Datura metel Administration Distorts Medial Prefrontal Cortex Histology of Wistar Rats

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Abstract

Datura metel is one of psychoactive substances of great ethno-pharmacological significance often abused because of its unrestricted availability, yet, little is known about its mechanisms of action. This work was therefore aimed at assessing the activity of ethanolic seed extract of D. metel on Nissl substances, astrocytes, axonal and neuronal integrity of the medial prefrontal cortex (mPFC) of Wistar rats. Eighteen Wistar rats averaging 175 g were used and randomly assigned to three groups: group A rats (n = 6) were administered extract of 100 mg/kg bw, group B rats (n = 6) received extract of 200 mg/kg bw, and group C-control (n = 6) took distilled water only for 14 days. The results revealed that D. metel is deleterious to the health of Wistar rats at a dose-dependent rate as observed in its actions on the medial prefrontal cortex at 100 mg/kg bw and 200 mg/kg bw. The histological study of the treated Wistar rats exhibited features of disoriented neuronal integrity such as, chromatolysis, reduced protein synthesis due to loss of Nissl substances and nuclei, neuronal loss as well as axonal injuries.

Keywords

D. metel, Nissl Substance, Prefrontal Cortex, Chromatolysis

1. Introduction

Substance abuse is a maladaptive pattern of use of a substance (drug) that is not considered dependent [1]. Substance abuse often includes problems with impulse control and impulsivity. Today, D. metel remains as a psy-
choactive plant of great ethno-pharmacological significance, especially in India, Southeast Asia and Africa [1] [2]. In Africa, D. metel is used for criminal telepathy and initiations. The seeds are also used to poison victims so they can be robbed [1] [2]. In Nigeria, it is abused by adding its decoction/tincture of leaves/fruits to drinks to achieve a “high”, as a substitute for marijuana because it is relatively cheap and readily accessible [3].

All Datura plants contain tropane alkaloids (such as scopolamine, hyoscyamine, and atropine), ylanginhaline A, and five known megastigmane sesquiterpenes, tannins, phlobatanins, cardiac glycosides, carbohydrates and flavonoids, primarily in their seeds and flowers [4]. Due to the anticholinergic substances it contains, Datura intoxication typically produces effects similar to those of an anticholinergic delirium, hyperthermia, sedation, tachycardia, bizarre (mental confusion), and possibly violent behavior, and severe mydriasis, and pronounced amnesia is another commonly reported effect [5].

Excessive doses can cause hallucinations, intoxication, and death. The window of toxic and medicinal dose is quite small. With medium doses, recovery can occur in 12 - 24 hours; however, with loss of memory and confusion that may last for days; no other psychoactive substance has received as many severely negative experience reports as Datura [4]. Children are especially vulnerable to atropine poisoning, and their prognosis is likely to be fatal [6]. D. metel is bitter tasting and is considered as an anesthetic, anti-asthmatic, antispasmodic, anti-tussive, hallucinogenic, and hypnotic [4] [6]. Its dried seeds are considered a more powerful soporific than the leaves.

The medial prefrontal cortex (mPFC) is involved in cognitive and executive functions [7] [8]. It is among those brain regions having the highest baseline metabolic activity at rest and one that exhibits decrease from this baseline across a wide variety of goal-directed behaviors in functional imaging studies [9]. This high metabolic rate and behavior suggest the existence of an organized mode of default brain function, elements of which may be either attenuated or enhanced [9]. Metel has long been noted for its intoxicating and narcotic properties as well as delirious state and death. An overdose causes a violent narcotic poisoning [4].

Hence, extract of D. metel may affect these functional roles of the prefrontal cortex. The psychological effect of Datura metel is well documented, and we have analyzed some biochemical parameters such as LDH, GPX, MDA, and SOD, which showed changes in the levels of the aforementioned bioassays essential for normal neural and glial physiology. We therefore aim to understand if there are histomorphological changes in the neurons and glia. This investigation seeks to understand how D. metel affects health as a neurotoxin or a drug. It will help determine its effects on the histological integrity of nucleic acids, Nissl substances, axonal and astrocyte integrity, which are essential for protein synthesis, and other cellular processes and mechanisms in the neurons and neuroglia of the medial prefrontal cortex. We tested the hypothesis that the ethanolic seed extract of D. metel distorts the histological integrity of the mPFC in Wistar rats.

