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Toxicity Study on the Effect of Ethanol Extracts of the Root and Leaf of *Datura stramonium* on the Brain and Non-Prostatic Acid Phosphatase Activity of Wistar rats

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ABSTRACT

Abuse of psychoactive compounds is a growing global challenge, especially in countries with high unemployment rates. Toxicity study was conducted on the effect of ethanol extracts of the root and leaf of a regularly abused psychoactive plant in Nigeria (*Datura stramonium*) on the brain and non-prostatic acid phosphatase activity of Wistar rats using sixteen (16) Wistar rats of average weights 135g that were assigned into four (4) groups A, B, C and D with four (4) rats each. Groups A, B, and C were given the same concentration (3.5% in chilled Coca-Cola) of root extracts, leaf extracts, and both leaf and root extracts respectively while group D (control) was administered with equal volume of chilled Coca-Cola drink. The animals were kept in separate cages and fed with normal rat Chaw with water provided *ad libitum*. Rats in the treatment groups were given approximately 3.7mg/Kg body weight of the root extract, leaf extract, and both leaf and root extracts respectively while rats in the control group were given just a chilled Coca-Cola drink. All the animals were sacrificed after day 14th of administration. *A* qualitative phytochemical screening on the ethanol extracts of the root and leaf of *D. stramonium* plant showed that it contains alkaloids, tannins, saponins, flavonoids, and steroids. Histological examination of the brain of rats administered ethanol extracts of the root and leaf of the plant showed some levels of toxicity (exemplified by shrinkage of nerve cells) as well as increased activity of serum level of non-prostatic acid phosphatase.

***Keywords*:** *Datura stramonium*, non-prostatic acid phosphatase, histology, brain.

Introduction

Plants and their extracts serve different roles worldwide including their use as food and for the treatment of diseases. In some cases, plants are used to either enhance sexual performance or as sedatives through their psychoactive properties. Little wonder the demand for plant-derived natural products is increasing globally. However, with this demand also comes the risk of abuse of some of these plant-derived natural products. Top of the list of most abused psychoactive plants is *Datura stramonium*, originally from Central America but now widespread all over Nigeria and arguably the most common weed in the world.[1] It is popularly called in English: Devil's trumpet, Downy thorn apple, or Jimson weed. Some tribes in Nigeria recognize and called the plant thus; "Zakami" in Hausa, "gegemu" in Yoruba, Ogoni people know it as "gegami" and the Igala native calls it Jegemi. It is widespread with high abundance in temperate, tropical, and subtropical regions.[2] It is a wild-growing flowering plant used as a medicinal herb in folklore medicine. From ancient civilization, it was traditionally used for religious visionary purposes throughout the world and used for witchcraft in medieval Europe.[3] The god lord Shiva was known to smoke *Cannabis* and *Datura*, and people still provide the small thorn apple during festivals and special days as offerings in Shiva icons at temples. An extract made from the leaves is taken orally to treat asthma and sinus infections and stripped bark is applied externally to treat swellings, burns, and ulcers.[4, 5]

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Other medicinal use of the plant includes the anti-inflammatory property of all parts of the plant, its ability to stimulate the central nervous system, respiratory decongestion, treatment of dental and skin infections, alopecia, and in the treatment of toothache and epilepsy.[6] It is a hallucinogenic plant that causes serious poisoning as sporadic poisoning of young adults and adolescents in the United States from ingestion of *D. stramonium* resulting in serious illness or death have been reported by the media in the 1990s and 2000s.[7] Consumption of any part of the plant may result in a severe anticholinergic reaction that may lead to toxicity and occasionally cause diagnostic difficulties. Cases of poisoning have also been reported after eating the berries. The wide distribution, the strong toxicity, and the potential for occurrence in foodstuffs are responsible for the numerous incidents of poisoning in humans.[8]

