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Research Article

Behavioral and Biochemical Indices of Neurotoxicity in Jimson weed Administered Rats

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ABSTRACT

This study examined the effect of alkaloid extracts from leaf and fruit of Jimson weed (*Datura stramonium* L; a toxic food contaminant of global reputation) on cognitive function, antioxidant status and activities of critical enzymes of monoaminergic and cholinergic systems of neurotransmission in rats. Alkaloid extracts were prepared by solvent extraction method. The gas chromatograph coupled with mass spectroscopic (GC-MS) characterization of the extracts was also carried out. Thereafter, rats were administered 100 and 200 mg/kg body weight (*p.o*) of extracts for thirty days. Prior to termination of the experiment, the rats were subjected to spontaneous alternation and hole-board behavioral tests. Thereafter, rats were sacrificed and activities of acetylcholinesterase (AChE), monoamine oxidase (MAO) and antioxidant enzymes (superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase), as well as malondialdehyde (MDA) and total thiol (TSH) contents of their brain homogenates were determined. The results showed that rats administered the extracts exhibited reduced memory index and also exhibited increased level of anxiety-like behavior. Furthermore, rats administered the extracts exhibited significantly reduced levels of brain AChE, MAO, SOD, catalase and GST activities, reduced amount of brain TSH and elevated brain MDA content compared to control. Results from the GC-MS characterization revealed the presence of hyoscyamine (atropine), scopolamine, amphetamine, cathine, phenlyephirine and Tropine trifluoroacetate among others in the extracts. Therefore, the alterations in cholinergic, monoaminergic and antioxidant systems in brain of rats administered fruit and leaf alkaloid extracts of Jimson weed could be one of the major biochemical mechanisms underlying their ability to induce impairment of cognitive functions which could be significantly linked to their constituent alkaloids.

Keywords*: Food contaminant; Neurotoxicity; Oxidative stress; alkaloid extract.*

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INTRODUCTION

Jimson weed (*Datura. stramonium* L) is a toxic plant that constitute as toxic food contaminant with global reputation as several reports have documented accidental poisoning of Jimson weed in animals and humans across the world (Al-Shaikh *et al*., 2005; Soni *et al*., 2012). Poisoning due to inclusion of Jimson weed plant parts in household materials such as toothpaste and accidental consumption as contaminant of edible vegetables has been reported in eastern part of United States (Pereira *et al*., 1994; Chang *et al*., 1999). In some European countries, Jimson weed poisoning as a result of consumption of contaminated commercial foods and beverages such as honey, Paraguay tea, hamburger and stiff porridge have been reported (Ramirez *et al*., 1999). In Nigeria, studies have reported Jimson weed abuse among adolescents to range from 3.8-40.1% (Fatoye *et al*., 2002; Abdulkarim *et al*., 2005; Oshodi *et al*., 2010). It is common among adolescents who consumed the plant, especially the leaf and seed for its hallucinating effects. It is also common to include the seed in alcoholic beverages to increase its intoxicating effects. All these toxicological properties have been largely attributed to its alkaloid constituents (Soni *et al*., 2012; Abdulkarim, *et al*., 2005).

Although some studies have reported the medicinal properties of Jimson weed (Soni *et al*., 2012; Altameme *et al*., 2015), concerns have often been about its neurological effects. This is more so because previous studies have reported symptoms of neurotoxicity including confusion, agitation, mydriasis, and hallucination in Jimson weed-intoxicated human subjects (Adegoke *et al*., 2013). However, Jimson

weed poisoning has been reported to arise with multiple symptoms and difficult to diagnose, often making management difficult (Shigenaga *et al*., 1994). Therefore, understanding the biochemical mechanisms of action of Jimson weed neurotoxicity in relations to its effect on cognitive functions is essential, especially for prompt and proper management of Jimson weed poisoning.

The brain is coordinated by series of neuronal activities that are systematically organized into interrelated systems of individually unique neurons, neurotransmitters, neuromodulators, receptor molecules and secondary messengers (Ademiluyi *et al*., 2016a). A distortion in this system often results in cascade of events which culminate in impaired neuronal functions and neurodegenerative processes. Among these systems include the cholinergic and monoaminergic systems of neurotransmission. The neurotransmitter-acetylcholine is central to the neuronal cholinergic transmission, where it acts as both neurotransmitter and neuromodulator (Kellogg *et al*., 2005). The biogenic amines or monoamines are oxidized by monoamine oxidase (MAO). In addition, MAO metabolizes toxic xenobiotic amines (Kalgutkar *et al*., 2001). Therefore, alterations in the activity of this enzyme could lead to several neurological disorders such as; serotonin and dopamine toxicity.

