European Institute of Knowledge & Innovation

Annals of Innovation in Medicine (AIM) ISSN: 2977-0335

Research Article

Neurological and biochemical impacts of *datura metel* hydroethanolic seed extracts on the hippocampus and cerebellar cortex of apparently healthy adult rats

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https://doi.org/eiki/10.59652/10.59652/aim.v2i4.320

Abstract:

Datura is a well-known toxic plant, and several cases of death due to Datura intoxication have been reported. It has been documented as a plant with hallucinogenic properties. This study aimed to determine the biochemical and neurological effects of hydroethanolic seed extracts of Datura metel on the hippocampus and cerebellar cortex of adult rats. Twenty-five adult rats were assigned into five (5) groups (A, B, C, D, and E). Group A served as the negative control, and B served as the positive control, administered with lead acetate. While groups C, D and E were treated with 150mg/kg, 300mg/kg, and 600mg/kg body weight of the seed extracts. The animals were humanely sacrificed after 14 days of exposure. Haematoxylin, eosin stain, and immunohistochemical staining were carried out for neurofilament proteins (NFP) and neuro-specific enolase (NSE). Brain tissues for biochemical analysis were homogenized, and the level of superoxide dismutase (SOD), catalase, malondialdehyde, and glutathione S-Transferase were measured. Results showed a non-statistically significant increase in SOD, catalase, and GST. However, there was a statistically significant decrease in the level of MDA. Oral administration of hydroethanolic seed extracts of Datura metel in adult rats created changes in the histology of the hippocampus and cerebellar cortex of the rats, such as perineural vacuolation and apparent reduction in neuronal cells. The results of the immunohistochemical investigation point to a dose-dependent increase in NFP, while NSE was markedly expressed.

Keywords: Neurological, biochemical impacts of Datura Metel, neurotoxicological study of Datura, Neurotoxicity

Received: 24 July 2024
Accepted: 20 Oct 2024
Published: 3 Nov 2024

1. Introduction

Substance abuse is rising worldwide, especially among youths (1, 2). Datura plants, though toxic, may also be abused by certain people. The datura plant is also used in traditional remedies and as a sexual enhancer. It has been utilized in Indian traditional medicine (Ayurveda) as a cure since old times. It is utilized in customs and for appealing to the Shiva Master (3). It is generally called "gegemu" or "ewe ikan" by the Yoruba people of Nigeria. It is used for various purposes due to its stimulating properties. It likewise has a large number of purposes, especially in medicine. It is utilized for the therapy of specific ailments like malignant growths, pain relievers, tranquilizers in epilepsy, flu, hack cure, asthma, wound mending, and skin diseases (3). Due to the presence of tropane alkaloids, like scopolamine, hyoscyamine, and atropine, in their blossoms and seeds, Datura has also been used as a poison (4). It was accounted for that there can be a 5:1 poison inconsistency among plants, and a given plant's

harmfulness relies upon the age, area, and the environmental conditions where it is developing. This variety renders Datura strikingly unsafe as a medication (5).

A few instances of death because of Datura's inebriation have been accounted for in articles of medicine. In a few European and Indian nations, Datura is known as a toxic substance used for self-harm and homicide. From 1950 to 1965, the State Substance Research centers in Agra, India, explored 2,778 deaths by ingesting Datura plants (6). Because of the compelling substance of anticholinergic substances, inebriation with Datura typically creates an outcome like that of an anticholinergic wooziness, hyperthermia, tachycardia, peculiar and plausible forceful way of behaving, and seriously expanded pupils. This is likewise trailed by a throbbing feeling of dread toward the light (photophobia), which can last various days (7).

Researchers and individuals might use or explore these plants for restorative and nourishing purposes. This is a result of their innate bioactive parts, which are valuable to people (8). In many parts of developing nations, individuals significantly rely upon traditional medications based on their socio-cultural norms (8). Further, individuals living in rural areas are more likely to use plant-based remedies due to poor access to healthcare (8).

Datura metel (DM) has a place with the family *Solanaceae*, the nightshade, which incorporates nearly 2,400 species (9). It has been reported as a plant with Stimulating properties, and the alkaloids present in the plant have been popular for herbal and clinical examination (10). The alkaloid content of various pieces of the Datura metel plant has been contemplated, and it was seen that the leaves, organic products, and roots contain around 38 alkaloids (11). Atropine, hyoscyamine, and scopolamine are the fundamental trophane alkaloids (12,13). Likewise, Megastimane, sesquiterpenes, tannins, phobatanins, cardiovascular glycosides, sugars, and flavonoids have additionally been identified in the extracts of the seeds and roots(14).

The stimulating elements of DM are accepted to be because of its high anticholinergic content- atropine. In this way, the daze, hyperthermia, sedation, tachycardia, unusual mental disarray, brutal way of behaving, and amnesia that individuals show as feeling "high" are predominantly anticholinergic impacts (7). DM mostly grows in warm regions of the world, for the most part in Nigeria, India, and Southeast Asia. The plant ordinarily develops anyplace as a weed, making its accessibility limitless. This significantly increases the chances of this plant being misused or mishandled (15). There are earlier investigations (16,17) featuring the impact of the plant on the mind, particularly on the cerebrum, prefrontal cortex, hippocampus, and visual pathways (18).

There has been a report about dose-dependent modifications on the cerebellar cortex and deterioration of the Purkinje cells. This agrees with the discoveries of a few preceding investigations (19). The leaf extracts of Datura metel destructively affected the tissues of the cortex of Wistar rats, which were likewise subject to concentration (10). The progressions seen in tissue morphology included perineuronal vacuolations of glial cells and pyramidal cells, which were demonstrative of looming cell death. There was likewise clear perinuclear dispersing among neurons and an associated decrease in the size of the neurons as assessed utilizing a stereological framework (18). The photomicrograph of the average prefrontal cortex of adult rats treated with Datura metel exhibited dose-dependent features of altered neuronal integrity with chromatolysis, reduced density of Nissl substances, neuronal loss, and injuries on the axon (19).

