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Original Research Article

SELECTIVE EFFECTS OF DATURA STRAMONIUM ON THE GRANULAR PARALLEL FIBRES AND PURKINJE CELLS OF THE CEREBELLUM IN WISTAR RATS

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ABSTRACT

Introduction: Datura stramonium (DS) is a tropical ubiquitous shrub which is often used to increase intoxication in some beverages and is also freely used as a hallucinogen. It is a depressant of the central nervous system, yet commonly smoked in like manner tobacco. The present study investigated changes induced by intoxication with DS on the purkinje cells and parallel fibres of the cerebellum in Wistar rats to further elucidate the effects of this drug on cerebellar structure.

Materials and Methods: The study was conducted on both male and female Wistar rats (200-250 g). They were placed into three batches and four groups were derived from each batch, with eight animals per group. Ethanolic dried seed extract of DS was diluted in normal saline and administered intraperitoneally (I.P.) at a dose of 750mg/kg and given to the treatment groups: once in batch 1, twice in batch 2, twelve hourly and thrice in batch 3, eight hourly per day respectively for 4 weeks, while the control groups received an equivalent of normal saline. The rats were euthanized and sections of the cerebellum were histologically processed in all groups. Silver impregnation stain for degenerating axons and neurons was used to elucidate the actions of DS on purkinje cells and the parallel fibres of the cerebellum.

Results: The result of IP administration of DS extract (750 mg/kg) given three times daily to the treated rats showed significant histological changes, which included atrophy of the parallel fibres but no significant changes in the purkinje cells of the cerebellum.

Conclusions: Intoxication of DS seed as a result of excessive ingestion may have a selective degenerative effect on the parallel fibres of the granule cells of the cerebellum while the purkinje cells are spared; the implication being motor dysfunction.

KEY WORDS: Datura stramonium, Cerebellum, Purkinje cells, Parallel fibres, Atrophy, Intraperitoneal.

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INTRODUCTION

Jimson weed is a common weed along roadsides, in cornfields, pastures and waste areas. It is a cosmopolitan weed of worldwide distribution. It has been used in some cultures to treat cough, chest pain, asthma, impotence as well as

diarrhea, and acts as an antipyretic for the treatment of fever. It has also been used to kill parasites [1]. In addition, it has been smoked in like manner as tobacco and used in many cultures worldwide in alcoholic beverages to increase intoxication [2].

The well-known alkaloids in this plant include: hyocyamine, atropine, hyoscine and scopolamine [3]. The toxins in Jimson weed are Tropane belladonna alkaloids which possess strong anticholinergic properties [3]. These act as competitive antagonists to acetylcholine on peripheral and central muscarinic receptors at a common binding site [4]. It has also been documented to contain gamma - 1 - glutamyl - 1- aspartate which is capable of impairing learning [5]. Poisoning results in widespread paralysis of parasympathetic innervated organs [6]. The cerebellum is a part of the brain that participates in many complex functions. It is involved not only in motor coordination and motor learning, but also plays a role in cognitive and affective functions [7]. The dorsal surface is characterized by a series of shallow ridges called folia (singular: folium), which run transversely (from side to side). In addition, there are deeper transverse fissures, revealed by making a sagittal slice through the cerebellum. These divide the cerebellum into 10 lobules. Because of the high density of neurons in its cortex, the cerebellum, which constitutes only about 10% of the total volume of the brain, contains more than 50% of the total number of neurons in the CNS [8].

Purkinje cells are the pivotal neurons of the cerebellar cortex, and the rest of the cortical mechanism serves to control their activity [7]. The axons of the granule cells bifurcate to form the parallel fibers [9]. In turn, each fiber interacts with many Purkinje cells. Because each mossy fiber excites numerous granule cells and the axons of these cells, the parallel fibers, synapse with numerous Purkinje cells, a single mossy fiber can elicit a graded excitatory response in a great many Purkinje cells through the parallel fibres of the granule cells [9].

