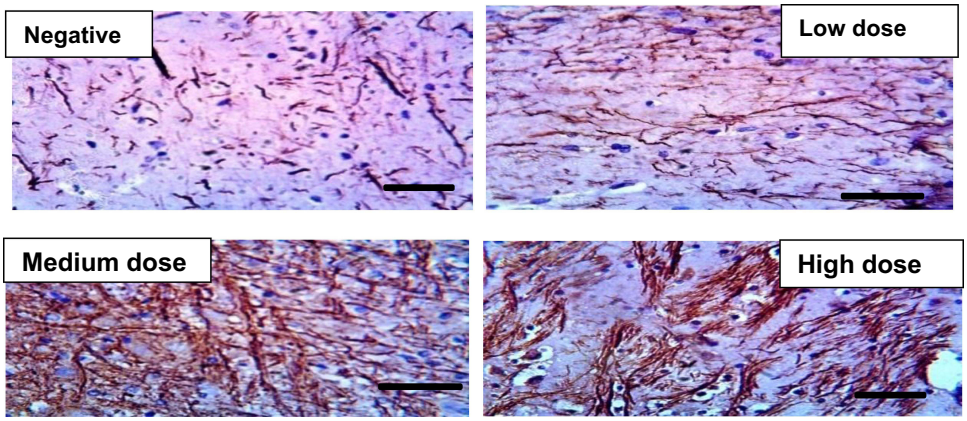

Figure 4 Brain tissue stained with Neurofibrillary protein antibody(NFP) x 400 magnification and Scale Bar of 50um showing positive dark brown deposits.

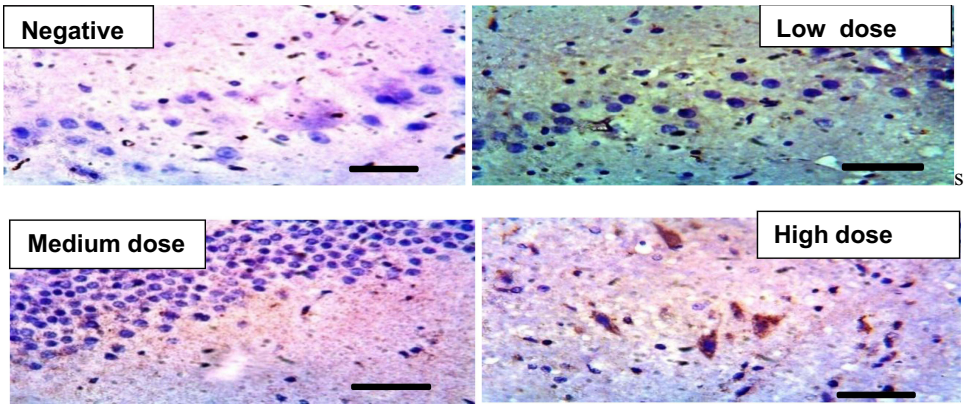

Figure 5 Brain tissue stained with Neurone specific Enolase antibody (NSE) x 400 magnification and Scale Bar of 50um showing positive dark brown deposits.

a dose-dependent increase in neurofilament proteins, which is in line with.[36] The dendrites and cytoplasm of neurons and neuronal endocrine cells contain NSE. The brain neuronal damage indicator NSE is highly sensitive. Neuron-specific enolase may leak from the cytosol into the extracellular space because of modifications in membrane integrity that occur after neuronal damage.[38,39] Figure 5 displays immunohistochemical labeling of neuron-specific enolase in the prefrontal cortex. The dendritic processes are dark brown. Neuron-specific enolase was not expressed in the low- and medium-dose groups and was over-expressed in the high-dose group as shown in Figure 5.

Conclusion

This study assessed the levels of SOD, Catalase, MDA and GST on adult rats exposed to different doses of *Datura metel root* extracts. The results showed that oral administration of *Datura metel* root extracts at high concentrations altered antioxidant enzyme levels, indicating that the body reacts to the elevated levels of oxidative stress. The current study indicates that Datura metel extract causes brain neurotoxicity through increased oxidative stress and changed proteins like neuron-specific enolase and neurofilament filament proteins, though the exact mechanism by which Datura metel root extracts induce neurotoxicity in the hippocampus and medial prefrontal cortex is still unknown. Findings from the hippocampus of rats orally administered *Datura metel* revealed degeneration of neurons in the hippocampus pyramidal cells. The Purkinje layer of the cerebellar cortex exhibits neuronal death and vacuolation. The study findings from Immunohistochemical staining revealed a mild expression of neurofilament proteins in the low dosage group when compared to that of the control group. The medium dose demonstrated moderate expression of neurofilament proteins, whereas high doses showed marked expression of NFP, and neuron-specific enolase (NSE) was moderately expressed in rats exposed-to high doses.

Limitation of Research of Recommendation

This study is limited to crude extraction. Pure extraction of the compounds should be use in future research. Molecular methods should be employ to highlight the molecular and cellular alteration.

Disclosure

The authors report no conflicts of interest in this work.

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