The developing interest in conventional medication weighs on toxicity risk and assessment of the different local preparations utilized in the treatment of diseases (20), hence the need for the current study. The study aims to evaluate the neurological and biochemical impacts of *datura metel* hydroethanolic seed extracts on adult rats' hippocampus and cerebellar cortex. Specific objectives are to investigate the effect of Datura metel seed extracts on antioxidant enzymes and body weight using regression analysis and descriptive statistics, respectively. Additionally, the effects on neurohistology will be ascertained using general tissue stain and immunohistochemical staining methods.

2. Materials and Methods

2.1 Experimental design

Twenty-five (25) healthy adult rats, weighing 180g to 303g, were obtained from the animal house of Niger Delta University (NDU) Wilberforce Island Amassoma, Bayelsa State. The animals were allowed to acclimatize for two weeks before the extracts were administered. The animals were allocated into five (5) groups: A, B, C, D, and E, each with five animals. The rats in group A served as the negative control, those in group B served as the positive control, while groups C, D, and E were treated with *Datura metel* seed extracts after inducement.

2.2. Ethical approval

Ethical approval was obtained from the ethical committee and Head of the Department of Medical Laboratory Science, Niger Delta University (NDU) Wilberforce Island Amassoma, Bayelsa State, Nigeria.

2.3. Extract preparation

Fresh seeds of the *Datura metel* plant were identified and authenticated at the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria (voucher number NDUP/24/02). Fresh seeds were dried in the hot air oven at 40^{o}C for a period of ten (10) days. Dried seeds were ground into powder using a metallic pestle and mortar weighing 255.42g. The minced seed material was weighed using an electronic weighing balance and soaked in 50% ethanol for 72 hrs. This was followed by shaking at intervals for 72 hours, then drained and sieved with filter paper. The substance was concentrated with a rotary evaporator that uses a vacuum to lower the boiling point of ethanol. The rotary evaporator consists of a condenser, water bath, chiller, concentrating flask, and solvent-receiving flask. The rotation of the concentrating flask at 30^{o}C permits heat circulation, which enables the evaporation of the extract into the condenser as vapor. The vapor was then reconverted to liquid into the receiving flask.

2.4. Animal treatment

Group A rats were given normal feed and water; group B was administered with lead while group C, D, and E were treated with seed extracts of *Datura metel* at the concentrations of 150mg/kg, 300mg/kg, and 600mg/kg body weight, respectively. The oral administration of the extract was for a period of fourteen (14) days.

2.5. Brain resection and processing

After anesthetizing the animal, the brain tissues were harvested, weighed, and transferred immediately to a 10% formal saline fixative. Tissues were subjected to routine tissue processing methods as adopted by (21,22). Brain tissues were dehydrated from low-grade alcohol to absolute alcohol, cleared in xylene, and impregnated with molten paraffin wax. The tissues were embedded using a tissue-tek embedding mold. Sectioning was also carried out using the rotary microtome (23).

2.6. Staining and microscopy

Tissue sections were stained using hematoxylin as adopted (24, 25). Sections were deparaffinized in two (2) changes of xylene and hydrated through descending grades of alcohol. Sections were stained with Harris hematoxylin 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 dibutylphyltalate polystyrene xylene (DPX). Tissue slides were then viewed under the microscope at ×10 and ×40 objective lenses.

2.7. Determination of antioxidant enzymes

After the brain tissue was cleaned with normal saline, 10 millimolar (mM) potassium phosphate buffer (pH of 7.4) containing 30mM potassium chloride was added. Following homogenization, it was centrifuged at 1000 rpm for 10 minutes. The biochemical (antioxidant) activities of glutathione S-transferases (GST), superoxide dismutase (SOD), malondialdehyde (MDA), and catalase were then measured using the supernatants.

Using a modified method (26) to determine malondialdehyde (MDA). A solution of 1.6 ml KCl buffer and 0.5 ml 30% TCA was added to 0.4 ml of the homogenate. After adding the thiobarbituric acid, the mixture was placed in an 80°C water bath for 45 minutes. Following cooling, it was measured calorimetrically and centrifuged at 3000 times the force of gravity (26).

For catalase estimation, 19 milliliters of distilled water were carefully combined with one milliliter of the homogenate. pH 7.0 phosphate buffer and 4 milliliters of hydrogen peroxide make up the test combination. At room temperature, a moderate swirling motion was used to quickly combine 1 milliliter of appropriately the diluted sample with the reaction mixture. At 60-second intervals, 1ml aliquot of the reaction mixture was removed and blown into a 2ml dichromate/acetic acid reagent (27).

The following approach was used to assess the activity of superoxide dismutase (SOD). 1 ml of the sample was diluted ten times by mixing it with 9 ml of distilled water. To initiate the reaction, 0.2 milliliters of the diluted sample were added to 2.5 milliliters of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The mixture was then rapidly mixed by inversion, and 0.3 milliliters of freshly prepared 0.3 millimeters of adrenaline was added. The glutathione-S-transferase (GST) activity was then calculated (28).

3. Results

3.1. Effect of hydroethanolic seed extract of datura metel on biochemical parameters

The Mean±SD of the negative control group, lead, low, medium, and high doses of the seed extract with regard to the concentration of SOD, as shown in Table 1, are 8.29±0.18, 5.59±0.59, 8.76±0.71, 8.76±0.71, and 9.29±0.46 respectively. A regression analysis was carried out on the concentration of SOD in the animals exposed to the seed extracts of *Datura metel,* which revealed B = 0.26 and R^2 = 0.182. Impliedly, a unit increase in the dosage of the seed will result in a 0.2 increase in the concentration of SOD of the study groups exposed to the seed extracts of *Datura metel.* In addition, R^2 =0.182 means that the model explains 18% of the variance in the concentration of SOD. See Table 1 for details. The result for superoxide dismutase showed a nonsignificant effect ($p>0.05$).

For catalase, the Mean±SD for the normal, lead, low medium, and high doses of the seed extract of *Datura metel* are shown as 5.56±.59, 2.35±.38, 5.62±.65, 6.08±.035, and 5.94±.36. A linear regression was applied to assess the effect of different doses of *Datura metel's* seed extract on catalase. From Table 1, the R^2 for the regression equation is 0.127, implying that 12% of the variance in catalase was predicted by the dosage of the seed extract. Also, the standardized beta (B) of 0.157 reveals that for every standard deviation increase in the dosage of the seed extract, catalase increases by 0.15. The effect was not statistically significant ($p>0.05$) and, thus, associated with mild cellular damage.

