Original Article

Changes in serum free sialic acid and histology of frontal cortex neurons in *Datura stramonium* treated rats

Peter Etim Ekanem¹, Regina Ekanem², Elizabeth A. Imbusi³

¹ Anatomy Unit, Biomedical Institute, College of Health Sciences, Mekelle University, Mekelle, Ethiopia.
² Public Health Department, School of Allied Health Sciences, Kampala International University.
³ Paediatrics Unit, Mekelle University Teaching Hospital, Mekelle, Ethiopia.

*Corresponding Author: Peter Etim Ekanem
Anatomy Unit, Biomedical Institute, College of Health Sciences, Mekelle University, Mekelle, Ethiopia.
E-mail address: etimakpan@gmail.com; Ph: +251925327490

Running Title: Histology of frontal cortex neurons in *Datura* treated rats

Received: 09 February, 2016; Revised: 23 March, 2016 Accepted: 16 April, 2016

Available online at http://www.thescientificpub.com
http://dx.doi.org/10.19046/abp.v03i02.01

Abstract

The human frontal cortex (FC) belongs to the cerebral cortex of the frontal lobe. It contains a network of neurons associated with the initiation of movement and is the substrate for functions that include: problem-solving, abstraction, imagination, planning and memory. In addition, it is also the site of the highest concentration of sialic acid (SA) in the human body. SA participates as an integral part of ganglioside structure in synaptogenesis and neural transmission. FC is one of the sites vulnerable to the toxic effects of certain drugs like *Datura stramonium* (DS) in the brain. DS is a known hallucinogen and a depressant of the central nervous system and it is commonly misused. The present study was designed to investigate potential effects of DS on the histology of the frontal cortex neurons and on serum free sialic acid (FSA) in Wistar rats. We found significant histological changes in both male and female rats treated with DS. There were also significant changes in the female FSA in the treated sub-groups compared to the controls, but no significant changes in male treated groups FSA compared to the controls. High doses of DS administration may have neuro-toxic effects that could lead to histological changes in frontal cortex neurons and also alter the level of female FSA. This may have an implication in neuronal deficits and neurological disorders.

Keywords: *Datura stramonium*, serum free sialic acid, frontal cortex neurons, Wistar rats

Introduction

Neurological disorders are diseases of the central and peripheral nervous system [1]. Some of these disorders can manifest as mental disorders or "psychiatric illnesses". According to WHO, [2], hundreds of millions of people worldwide are affected by neurological disorders and the use of psychoactive drugs has been implicated as one of the main causes of these diseases. The human cerebral cortex which also includes its subdivision, the frontal cortex, is one of the sites vulnerable to the toxic effects of drugs.

The frontal cortex is the part of the cerebral cortex that extends from the frontal pole to the central sulcus. It lies in the frontal lobe and includes: the motor area, premotor and prefrontal cortex as well as other important functional areas. The grey matter of the cerebral cortex is generally a convoluted, layered sheet of tissue, 2–3 millimetres thick in man but with a surface area of several hundred square centimeters [3]. It is a recent acquisition phylogenetically,
which is why it is also referred to as the neocortex [4]. Although its detailed cytological structure varies from region to region, it is generally recognized as consisting of six layers: Layer I, the most superficial layer, contains few nerve cell bodies but many dendritic and axonal processes, layer II contains many small neurons which establish intracortical connections and layer III contains medium-sized neurons which give rise to association and commissural fibres. Layer IV is the site of termination of afferent fibres from the specific thalamic nuclei while Layer V is the origin of projection fibres to extracortical targets, such as basal ganglia, thalamus, brain stem and spinal cord (in the primary motor cortex of the frontal cortex, this layer contains the giant Betz cells, which project fibres into the pyramidal tract). Layer VI also contains association and projection neurons [4].