2. Materials and Methods

2.1. Animals

Male Wistar rats (10 week old, 170 g average weight) were used for the laboratory experiment. They were housed in properly sanitized cages under natural (12 h) light and (12 h) dark cycles at room temperature in the animal holding of the Department of Anatomy, University of Ilorin. The animals were fed with rat pellets (Bendel Feed, Ilorin) ad libitum and water was given daily.

2.2. Extract Preparation

Fresh seeds of D. metel were collected at Ago-Oba, Ilorin, Nigeria. Fresh seeds were shade-dried grinded to powder weighing 102.52 g, and thereafter extracted in a Soxhlet extractor. The final yield (8.825 g) was a dark-brown sticky mass. This was stored in 4°C. A suspension of the extract was made in distilled water and administered orally to the animals with the aid of a metallic orogastric cannula. Extract could not be mixed with food because it is bitter and the rats would not take the food properly. Injection of the ethanolic extract was not used to avoid abscess formation. Orogastic cannula presented the best means to administer the extract for optimal result. The method used was in accordance with the work of Adekomi et al. [3].

2.3. Treatment of Animals

Animals in groups A, B, and C received 100 mg/kg bw/day of the extract, 200 mg/kg bw/day of the extract, and distilled water respectively for 14 days (A & B = treatment groups, C = control). Different animal brains in the
same group were used for the different histological staining due to the small size of the rat’s mPFC. Sectioning was done in the same area.

2.4. Termination of Treatment

After a 14 day treatment period, the animals were sacrificed by cervical dislocation. At euthanasia the brain was harvested and weighed using analytical weighing balance, and the mPFC was dissected under a dissecting microscope. Thereafter, the mPFC was fixed prior to histological processing.

2.5. Tissue Processing for Microscopy

The mPFC was fixed in formo-calcium fixative for Phosphotungstic Acid-Haematoxylin (PTAH)—astrocytes staining, and Ammoniacal Silver solution (MGE)—axons and nerve staining, formosaline fixative for H & E—general morphological study of the neurones and glia, and Carnoy’s fluid for Cresyl Fast Violet (CFV)—Nissl bodies staining and Feulgen DNA (FDNA)—for DNA staining. The mPFC were sectioned at 3 micrometer. Images were captured under NIKON microscope with objective magnifications of ×60 and ×100 (oil immersed), and eye piece magnification of ×10; making the final magnifications ×600 and ×100 respectively.

2.6. Cell Counting and Data Analysis

Image processing, average cell sizes and cell counting were measured using image J. Data was analyzed appropriately using R-studio. Data were expressed as Mean ± SD. Means were compared at 95% confidence interval (α = 0.05), using Welch two sample t-test (due to unequal variances and unequal sample means).

3. Results

Histological Observation

H & E stain is a routine stain used for general cytoarchitecture of a tissue. PTAH stain is used to show glial fibres (astrocyte) and gliosis in the central nervous system. Feulgen stain (FDNA) is used in histology to identify chromosomal material or DNA in cell specimens. DNA stains pink while the background is green. Cresyl violet (CFV) stain is used for the histological staining of neurones in the brain and spinal cord. It is used to demonstrate the Nissl substance in the neurones and cell nuclei.

Using modified Bilechowsky technique of ammonialcal silver impregnation, degenerating neurones in the central nervous system were selectively stained. It stains for nerve fibres, axons, plaque neurites and neurofibrillary tangles. The background appears yellow to brown while the axon, neurofibrillary tangles and plaque neurites were stain black to deep brown. Pyramidal neurones are found in the medial prefrontal cortex.

Figures 1-8 show the cytoarchitecture of the medial prefrontal cortex (mPFC) at day 14 of the treatment. The H & E stain presented a general neurocytology of animals in the treated groups (A & B), which appeared distorted compared to the control group (C). Histology of the animals in group A showed reduction in the density and size of neurones (pyramidal cells), and group B animals exhibited a reduced density but increase in size of neurones i.e. pyramidal cells (Figure 1 & Figure 2, Table 1). There was a significant increase in the astrocyte sizes of group B mPFC compared to control, while neurone sizes in group A animal decreases significantly compared to control.

CFV stain revealed central chromatolysis in group A animals which was pronounced in group B animals (Figure 4 & Figure 5). More so, some anoxic neurones were also observed in group B animals (Figure 3). FDNA stain in Figure 5 & Figure 6 showed lower nuclear density and distribution in groups A and B treated animals compared to control (C) indicating a lower Feulgen reaction. PTAH stain presented a higher astrocytic content in group A animals compared to the control, which contrasts to group B animals with a reduced astrocyte number compared to control (Figure 7, Table 1). The ammoniacal silver stain (MGE) revealed distorted axons in groups A & B animals (Figure 8).