Oxidative stress has been implicated in the pathogenesis and progression of many neurological disorders. Oxidative stress which often occur as a result of imbalance between endogenous antioxidant status and free radical production in favor of the latter, leads to cascade of events such as mitochondrial dysfunction and lipid peroxidation, which often lead to neuronal cell damage (Ide *et al*., 2001; Butterfield *et al*., 2002). Previous reports have shown that oxidative stress induced by free radical generation as a result of exposure to food and environmental toxicants such as toxins and pesticides, causing impaired brain antioxidant status are one of the mechanisms for the toxicity of such agents (Rossignol *et al*., 2012). Furthermore, oxidative stress has also been implicated in impaired cognitive functions. Series of experimental findings have shown that oxidative stress as a result of excessive free radical production and/or impaired brain antioxidant status have caused impairment in spatial and non-spatial working memories in rats (Tijsseling *et al*., 2014; Fujisaki *et al*., 2014).

Previous studies have attempted to study the toxicological implications of Jimson weed poisoning. In one study (Dugan *et al*., 1989), toxicological evaluation of Jimson weed seed were evaluated in a 90 day feeding study in rats. While this study reported that Jimson weed intoxicated rats exhibited reduced body weight, and impaired hematological indices, it did not report any biochemical mechanisms for these observations nor was there any focus given to neurological aspect of such toxicity. Others studies have however, evaluated the neurotoxicity aspect of Jimson weed in both *in vitro* (Ademiluyi *et al*., 2016a) and *in vivo* (Ademiluyi *et al*., 2016b; Ekanem *et al*., 2016) models, but with little or no correlation to physiological/behavioral aspects of the toxicity. Therefore, this study sought to evaluate the behavioral changes that accompany Jimson weed neurotoxicity in correlation with alterations in cholinergic, monoaminergic and antioxidant systems in brain of Jimson weed treated rats.

MATERIALS AND METHODS

Collection and preparation of samples: Jimson weed (*Datura stromonium* L) plant was harvested at the stage of opening of first capsule, from local farm settlement in Akure, Ondo State (South West) Nigeria. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State (South West) Nigeria. A sample voucher was deposited at the institute's herbarium (voucher number FHI 110111). Leaves and fruits of the plant were carefully separated, washed with water to remove dirt, and dried under shade for several days until a constant weight was obtained. Thereafter, the dried samples were pulverized in an electronic stainless steel blender, and stored in air-tight dark containers in the refrigerator at 40^{o}C for alkaloid extraction.

Chemicals and reagents: Chemicals and reagents used such as semicarbazide, benzylamine (99%), acetylthiocholine iodide (≥98%) and trichloroacetic acid (TCA; 98%) were sourced from Sigma-Aldrich, Inc., (St Louis, Missouri, USA). DNPH (2, 4-dinitrophenyl hydrazine, moist solid; min. 30% water) was source from ACROS Organics (New Jersey, USA). Methanol (99.8%) and acetic acid (99.5%) were sourced from BDH Chemicals Ltd., (Poole, UK). All other chemicals were of analytical grade while the water used for all analysis was glass distilled.

Preparation of alkaloid extracts: Alkaloid extract of samples were prepared according to the method of Harborne (Harborne, 1998), with slight modifications (Ademiluyi *et al*., 2016a). Briefly, pulverized samples were defatted with n-hexane for 24 hours. Thereafter, 10 g of defatted samples were extracted with 100 ml of 10% acetic acid in ethanol for 24 hours. This was followed by filtration, first using Muslin cloth and then filter paper (Whatman no. 1). The clear filtrate obtained was concentrated under vacuum at 45 ^{0}C in a rotary evaporator (Laborota 4000 Efficient, Heidolph, Germany). Subsequently, the filtrate was precipitated using concentrated ammonium hydroxide. The whole solution was allowed to settle and the precipitate was collected and rinsed with dilute ammonium hydroxide to obtain the alkaloid extracts. The extracts were collected, dried thoroughly at 45 ^{0}C and stored in the refrigerator at 4 ^{0}C for all subsequent analysis. The yield of extracts obtained were 3.92 g/100 g and 3.60 g/100 g of dried fruit and leaf samples of Jimson weed, respectively.