In addition to neuronal networks that are associated with the initiation of movement as well as to sensation from the body and the special sensory organs, the cortex is the substrate for functions that include: comprehension, cognition, communication, reasoning, problem-solving, abstraction, imagination, planning and memory. In the frontal cortex, the pyramidal cells, especially in the prefrontal cortex, have received wide attention because of its structure and connectivity [5]. The size, number of branches, and spine density of the basal dendrites were quantified and compared with those of pyramidal cells in the occipital, parietal, and temporal lobes. These analyses demonstrated that cells in the frontal lobe are significantly more spiny than those in the other lobes [5, 6]. Therefore, any drug or factor that affects the frontal cortex may also affect the pyramidal structure and ultimately alter its integrative role. Another important substance found in the frontal cortex is sialic acid which has been documented to play an important role in the neural pathway.

Sialic acids are a family of nine-carbon acidic monosaccharides that occur naturally at the end of sugar chains attached to the surfaces of cells and soluble proteins. In the human body, the highest concentration of sialic acid (as N-acetylneuraminic acid) occurs in the frontal cortex where it participates as an integral part of ganglioside structure in synaptogenesis and neural transmission [7]. Neural cell membranes contain 20 times more sialic acid than other types of membranes, indicating that sialic acid has a clear role in neural structure [8]. Increased levels of serum free sialic acid (FSA) have been implicated in liver cirrhosis associated with alcoholism. Therefore, a depressant of central nervous system like alcohol may trigger changes in the FSA levels in the frontal cortex. *Datura stramonium* (DS), a tropical ubiquitous drug, is a depressant of the CNS widely used to increase intoxication in alcoholic beverages. It can affect the structure of frontal cortex neurons due to its reported anti-cholinergic effect and can change FSA levels in the FC.

DS, also referred to as Jimson weed, is a typical example of a hallucinogen with wide and varied usage. It is useful in the treatment of cough, chest pain, asthma, epilepsy etc. In some cultures, it is used in alcoholic beverages to increase intoxication [9]. All parts of the plant are said to be toxic most especially the seed. The toxins in *Datura stramonium* are tropane alkaloids which possess strong anticholinergic properties [10]. The major alkaloids present include: hyosine, atropine, scopolamine and hyoscyamine [10]. *Datura stramonium* has been documented to cause permanent short term memory loss because it also contains a compound known as gamma - 1- glutamyl - 1- aspartate which is also said to impair learning [11]. Accidental exposure of adults to this plant and teenage intentional misuse by eating the seeds, drinking the plant as tea and smoking the seeds as cigarettes have resulted in severe poisoning [12-14]. The range of toxicity of DS seeds is highly variable and unpredictable because the concentrations of specific alkaloids vary with species, cultivation, environment, temperature moisture and storage [15]. This contributes to the danger of its misuse since the dosage cannot be predicted [16]. In this research, we hypothesized that since this drug is often misused by teenagers and could be ingested excessively in this process, it may induce changes in the serum free sialic acid as well as neuronal degeneration and neural loss in the FC, thus affecting the integrating functions of this vital part of the brain.

We investigated the effects of high doses of DS seed extract on the neurons of frontal cortex in male and female treated rats and also observed the changes it may induce on the FSA, with an implication for neurological disorders.

**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 (A.B.U.) and the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences of the same University. The seeds were air dried and extracted with 96.5% ethanol. Phytochemical analysis was carried out 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,
Histology of frontal cortex neurons in *Datura* treated rats