4. Discussion

The sections of the mPFC stained with haematoxylin and eosin (H & E); for general tissue morphology, Cresyl
Figure 1. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. H & E stain. ×600. Black arrow head—pyramidal cells, thick arrow head—neurones, thin black arrow—astrocytes, N—neutrophil.

Table 1. Neurone and astrocyte size (mean ± SD) and number (N) from PTAH and CFV stains using Imaje J (*asteriks indicate that P-value < 0.05, SD—standard deviation).

<table>
<thead>
<tr>
<th>Ptah Group</th>
<th>Count (N)</th>
<th>Total Area</th>
<th>Average Size</th>
<th>% Area</th>
<th>Mean</th>
<th>SD</th>
</tr>
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<tr>
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<td>5907</td>
<td>38.357</td>
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<td>89.158</td>
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<tr>
<td>A</td>
<td>206</td>
<td>6739</td>
<td>32.714</td>
<td>3.215</td>
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<tr>
<td>B</td>
<td>52</td>
<td>5837</td>
<td>112.250*</td>
<td>2.784</td>
<td>112.250*</td>
<td>53.92</td>
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<table>
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<th>Count</th>
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<th>Average Size</th>
<th>% Area</th>
<th>Mean</th>
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<td>53064</td>
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<td>82.387</td>
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<td>365.665</td>
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</tbody>
</table>

fast violet (CFV); for Nissl bodies, Feulgen DNA (FDNA); for deoxyribonucleic acid, Phosphotungstic acid-haematoxylin (PTAH); for astrocytes, and Silver impregnation (MGE); for axonal fibers revealed that oral administration of ethanolic seed extract of *Datura metel* administered to Wistar rats at 100 mg kg bw and 200 mg/kg bw for 14 days is deleterious and may cause reduced protein synthesis, oxidative stress, neuronal injury and astrocytosis, which may lead to loss of cognition. Results obtained from previous study of the biochemical parameters (under review) as well as brain—to body mass ratio, body weight, food and water intake further con-
Figure 2. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. H & E stain. ×1000, oil immersed. Thin white arrow—nucleus, blue arrow head—astrocytes, thin blue arrow—pyramidal cell, N—neutropil.

Figure 3. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. Cresyl fast violet stain. ×600. White arrow head—chromatolysis, black arrow head—neurone, thick black arrow—neurone, thin black arrow—astrocytes, N—neutropil.
Figure 4. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. Cresyl fast violet stain. ×1000, oil immersed. Thin white arrow—nucleus, blue arrow head—astrocytes, white arrow head—chromatolysis, N—neutrophil.

Figure 5. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. Feulgen DNA stain. ×600. Thick red arrow—DNA, N—neutrophil.
Figure 6. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. Feulgen DNA stain. ×1000, oil immersed. Thin red arrow—DNA.

Figure 7. Photomicrographs of the mPFC of groups (A)-(C) after 14 days treatment period. PTAH stain. ×600. Thin black arrow—astrocytes, N—neutrophil.
This study showed from the observation of the histological sections of the treatment groups A and B that oral administration of ethanolic leaf extracts of *Datura metel* brought about central chromatolysis of Nissl bodies (*Figure 3 & Figure 4*). It has been established that chromatolysis occurs as a result of trauma from free radicals production from exogenous agents or factors [10] [11]. Chromatolysis is the dissolution of the Nissl bodies in the cell body of a neuron [12]. It is an induced response of the cell usually triggered by axotomy, ischemia, toxicity to the cell, and cell exhaustion. Neuronal recovery through regeneration can occur after chromatolysis, but most often it is a precursor of apoptosis [12].

The event of chromatolysis is also characterized by a prominent migration of the nucleus to an eccentric position of the cell and an increase in the size of the nucleolus, nucleus, and cell body [13]. More so, histology of the treatment group B (*Figure 4*) presented anoxic neurons. Anoxic neurons are caused by hypoxia, ischemia, and hypoglycemia, thereby, resulting in irreversible neuronal injury. Injured neurons shrink, become eosinophilic due to condensation of mitochondria, and their nuclei become pyknotic. Hence, exposure of animals to *Datura metel* leads to chromatolysis which can result in apoptosis due to release of, or induction of free radicals. Increases in the levels of the bioassays of LHD (marker of tissue injury), MDA (reactive oxygen species marker), SOD (an antioxidant), from our previous study, affirmed the chromatolytic events in the mPFC neurone, neuronal injury, astrocytosis and oxidative stress.