More so, the Mean±SD in the concentration of MDA across the normal, lead, low dose, medium dose, and high dose of *Datura metel* is shown as 13.88±.08, 28.29±.24, 13.09±.09, 12.46±.34, 12.25±.33. The beta coefficient for the regression analysis revealed that for a unit increase in dosage of the seed extract, MDA decreases by 0.4 units, i.e., B = -0.42, and the R square change (R^2) of 0.70 describes that 70% of the change in the dependent variable (MDA) was predicted by the independent variable (Seed). Additionally, this effect also showed statistical significance with p = 0.035 at a 95% confidence interval.

On assessing the mean difference in the level of GST in the subjects administered with the different doses of the ethanolic extract of the seed, the Mean±SD of the normal and lead = 2.05±0.16 and 2.03±.09. While the Mean±SD = 1.99±0.02, 2.05±0.09, and 2.04±0.17 for the low medium and high doses, respectively. Furthermore, the standardized beta (B) = 0.02 with R^2 = 0.05 and p = 0.67. The analysis indicates that a unit increase in the concentration of the seed could result in a 0.02 increase in GST. Additionally, the R^2 suggests that the seed extract has a 5% impact on the GST level. See the table below for details. The result was not statistically significant ($p>0.05$); however, the administered extract provoked neuronal damage.

Table 3.1: Effect of Hydroethanolic Seed Extract of *Datura Metel* on Biochemical Parameters.

Parameter	Normal	Lead	Low ($\bar{X}$±SD)	Medium ($\bar{X}$±SD)	High ($\bar{X}$±SD)	B	R^2	P-value	Remark
SOD (U/mg)	8.29±.18	5.59±.59	8.76±.71	8.76±.71	9.29±.46	.263	.182	0.398	N/S
Catalase (U/mg)	5.56±.59	2.35±.38	5.62±.65	6.09±.04	5.94±.36	.157	.127	0.488	N/S
MDA (µMol/mg)	13.88±.08	28.29±.24	13.09±.09	12.46±.33	12.25±.33	-.420	.7090	0.035*	Sig
GST (µMol/mg)	2.05±.16	2.03±.09	1.99±.02	2.05±.09	2.04±.17	.023	.048	0.678	N/S

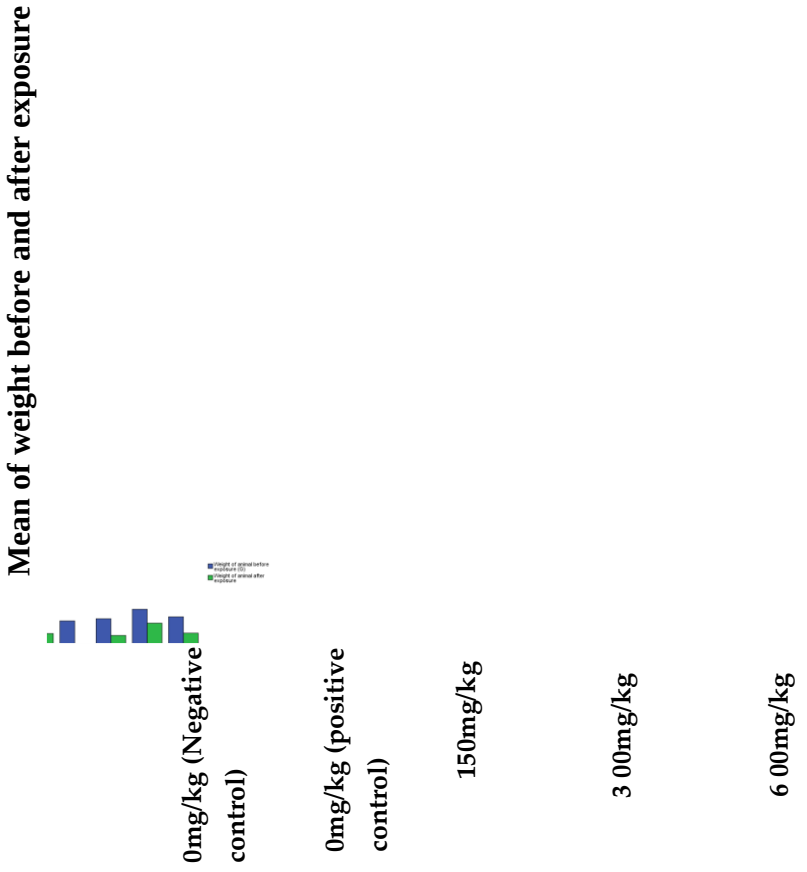

Figure 1: Bar chart showing mean weights of animals before and after extract's administration.

Key: Weight of animals before extract administration (G)

Weight of animals after extract administration (G)

a. Histomorphological evaluations on the hippocampus

photomicrographs of brain tissues exposed to hydroethanolic seed extracts of *Datura metel* stained with hematoxylin and eosin are shown in plate 1. Photomicrograph of the transverse section of the hippocampus tissue shows the CA3 neuronal population, dentate gyrus, and pyramidal cells stained with hematoxylin and eosin at x100 and x400 magnification, respectively. The neuronal population decreases from moderate dose to severe degeneration in the high-dose group. The substance administered showed neuronal damage at higher concentrations.