Animal preparation for experimentation

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 A.B.U., Zaria. The university's rules and regulations governing animal handling were strictly adhered to and the experiment was conducted in accordance with ethical committee guidelines. A total of 24 experimental Wistar rats were randomly assigned to three groups of 8 animals per group. Each group had 4 males and 4 females which were further sub-divided into two sub-groups. Sub-groups A and B contained male control and treated rats, while C and D contained female control and treated rats respectively. Sub-groups A and C served as controls for both male and female rats and received normal saline. Bania et al. (2004) [17] 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 was used as a baseline for the calculation of the treatment doses. To induce intoxication, 7.5mg/kg was increased tenfold since LD₅₀ of this particular DS was found to be above 5000mg/kg. Sub-groups B and D were treated with different doses of DS extract. Group 1 received 750 mg/kg i.p once daily, the second group received 750 mg/kg, i.p twice daily and group 3 received 750mg/kg, i.p thrice daily. This treatment was administered for 4 weeks to observe whether chronic use of DS at high doses could induce changes in serum FSA and in the neurons of the FC. Blood samples were collected from both the control and treated rats for free serum sialic acid (FSA) assay. The animals were thereafter sacrificed by decapitation. The brain was dissected and the frontal cortices were carefully removed, sliced and fixed in 10% formalin for histological processing.

Histology

This part of the work was carried out in the histology unit of the Anatomy Department, A.B.U., Zaria, Nigeria. 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 a (LeitzWetzlar) microtome, 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 [18]. The tissues were observed under an upright microscope (Olympus x1000) with an attached digital camera. Sections of the frontal cortex were observed in male and female treated rats and compared with the control.

Free serum sialic acid assay

This work was carried out in Biochemistry department of A.B.U, Zaria. To determine the effect of the *Datura* seed extract on the serum free sialic acid, the method described by Aminoff (1961) [19] was used. Blood samples were collected from the control and treated rats into clean test tubes. The blood samples were then centrifuged at 1000 rpm for 15 minutes until the blood cells settled at the bottom of the tube while sodium periodate was added. 100 µl of Sodium Arsenite solution was added to destroy excess periodate in the serum, followed by the addition of 1 ml of thiobarbituric acid. The test tubes with their contents were placed in boiling water for 10 minutes until a pink color developed and 2 ml of acid: butanol was added at a concentration of 1:19 (Hydrochloric acid to Butanol-1) to extract the sialic acid at color phase. Measurements were performed at 549 nm using quartz cuvettes on the spectrophotometer Shimadzu UV-1202 (Shimadzu Europa GmbH, Duisburg, Germany). The amount of FSA was calculated using the following equation:

\[
\mu \text{ mol of FSA} = \frac{V \times \text{OD}_{549}}{68}
\]

Where \( V \) is the final volume of the solution and \( \text{OD}_{549} \) is the optical density at 549 nm [20].

Statistical Analysis: The values of serum total sialic acid were processed using their means and standard error of means (SEM). All groups were analyzed using unpaired student T-test in Excel 2007 software package. The confidence interval was set at 95% and \( p \) was considered significant at ≤ 0.05.

Results

Effects of treatment on the histology of the frontal cortex

The histology of both the control and treated rats of FC was examined under the microscope. Figure 1 shows a general view of the FC in low magnification and Fig. 2 shows the magnified view of the FC in the control male rats. Fig. 4 and 5 also show similar sections in the female control rats. The large cell bodies of the neurons and their thick and numerous neurites can be observed in the magnified sections of FC in both male and female control rats. There were no significant changes in the treated
batches administered 750mg/kg once and twice daily for 4 weeks in both male and female rats. The frontal cortices of both male and female treated rats in the sub-groups that received 750mg/kg thrice daily for four weeks seen in Figures 3 and 6 showed significant changes. The changes observed included: pyknotic cells, reduced size and fibres of neurites and cytoplasmic vacuolization. These changes indicated shrinkage and condensing which characterize dying cells.

Effect of Datura stramonium seed extract on serum free sialic acid

There was a significant increase in the value of serum FSA in group 1 female treated rats, which received 750 mg/kg of DS seed extract, i.p. once daily for 4 weeks, compared to the control ($p<0.001$). There was no significant difference in the male treated rats in the same group as shown in Fig. 7.

There was no significant difference for both male and female treated sub-groups in group 2, compared to the control, both of which received 750 mg/kg of DS seed extract, i.p. twice daily for 4 weeks.