This connection in the cerebellum can be damaged by injuries, ischemia, hemorrhage, tumors, inflammation, intoxication and inheritedneurodegenerative conditions [10].

Manifestations of cerebellar dysfunction include motor deficits as well as mental and behavioral abnormalities known in humans as cognitive-affective syndrome [9].

DS has been documented to cause acute intoxication in both grazing animals and humans physiologically, who intentionally smoke the seeds of DS or use them in beverages to increase intoxication [11,2]. The exact concentration of specific alkaloids is a major challenge because it varies with species, cultivation, environment, temperature, moisture and storage. Therefore, their range of toxicity is highly variable and unpredictable [12]. Signs of cerebellar dysfunctions include dyssynergia, disequilibrium and other symptoms of intoxication have been reported in excessive consumption of DS seeds [13].

We hypothesized that intoxication, as a result of excessive ingestion of DS, can affect the histology of the purkinje cells and granular cell parallel fibres which form the major excitatory input of the cerebellum, leading to cerebellar dysfunctions. Many works have been carried out physiologically and pharmacologically to describe the effect of this drug but no work has been done on the effect of DS seed extract on the morphology of the cerebellum histologically to observe the effect of DS seed on the purkinje cells and purkinje fibres. Therefore, in the present context this, research is aimed at elucidating the effect of DS extract on the histology of the purkinje cells and parallel fibres of the cerebellum.

The rationale of this study is to educate the general populace of the potential danger in the misuse of this drug and possible legislation by the government to minimize the use of this drug as a way of mitigating its adverse effect on the population.

MATERIALS AND METHODS

Plant Material handling: Datura stramonium seeds were harvested in Samaru, Zaria, Nigeria and identified in the herbarium of Biological Sciences, Ahmadu Bello University and the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, A.B.U., Zaria. The seeds were air dried, extracted with 96.5% ethanol and phytochemical analysis conducted

to identify the chemical composition of the extract. Dragendorff's reagent was used to confirm the presence of the major alkaloids and thin layer chromatography (TLC) was used to identify scopolamine, hyoscine, atropine and hyocyamine as the major alkaloids.

Animal treatments: Adult male and female Wistar rats, weighing 200 – 250 g were used in this study. The animals were obtained from the animal houses of the Anatomy department and the Veterinary Faculty both of ABU, Zaria. The rules and regulations governing animal handling of Ahmadu Bello University were strictly adhered to and the experiment was conducted in accordance to the ethical committee guidelines. A total of 96 experimental animals were randomly assigned to three batches which were further subdivided into four groups of eight rats each: A, B, C and D. Groups B and D were treated with different doses of DS extract. Bania et al. (2004) [13] proposed a pretreatment dose of 7.5 mg/kg atropine equivalent of Datura stramonium seed extract as a protective agent in severe organophosphate toxicity as a safe dose. This dose was increased 10 fold to induce intoxication after establishing the LDso to be more than 1600mg/kg. The rationale for this is its addition to beverages to increase intoxication without determining its safety or percentage composition of its alkaloids. This was used as a reference for the calculation of the treatment dose in each batch of treated rats. Batch 1 received 750 mg/ kg, i.p once daily; batch 2 twice daily and batch 3 thrice daily respectively. Groups A and C served as controls for both male and female rats in each batch and received an equivalent amount of normal saline. The animals were treated for 4 weeks, after which they were sacrificed by decapitation. Brains were carefully removed and the cerebellum dissected and processed histologically.

Tissue processing for microscopy: The brain tissues were dehydrated in different grades of alcohol and cleared in xylene using an automatic processing machine (Shandon Southern Duplex Processor). The tissues were then infiltrated with paraffin wax and blocked in the coronal plane. Serial sections of the blocks were taken at 8µm with (Leitz Wetzlar) microtome. The sections were mounted on glass slides and allowed to dry overnight. The staining technique employed was Glee's ammonia silver impregnation method for neurons and degenerating axons in paraffin sections [14]. The parallel fibres stained black while the purkinje cells stained dark brown. Tissues were then observed under an upright life science microscope (Olympus x1000) with an attached digital camera. Sections of cerebellum were observed at low and high magnification for changes in the purkinje neurons and parallel fibres of the granular cells of the cerebellum.