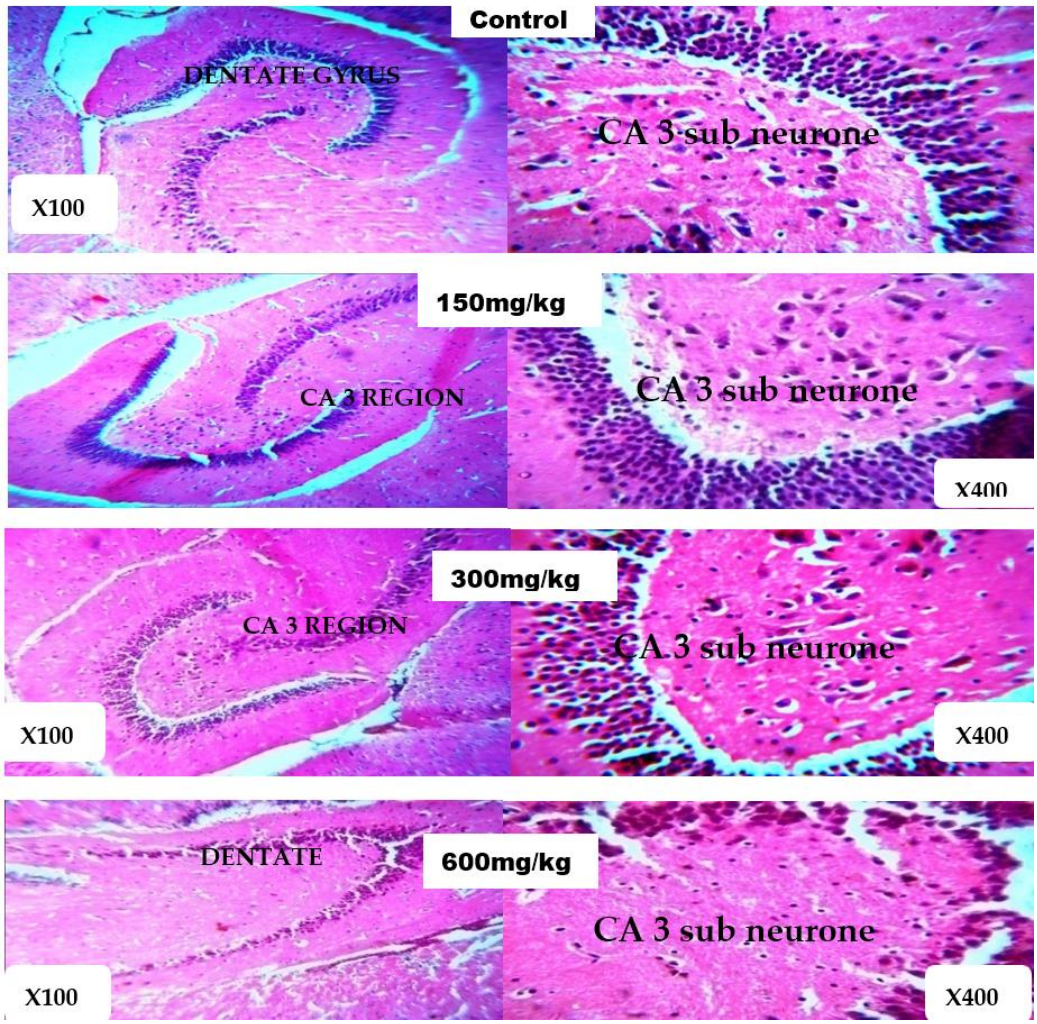

Plate 1: Photomicrographs of brain tissues (hippocampus) exposed to hydroethanolic seed extracts of Datura metel and stained with hematoxylin and eosin at x100 and x400. The neuronal population decreases from moderate dose to severe degeneration in the high-dose group.

b . Histological Results from the cerebellar cortex

Plate 2 is the cerebella cortex of normal rats, showing the granular cell layer, molecular layer, and Purkinje layer. The Purkinje layer shows five active Purkinje cells and one death per high-power field. The granular cell layer shows an even distribution of cells. Conversely, in the histology of the cerebella cortex of animals exposed to *Datura metel* seed extracts, histology shows an eosinophilic granular cell layer, molecular layer, and Purkinje layer. The Purkinje layer shows **1** active Purkinje neuron and **4** deaths per high-power field. The neurone shows

vacuolation. There is a proliferation of granular layer material into the molecular layer. See plate 3 for details.