In group 3, which received 750mg/kg of DS seed extract, i.p. thrice daily for four weeks, there was significant decrease in the serum FSA in the female treated sub-group with ($p<0.01$) compared to the control. There was no significant difference in the male treated sub-group compared with the male controls in this batch.

**Figure 1:** A coronal section of the male control FC, showing a general view in low magnification. Silver stain (x40).

**Figure 2:** A coronal magnified section of FC in control male rats showing large cell bodies (yellow arrow) and thick numerous neurites (red arrow). Silver stain (x400).

**Figure 3:** A coronal section of FC in male rats (batch 3), administered 750mg/kg of DS seed extract i.p. thrice daily for 4 weeks, showing pyknotic cell bodies (yellow arrow), reduced size and fibres of neurites (red arrow) and cytoplasmic vacuolization (green arrow). Silver stain. (x400)

**Figure 4:** A coronal section of the female control FC, showing a general view in low magnification. Silver stain. (x40).

**Figure 5:** A coronal magnified section of the control female FC, showing prominent cell bodies (yellow arrow) and thick numerous neurites (red arrow). Silver stain (x400).
Histology of frontal cortex neurons in Datura treated rats

Studies in several animals have revealed extensive axonal degeneration detectable in the frontal cortex of rats treated with Datura stramonium seed extract and also observed a selective degenerative effect on the granular cell parallel fibres and Purkinje cells of the cerebellum in Wistar rats [27]. In another study, high doses of DS seed extract had a selective degenerative effect on the granular cell parallel fibres and Purkinje cells of the cerebellum in Wistar rats [27].

The treated sub-groups in groups 1 and 2 which received 750mg/kg once and twice daily respectively did not show major histological changes in the FC neurons and neurites compared with the controls. DS has been shown to have some beneficial uses and is widely used in several cultures to treat several ailments. The drug is used in eye drops to induce mydriasis (pupillary dilation) and cycloplegia (paralysis of the eye focusing muscle). It is also used primarily in the treatment of the eye disorders uveitis, iritis, iridocyclitis etc [21]. In dosage form, hyoscine 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 [22]. Bania et al (2004) [17] propose a pre-treatment dose of 7.5mg/kg atropine equivalent of DS seed extract as a protective agent in severe organophosphate toxicity. This could imply that lower dosages may not change the histology of the FC neurons as dramatically observed in group 3. Several studies have shown that administering alkaloids at low doses may not produce remarkable histological changes. Gidado et al. (2007) [23] observed the effect of acute, subacute and chronic administration of alkaloids (atropine and scopolamine), the main constituents of the active component of DS, administered at a dose of 100 mg/kg of total alkaloids in the seeds of DS. This did not produce remarkable changes in general appearance and no deaths occurred in any experimental group. Bouzidi et al. (2011) [24] 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 observed that these did not produce death, however, diarrhoea and hypoactivity were observed. Also the relative weight of the liver was significantly less than that of the control group. Therefore, even in low doses, DS may still produce some form of toxicity which may not be easily observed or detected.

Both male and female treated rats in group 3, which received 750 mg/kg of DS seed extract, i.p. thrice daily for 4 weeks showed: cell pyknosis, reduced size and number of neurites and cytoplasm vacuolization in the neurons of FC (Figs.3 and 6). These events could actually contribute to the clinical signs of DS seed intoxication documented in several studies. These signs include: headache, nausea, vomiting, blurred vision, dilated pupils, hot dry skin, dizziness, dryness of the mouth, difficulty in swallowing and CNS stimulation [22]. The common side effects are related to anticholinergic effects in parasympathetic post synaptic receptors which has resulted in hallucinations and delirium, especially with higher doses. Anterograde amnesia and submissive behavior have also been reported [21]. Maibam et al. (2012) [25] administered DS leaf extract at a certain concentration and found that it induced neurotoxicity in the cerebral cortex. Their observations in electron photomicrographs showed damaged and ruptured mitochondria as well as non-uniform broken nuclear membranes of the cells in the two experimental groups when compared with the control groups. Ekanem et al. (2015a) [26] observed that administration of DS seed extract at high doses led to pyramidal cell degeneration and also changed cyclic response element binding (CREB) protein levels in the hippocampus of Wistar rats. In another study, high doses of DS seed extract had a selective degenerative effect on the granular cell parallel fibres and Purkinje cells of the cerebellum in Wistar rats [27].