RESULTS

Plate 1: A coronal section of control male rat cerebellum showing layers of the cerebellum. I: white matter; ii: granular layer; iii: purkinje layer, iv: molecular layer. Silver stain. ×40

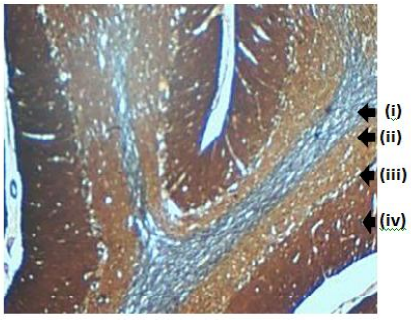


Plate 2: Coronal section of control male rat cerebellum showing purkinje cells (ii) and parallel fibres of granule cells (i). Silver stain. ×400

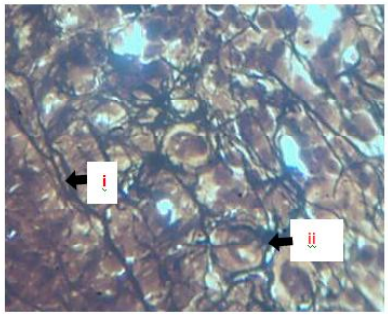
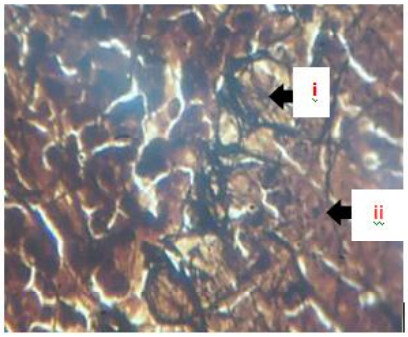


Plate 3: Coronal section of treated batch 3 male rat cerebellum showing axonal atrophy of parallel fibres of the granule cells(ii) of the cerebellum and pukinje cell surrounded by climbing fibes(i). Silver stain. ×400



In batches 1 and 2, male and female treated rats showed no significant histological changes in the granule cells of their parallel fibres and purkinje cells of the cerebellum as compared to the control.

In batch 3 treated groups, there was significant atrophy of the parallel fibres of the granule cells. The fibers appeared markedly reduced in their sizes and fewer in microscopic observations though stereometry conducted to measure the differences between the two sexes.

No signicant histological changes were observed in the purkinje cells when compared to the control groups.

Plate 4: Coronal section of control female rat cerebellum showing layers of the cerebellum. I: molecular layer; ii: purkinje cell layer; iii: granular layer; iv: cerebellar white matter Silver stain. ×400

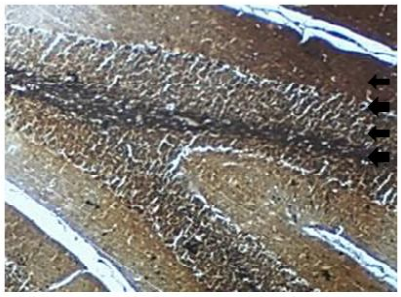


Plate 5: Coronal section of control female rat cerebellum showing parallel fibres of the granule cells(i) and purkinje cells(ii) surrounded by climbing fibre. Silver stain . ×400

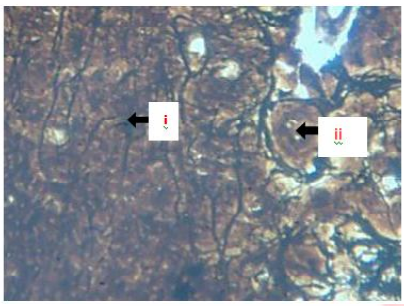
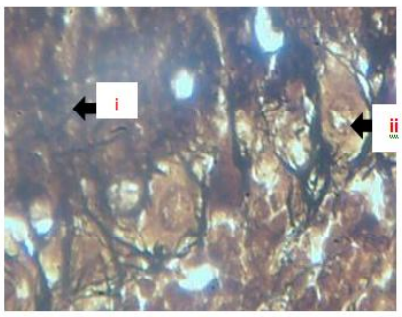