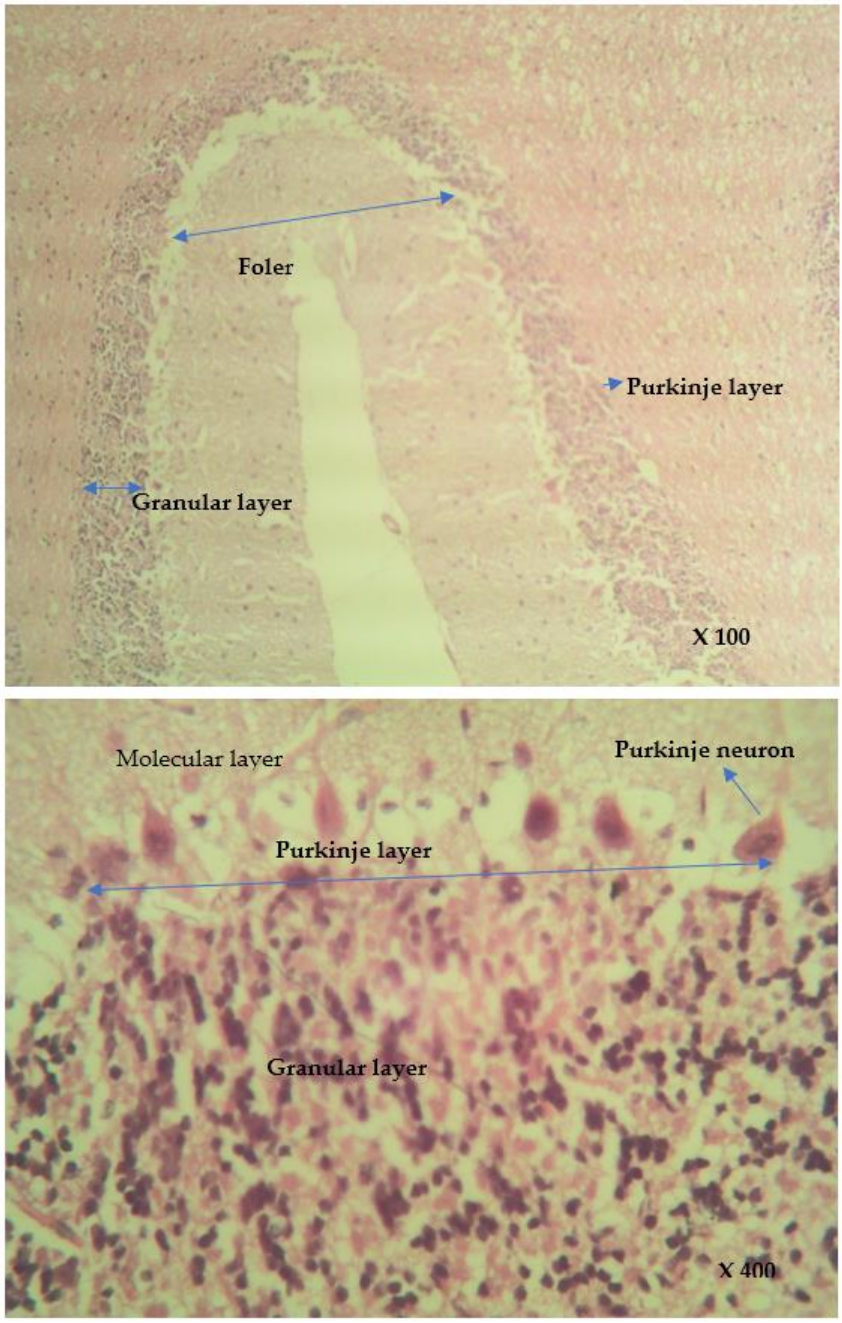

Plate 2: Photomicrograph of the cerebellar cortex from normal rat and stained with haematoxylin and eosin.

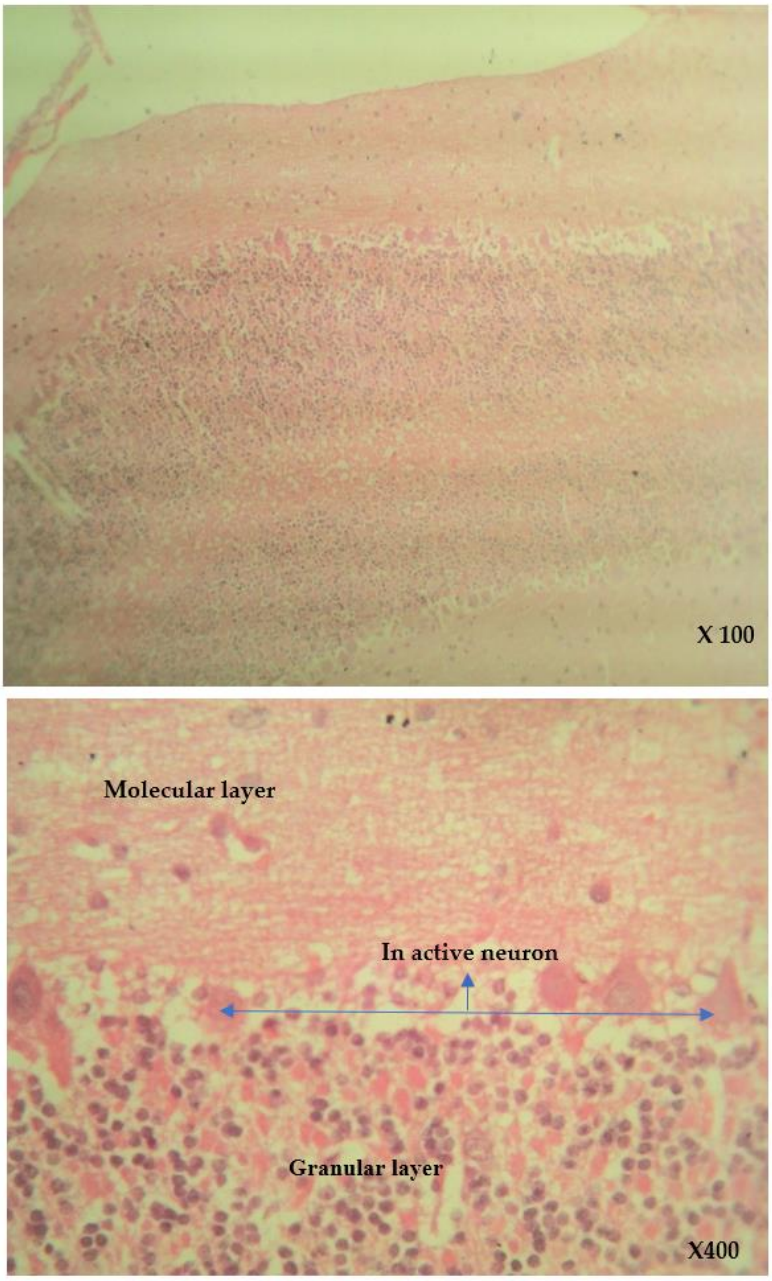

Plate 3: Photomicrograph of cerebellar cortex after exposure to Datura metel and stained with hematoxylin and eosin stain (x100 and ×400). The Purkinje layer shows **1** active Purkinje neuron and **4** deaths per high-power field. The neurone shows vacuolation. There is proliferation of granular layer material into the molecular layer and eosinophilic granular cell layer.

c. Immunohistochemical Expression of Neurofilament Proteins

In comparison to the control rats, the number of neurons declined from low dose to severe degeneration at high dose. Neurofilament proteins are expressed moderately at the medium dose and significantly at the high dose.