One of the most investigated phytochemicals from this plant is the tropane alkaloid purportedly found in anticholinergic drugs like atropine and scopolamine. Because of the high amount of alkaloids in the seed of the plant,[9] one can consider using it as an alternative to atropine for the treatment of the muscarinic symptoms of organophosphate toxicity and some of the central anticholinergic effects.[10]

Despite the general knowledge that all parts of the plant perform different roles through the phytochemicals present in them, there have been no comparative studies on the different parts of the plant. Moreover, even though the role of plant extract on the central nervous system has been reported, histological examination of the impact of the plant on the brain has not been reported. This study, therefore, provides essential information on this aspect including the effect of the plant on non-prostatic acid phosphatase activity.

Materials and Methods

Plant extract preparation

The leaves and roots of *Datura stramonium* were collected in June, 2015 from the wild within Anyigba and Abejukolo ife, Kogi State and were identified by Professor S.S Usman of Biological Science

department (botany option), Kogi state University, Anyigba, Nigeria; samples were also deposited in the department after taxonomic classification. The samples were meshed with an enamel mortar and pestle and placed in two different plastic containers. One liter of ethanol was added to the containers containing the pulverized samples. This led to the separation of the solution into two layers which were left to stand for 24 hours (cold extraction).
This was followed by vacuum filtration with a high vacuum pump. Thereafter, the filtrate was concentrated by evaporation for about a week and the crude extract was collected in a beaker, covered with foil paper, and labeled accordingly for further use.

Phytochemical screening
Phytochemical screening was performed on the extracts from the root of the plant as described previously[11] and shown in Table 1.

Animal grouping
Wistar rats of average weight 135g were obtained from the animal house, Federal University, Lokoja after approval, and the animals were handled according to international guidelines and regulations. The rats were divided into four groups A, B, C, and D made up of four rats each and within the acceptable sample size for animal experiments.[12] The treatment groups were groups A, B, and C while group D served as the control. Each of the treatment groups was given 3.5% of the extracts dissolved in coke which was prepared by dissolving 0.5mg of the extract in 15ml of chilled coke (wt/vol) while the control group was given only the chilled coke.
Ethanol extracts of *Datura stramonium* were administered to each of the treatment groups as follows:
Group A was given 3.5 % (wt/vol) of the ethanol root extract dissolved in chilled coke,
Group B was given 3.5 % (wt/vol) of the ethanol leaf extract dissolved in chilled coke while Group C was given 3.5 % (wt/vol) of both the ethanol root and leaf extracts and the control group D was given an equal amount of chilled coke.
Ethanol extracts of *Datura stramonium* were administered to each of the treatment groups once daily with 0.5 ml of the respective solutions administered to the animals in group A and B while the animals in group C were given 0.5 ml each of solutions A and B (altogether 1 ml). The control group was administered 0.5ml of chilled coke. The extracts were administered orally for a period of 14 days.

Collection of blood and tissue preparation
The animals were sacrificed twenty-four hours after the last dose by jugular puncture[13] after mild anesthesia with chloroform and the blood from the animals was collected using a 25G needle into plain EDTA bottles and centrifuged at 10000 rpm for five minutes. The serum obtained was used for further analysis. The organ (brain) was carefully extracted from the animals and fixed in 10% buffered formalin. The brain was then taken for histological examination as described previously.[11]

Determination of non-prostatic acid phosphatase (ACP) activity
1.0ml of blood sample (serum) was measured into a test tube and 0.5ml of sample 1 (sodium citrate pH 5.2) was added. The mixture was equilibrated in a water bath for 10 minutes at 37^0C. Sample 2 (α-napthyl-phosphatate) was added. The mixture was incubated for five minutes. The initial absorbance at 450nm was read at one-minute intervals thereafter for 3 minutes. The difference between the absorbance and average absorbance differences per minute (ΔA/min) was then estimated.[14]
The enzyme activity was calculated using ΔA/min×743

Data analysis
Statistical and image analysis was conducted using graph pad prism 9 and image J respectively.