Plate 6: Coronal section of the treated batch 3 female rat cerebellum showing atrophy of the parallel fibres of the granule cells(i) and purkinje cell (ii) surrounded by climbing fibres. Silver stain. ×400



DISCUSSION

Batch 1 and 2 treated rats did not show any significant changes in the parallel fibres of the granular cells and purkinje cells of the cerebellum.

Gladado et al. (2007) [20] carried a study on the effects of acute, subacute and chronic administration of alkaloids atropine and scopolamine, the main constituents of the active principle of *D. stromonium*, with toxic properties, in male Albino Wistar rats. After acute *i.p.* administration of dose 100 mg/kg of total alkaloids in the seeds of *D. stramonium*, there were no remarkable changes in general appearance and no deaths occurred in any experimental group. Bouzidi et al. (2011) [21], conducted a chronic study using synthetic alkaloids administered *i.p.* at daily doses of 4.2 mg/kg of atropine and 1.6 mg/kg of scopolamine. They did not produce death. However diarrhoea and hypoactivity were observed. The relative weight of liver was significantly less than that of the control group. This shows that doses less than 2250mg, DS seed extract may not induce any significant changes in the parallel fibres of the granular cells and purkinje cells of the cerebellum. Bania et al. 2004 [13] proposed a pretreatment dose of 7.5 mg/kg atropine equivalent of Datura stramonium seed extract as a protective agent in severe organophosphate toxicity. This dose of 750mg/kg once and twice daily may not have been toxic to batches 1 and 2 respectively, hence the normal appearance of the parallel fibres of the granule and purkinje cells observed in these batches compared to the control. This also confirmed the fact that 7.5 mg/kg may be safe and even beneficial. In dosage form, hyoscine one of the alkaloids exists as tablets and drops, elixir, and injection and is used for the treatment of bladder spasms, peptic ulcer disease, diverticulitis, colic, irritable bowel syndrome, cystitis and pancreatitis [15]. It has also been reported to treat impotence and diarrhea, kill parasites and act as analgesic [1].

Batch 3, which represented excess treatment dose of DS could have caused the atrophy of the parallel fibres observed in this batch for both male and female rats. Flegel et al. (2007) [16] showed in their work that Bavarian mountain

dogs which presented with chronic signs of cerebellar dysfunction were diagnosed with cerebellar hypoplasia. On histopathologic examination, a selective loss of cerebellar granule cells with sparing of Purkinje cells was evident. A degenerating neurons' experiment conducted by O'Hearn and Molliver (1993) [17], with indole alkaloids; ibogaine and harmaline confirmed degeneration of the purkinje cells of the cerebellum while the granular cells were spared. They concluded that both ibogaine and harmaline have selective neurotoxic effects which lead to degeneration of Purkinje cells in the cerebellar vermis. This shows that DS seeds may have a selective degenerative effect on the granular cells and their parallel fibres while the purkinje cells may be spared. Binev, et al. (2006) [11] described the spontaneous intoxication of DS in horses; Nelson et al. (1982) [18] described intoxication in cattle and Forrester (2006) [18] documented intoxication in humans. The most reported clinical effects were hallucinations, tachycardia, agitation, mydriasis, confusion and memory loss.

CONCLUSION

Excessive ingestion of DS seeds may have triggered a series of cascading events which selectively affected the granule cells and their parallel fibres, resulting in the parallel fibres' degeneration and atrophy. This could lead to cerebellar dysfunctions like disequilibrium, dyssynergia, hypoplasia and other abnormalities. These may impair the motor, learning and memory roles of the cerebellum, and burden the families of the patients and the society at large.

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Conflicts of Interests: None

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