In contrast to the control animals, photomicrographs (*Figure 5 & Figure 6*) of animals treated with *D. metel* demonstrated a reduced nuclear density and Feulgen reaction which was lowest in animals in group B. This showed that the DNA in the treated animals was compromised, suggesting an alteration in its integrity. Nuclear degradation will cause impairment in the normal functioning of the neurons of the mPFC because DNA is required for replication and transcription. Transcription is the first element that initiates protein synthesis and if lost, as observed in *Figure 7*, will to decreased protein synthesis. This result corroborates report of Adekomi *et al.* (2010). MDA forms adduct with DNA, thereby damaging the DNA. Increase in antioxidant level (SOD) was
as a result of increase in ROS (MDA).

Astrocytosis was observed in the histology of the mPFC of the treated animals, groups A and B; that received 100 mg/kg bw and 200 mg/kg bw of the extract which expressed a higher astrocytic content compared to the control (Figure 7). Animals in group B showed the highest level of astrocytic reaction. Astrocytes perform many functions including, nutrient supply and homeostasis of ions to the neurones, and repair and scarring process following neural traumatic injury [14]. Astrocytosis (or astroglyosis) is an abnormal increase in the number of astrocytes due to the destruction of nearby neurones.

The increased number and size of astrocytes in the treatment groups A and B respectively is an indicator of lesions of the neurones of the medial prefrontal cortex. Some plaques were also observed. Amyloid plaques are characteristic of neurodegenerative diseases such as Alzheimer’s disease. They contain mostly insoluble deposits of amyloid-beta peptide and cellular material outside and around neurons. Tangles (neurofibrillary tangles) are aggregates of the microtubule-associated protein tau which has become hyperphosphorylated and accumulate inside the cells themselves [15]. This shows that D. metel possesses neurodegenerative potentials.

Figure 8 depicts the axonal assemblage. The control animals had the highest number of large axonal fibres with a normal alignment which contrasts with the animals in the treated groups A and B with fewer and smaller axonal fibres as well as distortion of alignment that was more pronounced in group B treated animals with the smallest axons. There was manifestation of some plaques in the histology of group B animals (Figure 8). It has been suggested that axonal atrophy is correlated to loss of axonal neurofilaments and chromatolysis of the perikaryon [13]. Loss of oligodendrocytes induces axonal loss and astrogliosis. Hence, the axonal atrophy observed in the treatment groups A and B is an indicator of axonal neurofilaments and myelin loss. It also confirms that the axonal injury initiated central chromatolysis (axonal reaction) since chromatolysis is vague in the control animals.

From our previous study, weight loss was evident in the Datura treated rats. Moreover, food and water intake were lower compared to the control rats. The anticholinergic (specifically, antimuscarinic agents) present in D. metel extract is antispasmodic [4]. This antispasmodic property is responsible for indigestion and constipation in the treated rats. We also showed lower brain-to-body mass ratio in the Datura administered animals. According to Miller et al. (2002), low fluid intake is linked to indigestion, constipation fatigue, and mood swings. The little quantity of food taken by the treated animals followed by little amount of water taken is responsible for constipation and mood swings in the animals.

D. metel has may have effects on the feeding center of the hypothalamus. Leptin (with major receptors in the hypothalamus) plays important role in control of food intake and metabolic rate, as body fat mass increases, so does the concentration of leptin [16] [17]. Also, ghrelin is a major stimulator of appetite in the GIT and this is inhibited by the extract. Hence, leptin and ghrelin are implicated in reduced food intake and weight loss. The brain-to-body mass ratio is a rough estimate of the intelligence of animals; a more complex measurement of intelligence in animals is encephalization quotient [18]. The larger the brain, the more brain weight and space is available for more complex cognitive task. Overall brain size best predicts cognitive function in non-primate humans [19]. Hence, the delirious effect of the ethanolic seed extract of D. metel is could contribute to reduced cognitive function and intelligence.

5. Conclusion

This study has demonstrated the histological distortion of the medial prefrontal cortex of ethanolic seed extracts of Datura metel treated to Wistar rats for a period of 14 days. With consideration to the observed results of D. metel oral administration, it may be concluded that D. metel has deleterious actions on the medial prefrontal cortex at 100 mg/kg bw and 200 mg/kg bw, at a dose-dependent rate. Future research on Datura metel should focus on revealing its biochemical and molecular underpinnings that caused the histological disruption of the medial prefrontal cortex. Moreover, studies should focus on how Datura plant affects the hypothalamic-gut axis.

References