Results and Discussion

Histological examination of the brain tissue
Histological examination was performed on the impact of ethanol extracts from different parts of *D. stramonium* (root and leaf) on the brain of Wistar rats as well as the impact on non-prostatic acid phosphatase activity. All the test groups showed varying degrees of toxicity with the leaf extracts affecting the astrocytes more than the root extract as demonstrated by the area of infiltrated cells (Figure. 1). The toxic effect could be due to the presence of alkaloids in the plant extract. Halpern had shown in 2004 that all parts of the plant contain lethal levels of tropane alkaloids scopolamine, hyoscyamine, and atropine which block the neurovegetative cholinergic system by competitively antagonizing the central and peripheral muscarinic cholinergic receptors.[15] This is consistent with our phytochemical screen of the root extracts that qualitatively shows the presence of alkaloids (Table 1). Based on their toxic effect, they are grouped under anticholinergics or deliriants with fatal consequences like mydriasis, dry skin, and tachycardia among others when ingested. As anticholinergics, they inhibit cholinesterases resulting in the accumulation of excessive choline at the synaptic cleft and overstimulation of cholinergic neurons.[16] It is this excessive stimulation of the cholinergic neurons that is believed to be responsible for neurodegeneration syndromes like hallucination, psychosis, tachycardia, severe mydriasis, delirium, hyperthermia, bizarre behavior and ultimately death.[17] Apart from their anticholinergic effect as a neurotoxic mechanism, *D. stramonium* extracts have also been shown to affect the monoaminergic system of neurotransmission by inhibiting the two isoforms of monoamine oxidase (MAO isoforms A and B) which are involved in the oxidative deamination of biogenic amines such as tyramine, dopamine, serotonin, noradrenaline, etc., leading to hypertensive crises and serotonin toxicity.[18,19] *D. stramonium* also exerts its neurotoxic effect by impairing the purinergic system of neurotransmission through its impact on enzymes such as the ecto-nucleotide pyrophosphatase/phosphodiesterase, ecto-nucleoside triphosphate diphosphohydrolase, ecto-5'-nucleotidase, alkaline phosphatase, and Na^+/K^+ATPase which are involved in the system. When the plant affects Na^+/K^+ATPase, it leads to an imbalance in Na^+/K^+ equilibrium resulting in a polarization of nerve endings and Ca^{2+} influx into brain cells leading to swelling of the neurons and the release of excess neurotransmitters.[20] Inhibition of ecto-nucleoside triphosphate diphosphohydrolase by *D. stramonium* impairs neuronal adenosine triphosphate (ATP) hydrolysis. This mechanism has been shown to result in the accumulation of large amounts of extracellular ATP, overstimulation of P_2 purinergic receptors, and by implication, impairment of the purinergic neurotransmission system. Furthermore, ecto-5'-nucleotidase inhibition by *D. stramonium* has been implicated in extracellular adenosine levels depletion, resulting in adrenergic neurotransmission and memory impairment.[21]
Another mechanism of neurotoxicity by *D. stramonium* is by altering the gene expression of the cAMP response element-binding protein (CREB). Elevated CREB gene expression observed in male rats was suggested to be due to excessive expression of the inactive form of CREB protein or the CREB-2 isoform. This process has been implicated in the neurodegeneration of the frontal cortex and hippocampal neurons. At the same time, the depleted CREB gene expression observed in female rats was associated with neurodegeneration via excessive inhibition of CREB function and expression in pyramidal neurons, which results in excessive loss of cornu ammomis (CA) 1 subfield neurons. Similar neurodegeneration was observed in the frontal cortex of female rats, where depletion in CREB expression and its integrative role was observed during *D. stramonium* exposure.[22] Consequently, the release of free radicals and impairment of the anti-oxidant system have also been reported as a mechanism of neurotoxicity by *D. stramonium*. Release of excessive free radicals leads to a high level of lipid peroxidation, Na^+K^+ATPase impaired function, activation of glial cells, misfolded proteins, membrane configuration derangement, cellular apoptosis, and dysfunction of cellular mitochondrial field field field field[23] while its impairment of the antioxidant system occurs by depletion of the system leading to loss of cognitive functions, neuronal cell death and depletion of neuronal integrity.[24]

Commented [PF1]: check

Though our study did not directly investigate any of the above mechanisms of neurotoxicity, we believe that the observed toxicity could be through any of the above-mentioned mechanisms or a combination of both.