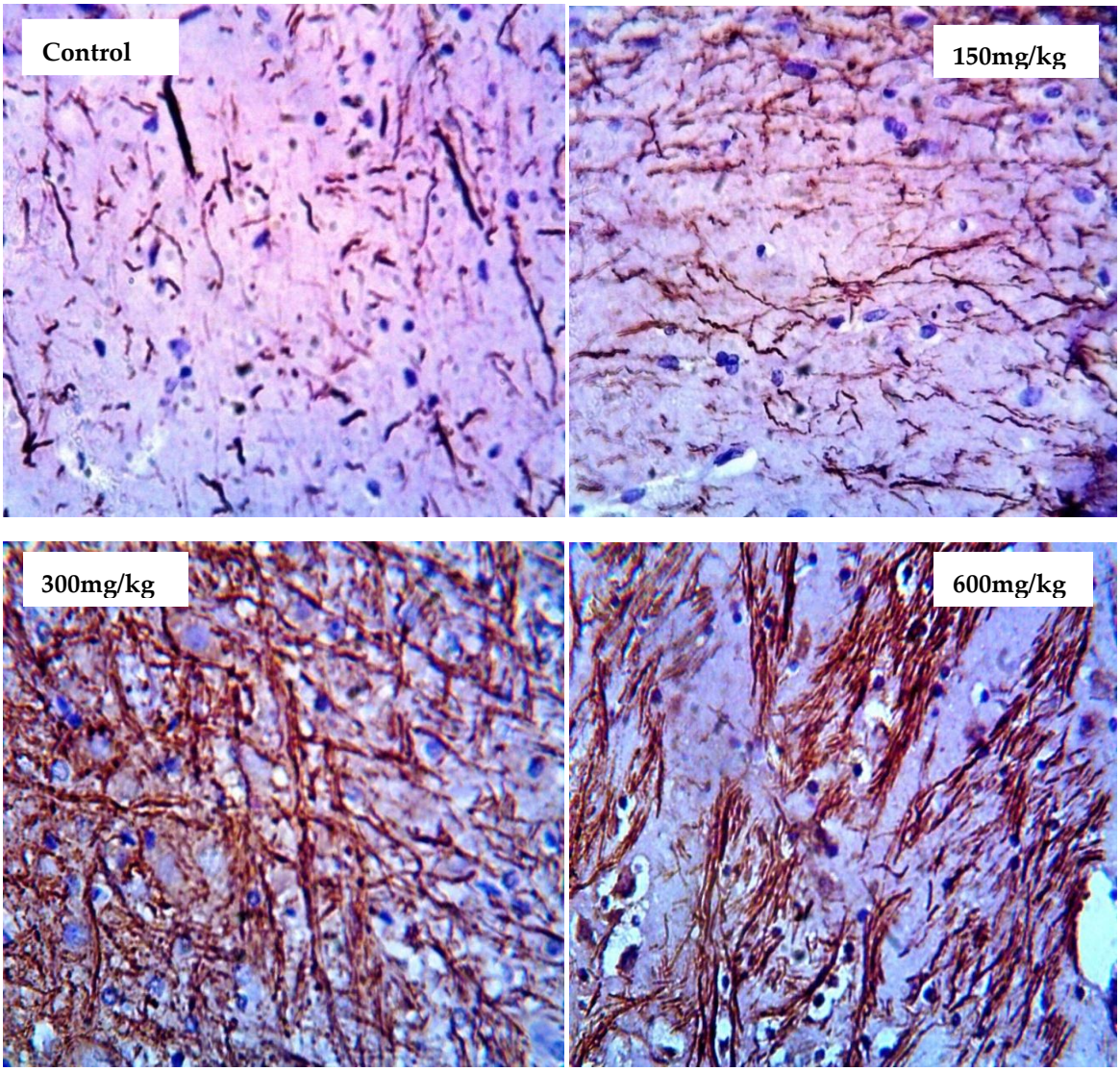

Plate 4: Expression of Neurofilament Proteins. Neurofilament proteins are significantly expressed in the medium-dose and high-dose groups.

3.2. Immunohistochemical Labelling of Neuron-specific Enolase (NSE)

The photomicrographs in plate 5 below demonstrate how neuron-specific enolase was expressed in the adult rats prefrontal cortex. The brown stain is present in the dendritic processes. There was no expression of NSE in the animals exposed to the low dose and medium doses. Compared to the control group, those animals exposed to the high dose displayed mild expressions of neuron-specific enolase.