Studies in several animals have revealed extensive axonal degeneration detectable in the frontal cortex of rats

Discussion

The treated sub-groups in groups 1 and 2 which received 750mg/kg once and twice daily respectively did not show major histological changes in the FC neurons and neurites compared with the controls. DS has been shown to have some beneficial uses and is widely used in several cultures to treat several ailments. The drug is used in eye drops to induce mydriasis (pupillary dilation) and cycloplegia (paralysis of the eye focusing muscle). It is also used primarily in the treatment of the eye disorders uveitis, iritis, iridocyclitis etc [21]. In dosage form, hyoscine 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 [22]. Bania et al (2004) [17] propose a pre-treatment dose of 7.5mg/kg atropine equivalent of DS seed extract as a protective agent in severe organophosphate toxicity. This could imply that lower dosages may not change the histology of the FC neurons as dramatically observed in group 3. Several studies have shown that administering alkaloids at low doses may not produce remarkable histological changes. Gidado et al. (2007) [23] observed the effect of acute, subacute and chronic administration of alkaloids (atropine and scopolamine), the main constituents of the active component of DS, administered at a dose of 100 mg/kg of total alkaloids in the seeds of DS. This did not produce remarkable changes in general appearance and no deaths occurred in any experimental group. Bouzidi et al. (2011) [24] 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 observed that these did not produce death, however, diarrhoea and hypoactivity were observed. Also the relative weight of the liver was significantly less than that of the control group. Therefore, even in low doses, DS may still produce some form of toxicity which may not be easily observed or detected.

Both male and female treated rats in group 3, which received 750 mg/kg of DS seed extract, i.p. thrice daily for 4 weeks showed: cell pyknosis, reduced size and number of neurites and cytoplasm vacuolization in the neurons of FC (Figs.3 and 6). These events could actually contribute to the clinical signs of DS seed intoxication documented in several studies. These signs include: headache, nausea, vomiting, blurred vision, dilated pupils, hot dry skin, dizziness, dryness of the mouth, difficulty in swallowing and CNS stimulation [22]. The common side effects are related to anticholinergic effects in parasympathetic post synaptic receptors which has resulted in hallucinations and delirium, especially with higher doses. Anterograde amnesia and submissive behavior have also been reported [21]. Maibam et al. (2012) [25] administered DS leaf extract at a certain concentration and found that it induced neurotoxicity in the cerebral cortex. Their observations in electron photomicrographs showed damaged and ruptured mitochondria as well as non-uniform broken nuclear membranes of the cells in the two experimental groups when compared with the control groups. Ekanem et al. (2015a) [26] observed that administration of DS seed extract at high doses led to pyramidal cell degeneration and also changed cyclic response element binding (CREB) protein levels in the hippocampus of Wistar rats. In another study, high doses of DS seed extract had a selective degenerative effect on the granular cell parallel fibres and Purkinje cells of the cerebellum in Wistar rats [27].
following administration of d-amphetamine sulfate [28]. Fowler A-K. (2014) [29], using histological analysis combined with stereological technique, demonstrated that the prefrontal cortex (PFC) is vulnerable to chronic alcohol-induced oxidative stress and neuronal cell death. Excessive oxidative stress and subsequent DNA damage can be responsible for neuronal apoptosis and neuronal dysfunction associated with different neurological pathologies [30- 32]. This confirms that the observed changes in the FC neurons in this work may have been caused by the administration of the high doses of DS seed extract to both male and female Wistar rats.