D. stramonium extracts affect non-prostatic acid phosphatase activity

The result of the effect of the extracts on non-prostatic acid phosphatase activity showed a significantly increased ($p<0.0001$) activity of the enzyme in the test groups. Apart from the prostate gland where the large activity of ALP is found, the enzyme is also found in other body organs such as bone, liver, spleen, and kidney, and even in some cells and organelles like platelets, red blood cells, and lysosomes. Increased activity of this enzyme in these organs can serve as a diagnostic marker for tissue damage. We believe that this result further buttresses the toxic effect of the extracts. ALP has also been implicated in the enhancement of the toxicity of extracellular tau protein leading to the progression of Alzheimer's disease.[25]

Altogether, this report has shown that ethanol extracts of the root and leaf of *D. stramonium* are toxic to the brain of Wistar rats and also cause tissue damage that increased the serum level of non-prostatic acid phosphatase activity.

Table 1: Phytochemical test

Phytochemicals	Inference
Alkaloid	+
Tannin	+
Saponin	+
Flavonoid	+
Steroid	+

+ = present

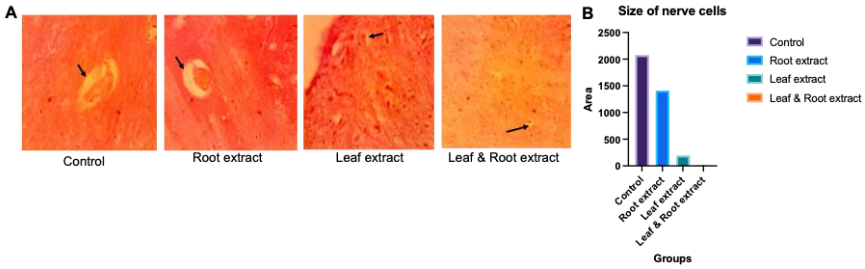

Figure 1: Histological examination of the brain tissue (A)Histology of the brain tissue of Wistar rats after formalin fixation **(B)** Bar chart showing the area of nerve cells for the different histological images. The analysis was conducted using image J.

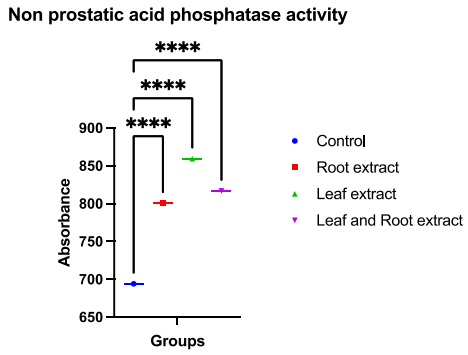

Figure 2: Determination of non-prostatic acid phosphatase activity. The graph shows the non-prostatic acid phosphatase activity in the experimental animals. Statistical analysis was conducted using graph pad prism 9 and comparison was done using one-way ANOVA.

Conclusion

It was observed that the extracts were toxic to all the treatment groups as shown by infiltration of the brain cells and increase in serum levels of non-prostatic acid phosphatase. This observation is attributable to neurotoxicity and tissue damage, perhaps caused by phytochemicals such as alkaloids. Further studies on the tissue damage caused by the extracts are required as this can help design biosensors to examine *D. stramonium* poisoning in foods and raise awareness of the danger of continuous abuse of the plant for recreational purposes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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