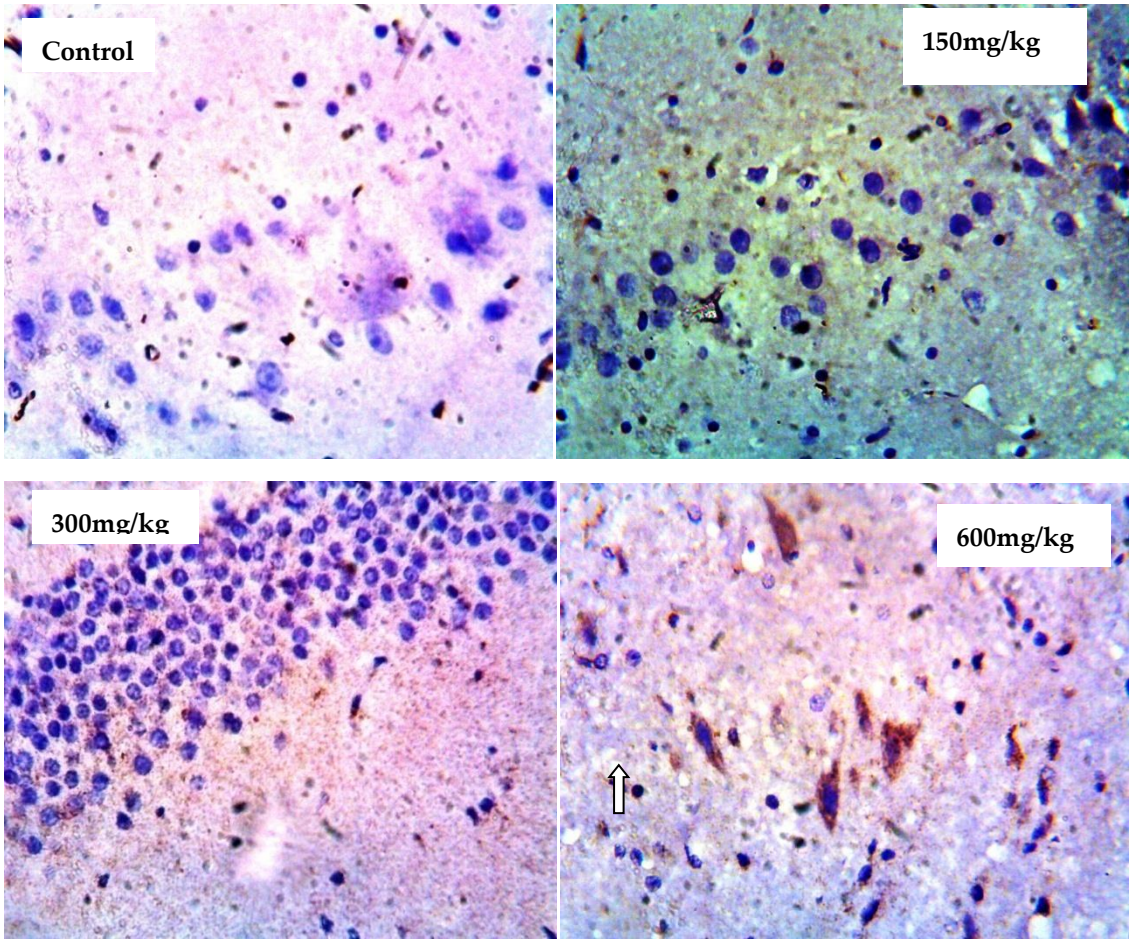

Plate 5: Photomicrograph displaying neuron-specific enolase enzyme (NSE) expression in rats' brain tissue after exposure to varying Datura metel dosages. Moderate expression is shown at high doses, while lost expression is seen at lower concentrations. The expression of the NSE varies with the severity of the injury. Dark brown deposits are a sign of positivity.

4. Discussion

4.1. Effect of hydroethanolic seed extracts of datura metel on superoxide dismutase (SOD)

Objective 1 was to investigate the effect of *Datura metel* seed extracts on antioxidant enzymes using regression analysis. From Table 1 above, the Mean±SD of the negative control group, lead, low dose medium, and high dose of the seed extract in relation to the concentration of SOD are 8.29±0.18, 5.59±0.59, 8.76±0.71, 8.76±0.71, and 9.29±0.46 respectively. A regression analysis was carried out on the concentration of SOD in the animals exposed to the seed extracts of *Datura metel,* which revealed B = 0.26, R^2 = 0.182. Impliedly, a unit increase in the dosage of the seed will result in a 0.2 increase in the concentration of SOD in the subjects exposed to the seed extracts of *Datura metel.* In addition, R^2 =0.182 means that the model explains 18% of the variance in the concentration of SOD. The study findings explain that the concentration of SOD in the brain increases with an increase in the dose of the seed extract. This result also supports other reports that the aqueous extracts of *Datura metel* contain phytochemicals that could increase antioxidant biomolecules during stress (29). In the study, the aqueous extract of the plant displayed antioxidant activity and considered the plant as a natural source of antioxidants in cellular stress (29). Moreover, methanolic and hydroalcoholic seed extracts of *Datura metel* possess slightly high antioxidant activities (30). SOD levels are commonly indicative of the body's antioxidant defense status and are utilized as markers for various disease states and stress. Under conditions of stress, the level of cellular SOD generally increases (31). This situation aims to increase the cells' capacity to cope with oxidative stress to prevent severe damage (32). While this increase is observed as a rapid

adaptive response in acute stress situations, a continuous rise in SOD activity during chronic stress can lead to the depletion of intracellular antioxidant defense systems and potentially to an increase in oxidative damage (33).

4.2. Effect of hydroethanolic seed extracts of datura metel on catalase

The enzyme catalase increases gradually in response to cellular injury, known as oxidative stress. Deficiency or malfunction of catalase, especially in conditions of stress, is postulated to be related to the pathogenesis of many age-associated degenerative diseases like diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia (34).

For catalase, the Mean±SD for the normal, lead, low, medium, and high doses of the seed extract of *Datura metel* is shown as 5.56±.59, 2.35±.38, 5.62±.65, 6.08±.035, and 5.94±.36. A linear regression was applied to assess the effect of seed extracts of 150mg/kg, 300mg/kg, and 600mg/kg of *Datura metel* on catalase. From Table 1, the R^2 for the regression equation is 0.127, implying that 12% of the variance in catalase was predicted by the dosage of the seed extract. Also, the standardized beta (B) of 0.157 reveals that for every standard deviation increase in the dosage of the seed extract, catalase increases by 0.15. This indicates that there was no significant increase in the level of catalase in the group introduced to the ethanolic seed extracts of *Datura metel* with a p-value greater than 0.05.

4.3. Effect of hydroethanolic seed extracts of datura metel on MDA

MDA is an oxidative stress marker that can be used to measure lipid peroxidation. MDA was quantitatively calculated. Malondialdehyde is generated through lipid peroxidation and represents a form of reactive aldehyde. Lipid peroxidation occurs because free radicals oxidize polyunsaturated fatty acids in the cell membrane. MDA is an end product of this process and can indicate oxidative stress in the cell (35). MDA is a marker of oxidative stress, and because it arises from the peroxidation of lipids in cell membranes, it tends to be higher in tissues rich in lipids, such as the brain, liver, heart, and kidneys (32). MDA, as a by-product of the lipid peroxidation of polyunsaturated fatty acids, serves as a reliable indicator of oxidative stress. Its accumulation can occur as a result of an increase in free radicals and ROS within cells. MDA can react with proteins, genetic material, glycoproteins, and other cellular components to form adducts. These interactions can lead to cellular structure and function disruptions, contribute to cellular aging, and are implicated in the pathogenesis of various diseases due to the damage they cause to essential biomolecules (36).

Biochemical assay analysis for markers of oxidative and antioxidant activities showed dose-dependent significant changes in MDA following the administration of the seed extracts. The Mean±SD in the concentration of MDA across the normal, lead, low dose, medium dose, and high dose of *Datura metel* is shown as 13.88±.08, 28.29±.24, 13.09±.09, 12.46±.34, 12.25±.33. The beta coefficient for the regression analysis revealed that for a unit increase in dosage of the seed extract, MDA decreases by 0.4 units, i.e., B = -0.42, and the R square change (R^2) of 0.70 describes that 70% of the change in the dependent variable (MDA) was predicted by the independent variable (Seed). Additionally, this effect also showed statistical significance with p = 0.035 at a 95% confidence interval.