The mechanism by which DS seed extract exerts its effect on the FC neurons is not known. Yagihashi et al. (1990) [33] asserted from their work that axonal atrophy is related to a proportional loss of axonal neurofilaments in diabetic nerves. They declared that the universal reduction of axonal size in diabetic nerves may be accounted for by impaired supply of neurofilaments or reduced neurofilament synthesis. Such cytoskeletal defects may, in turn, lead to distal axonal degeneration or contribute to the susceptibility of neurons to various external noxious substances, including ischemia and hypoglycemia [33].

The serum free sialic acid (FSA) showed significant changes in groups 1 and 3 treated females but no significant changes in the male treated sub-groups compared to the controls. The differences in the concentration of FSA between the male and female treated rats may be due to the fact that FSA varies in concentration widely between species and in different organs within the same organism. The human adult cerebral cortex, particularly, the frontal cortex and cerebellar grey matter have approximately 3 times more FSA than the corresponding white matter [34]. Moreover, the sialic acid concentration in the left lobe of the brain cortex is 22% higher than that of the right lobe [35] possibly because the different brain regions perform different neurological functions [36].

The results in group 1 female treated rats (750mg/kg once daily) showed a pattern similar to that observed in chronic alcoholism. Ewa et al. (2014) [20] showed that FSA concentrations were significantly higher in toxic hepatitis than in nonalcoholic cirrhosis. This difference could be explained by the pathogenesis of toxic hepatitis due to excessive consumption of alcohol in about 70% of patients examined. They suggested that differences in FSA concentration between these diseases are the result of aberrant glycosylation in alcohol abusers.

The most common disturbances in glycosylation rely on the increase of enzymatic activity that cuts off sialic acid residues from serum glycoproteins and/or on the decrease of enzymatic activity that binds sugar residues to oligosaccharide chains. These processes may result in increased (FSA) concentration in the blood and compromise the degree of synaptogenesis in the brain. The decrease in FSA observed in group 3 treated female rats may have been due to decreased enzymatic activity in FC. The result of administration of DS seed extract in this batch also shows that it might have a neuro-protective effect on the frontal cortex in the treated females at higher doses, though this is not consistent with the histological changes observed in the neurons of the FC in the treated females. When compared with the male Wistar rats that did not express any significant changes in the FSA, this could indicate that DS seed extract may not affect the activity of the enzyme which catalyzes glycosylation of sialic acid. This calls for more studies to be carried out on the activity of FSA in the frontal cortex in response to administration of drugs such as DS. Taken together, it is likely that DS administration may affect FSA concentration in the female treated rats more than males and may have both neuro-toxic and neuro-protective effects in the female treated rats.

Conclusion

In conclusion, ethanolic extract of DS seeds given chronically at high doses may produce changes in the FC neurons of both male and female rats which include: pyknosis of the cell, reduced size and number of neurites as well as cytoplasmic vacuolization. Serum free sialic acid was found to vary more in the female than in the male treated rats but these changes were not consistent with the histological observations in this area. Therefore, the misuse of DS seeds or over consumption of these seeds may lead to degeneration of frontal cortex neurons, which may also lead to neurological disorders and impairment of important vital integrative functions controlled by the frontal cortex.

Acknowledgement

The researchers wish to acknowledge the staff of the Histology Unit of the Anatomy department and the biochemistry laboratory of Ahmadu Bello University, Zaria, for their support and provision of technical assistance in carrying out this research work. We also acknowledge the supportive role of Dr. Kendi Nyaga in reviewing and formatting manuscript for publication.

Conflict of Interest

Authors declare that there is no conflict of interest to reveal.

Financial assistance: None declared.
References


Histology of frontal cortex neurons in Datura treated rats

Advances in Biomedicine and Pharmacy Vol. 3 (2) 2016


© 2016 Reproduction is free for scientific studies

This work is licensed under a Creative Commons Attribution 4.0 International License.