4.4. Effect of hydroethanolic Seed Extracts of Datura metel on glutathione-s-transferase (GST)

It is speculated that this enzyme GST is induced under conditions of oxidative stress as a protective mechanism (37). On assessing the mean difference in the level of GST in the subjects administered with the different doses of the ethanolic extract of the seed, the Mean±SD of the normal and lead = 2.05±0.16 and 2.03±.09. While the Mean±SD = 1.99±0.02, 2.05±0.09, and 2.04±0.17 for the low medium and high doses, respectively. Furthermore, the standardized beta (B) = 0.02 with R^2 = 0.05 and p = 0.67. The analysis shows that a unit increase in the concentration of the seed resulted in a nonsignificant influence on GST. Additionally, the R^2 implies that the seed extract exerted only a 5% impact on the GST level. No significant difference was noted when the controls were contrasted with the treated groups. However, it was reported that the administration of the seed and leaves extracts of the species '*Datura metel*' caused significant ($p<0.05$) decreases in catalase and glutathione

peroxidase, glutathione and malondialdehyde on cyclophosphamide-induced oxidative stress in albino rats compared to the control group (38).

4.5. Effect of Datura metel seed extracts on body weight

Objective 2 was achieved by comparing the mean weight of animals before and after extract administration. A descriptive analysis of the weight of animals conducted after exposure revealed that the weight of animals reduced after exposure to *Datura metel*. Comparing the initial weight before exposure, there was a general decrease in final body weight across the treatment groups. This is in agreement with a previous study that the final body weight of animals exposed to *Datura metel* showed a reduction in weight at the conclusion of the experiment (39).

4.6. Hippocampal observations

The effect of the seed extracts of *Datura metel* on the hippocampus and cerebral cortex was accomplished through hematoxylin and eosin staining, and the architectural details of the hippocampus were assessed. The primary cell type of the hippocampus is the excitatory pyramidal neuron, which incorporates contextual, spatial, and emotional information and relays output to various cortical and sub-cortical structures in the brain (40). Plate 1 is the photomicrographs that represent the control animals and the photomicrographs of the low, medium, and high doses of the seed extract at ×100 and ×400 objective lenses, respectively. The neuronal population of the animals exposed to the seed extracts decreased from the low dose (150mg/kg) to medium (300mg/kg) and high dose (600mg/kg). As compared with the normal, this is indicative that hydroethanolic seed extracts of *Datura metel* could result in neural damage, especially at high concentrations. Similarly, the hippocampal pyramidal cells were characterized by loss of cellularity, pyknosis, and chromatolysis. Also, emphasis was made on neuronal distortion by visualizing the entire neuronal morphology and quantifying the cell body diameter, areas, perimeter, length, width, and distance from the cell bodies. Neuronal distortion in the pyramidal cell bodies was reported, as well as loss of dendritic and axonal arborizations in all treated groups. This loss of dendritic arborizations may impede synaptic transmissions and thus affect memory (41).

4.7. Observations in the cerebellar cortex

Oral administration of hydroethanolic extracts of *Datura metel* was found to induce neurotoxicity in the cerebellar cortex, as observed in plate 3 above. The photomicrograph of the cerebellar cortex of the normal group shows a granular cell layer, molecular layer, and Purkinje layer. The Purkinje layer of the negative control group shows 5 active Purkinje cells and one death per high power field. The granular cell layer shows an even distribution of cells. On the contrary, the histology of the cerebellar cortex obtained from the animals administered with the seed extracts is shown in Plate 3. The Purkinje layer shows 1 active Purkinje neuron and 4 deaths per high-power field. The neurons show vacuolation, and there is a proliferation of the granular layer material into the molecular layer compared with the normal group.

4.8. Expression of neurofilament proteins

Neurofilaments are mostly found in axons, where they are necessary for impulse transmission across axons, axon maintenance, and radial growth of axons during development (42,43). Neurofilaments can accumulate greatly within the cell bodies and proximal axons of afflicted neurons in some pathological situations (44). The Histological plates above display immunohistochemical staining for neurofilament protein expression. The results of the study showed that the low group had milder expression of neurofilament proteins. Neurofilament proteins are moderately expressed at the medium dose and significantly expressed at the high dose. An indicator of damage that rises with dose from low to high is the production of neurofilament proteins. The results of the investigation point to a dose-dependent increase in neurofilament proteins.

4.9. Neuron-specific enolase (NSE) expression

An enzyme called neuron-specific enolase (NSE) is involved in the brain's glycolytic energy cycle. Upon damage, it is released from the neuron. Proteins known as neuron-specific enolases (NSEs) are highly expressed in neurons. Plate 5 displays immunohistochemical

labeling for neuron-specific enolase in the cerebellar cortex. The color of the dendritic processes is dark brown. Neuron-specific enolase was not expressed in the animals, given the low or medium dose of the extracts. When comparing the high dose to the lesser concentrations and the control, neuron-specific enolase expression was moderate at a lower dose. According to (45), NSE is found in the cytoplasm and dendrites of neurons and neuronal endocrine cells. NSE is a sensitive indicator of brain neuronal damage. Changes in membrane integrity following neuronal injury might trigger leakage of neuron-specific enolase from the cytosol into extracellular space. NSE is thought to be more concentrated in the grey matter and less concentrated in the white matter (46). The extract's capacity to induce NSE expression is indicative of neuronal damage.

5. Conclusion

Biochemical analysis on the effect of seed extracts of *Datura metel* showed changes in the levels of SOD, Catalase, MDA, and GST. Administration of the seed extracts of *Datura metel* revealed that a unit increase in the dosage of the seed would result in a 0.2 increase in the concentration of SOD in the subjects exposed to the seed extracts of *Datura metel.* The study findings explain that the concentration of SOD in the brain increases with an increase in the dose of the seed extract. However, there was no significant increase in the level of catalase in the group introduced to the ethanolic seed extracts of *Datura metel* with a p-value greater than 0.05. Also, the study revealed that oral administration of hydroethanolic seed extracts of *Datura metel* brought about a 70% decrease in MDA levels in the brain. The assessment on GST tells that a unit increase in the concentration of the seed resulted in a nonsignificant influence on GST. Also, oral administration of hydroethanolic seed extracts of *Datura metel* to adult rats created changes in the histology of the hippocampus and the cerebellar cortex of adult rats. The study result revealed an apparent reduction in neuronal cells. The results of the study point to a dose-dependent rise in neurofilament proteins. The extract's capacity to induce neurofilament proteins (NFP) and neuron specific-enolase (NSE) expression is indicative of neuronal damage. The histological changes are indicative of degenerative processes in the tissues. *Datura metel* is, therefore, potentially toxic to the hippocampus and cerebellar cortex.

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