Experimental animals: Albino rats weighing 200-210 g were used for this study. Rats were maintained at 25 ^{0}C, on a 12 h light/12 h dark cycle, with free access to food and water. They were acclimatized under these conditions for 1-2 week before the experiment. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental Design: After a period of two weeks acclimatization, the rats were randomly divided into six groups (n=6). Group 1 (Control) served as the control group

and received distilled water by gavage throughout the experiment; Group 2 (JW L1) were administered 100 mg/kg body weight of Jimson weed leaf alkaloid extracts (p.o) once daily; Group 3 (JW L2) were administered 200 mg/kg body weight of Jimson weed leaf alkaloid extracts (p.o) once daily; Group 4 (JW F1) were administered 100 mg/kg body weight of Jimson weed fruit alkaloid extracts (p.o) once daily; while Group 5 (JW F2) were administered 200 mg/kg body weight of Jimson weed fruit alkaloid extracts (p.o) once daily. The experiment lasted for thirty (30) days; rats were subjected to behavioral studies, thereafter sacrificed and brain tissue prepared as described below. During the course of the experiment, all animals had free and equal access to food and water. The choice of dose for the alkaloid extracts (100 and 200 mg/kg) were based on previously findings on LD_{50} of Jimson weed leaf and seed extracts which were reported to be above 3,000 mg/kg in rats (Ekanem *et al*., 2016; Mahmood *et al*., 2015).

Behavioral studies

Y-Maze (Spontaneous alternation) Test: This was done to analyze the spatial working memory of the animals. The Y-maze test was performed as described by Wall and Messier, (2002). The maze was made of 3 identical arms, 40 cm long, 35 cm high and 12 cm wide, positioned at equal angles and labelled A, B, and C. Rats were placed at the end of one arm and allowed to move freely through the maze during a 5 min session. Spontaneous alternation was examined by visually recording the pattern of entrance into each arm in the maze for each rat. Arm entry was considered to be complete when the hind paws of the rat were completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet set (i.e., ABC, BCA...). Accordingly, the frequencies of alteration between the arms recorded was plotted to determine the memory index (percentage of correct alteration) as described by Akinyemi *et al*., (2017).

Hole-board (Head-dipping) Test: The anxiety-like behaviors of the rats were analyzed using the hole-board test according to the previously reported method (Masood *et al*., 2008). Briefly, the hole-board apparatus was made up of (60 x 60 x 35 cm) wooden box, constructed with its floor consisting of four equally spaced holes of 4 cm diameter. To carry out the test, each animal was carefully placed at the center of the box and allowed to freely explore the apparatus for 5 min. Thereafter, the number of head dips in the holes were recorded and analyzed. The experiment was carried out in a quiet room away from any audio or visual disturbances.

Bioassays

Preparation of Tissue Homogenate: At the completion of the behavioral studies, the rats were immobilized by cervical dislocation and the whole brain tissues were rapidly isolated, rinsed with cold saline, placed on ice and weighed. These tissues were subsequently homogenized in appropriate buffer (1:5 w/v) with about 10-up-and –down strokes at approximately 1200 rev/min in a Teflon glass homogenizer (Mexxcare, mc14 362, Aayu-shi Design Pvt. Ltd. India). The homogenates were centrifuged for 10 min at 3000 x g in a refrigerated centrifuge (KX3400C, KENXIN Intl. Co., Hong Kong) at 4 ^{0}C to yield pellets that were discarded, and supernatants (homogenates) which were used for all enzyme assays. The total protein content of the homogenate was determined according to Lowry *et al*. (1951), with bovine serum albumin (BSA) used as standard.

Determination of acetylcholinesterase (AChE) activity : The AChE activity assay was determined using the modified colorimetric method of Ellman (Ellman *et al*., 1961) as previously described by Akinyemi *et al*., (2016). The AChE activity of the brain homogenates were determined in a 2 ml final reaction mixture containing 0.1 M phosphate buffer (pH 8.0), 3.3 mM of 5,5′-dithio-bis (2-nitrobenzoic) acid (DTNB), sample homogenates and 0.1 M phosphate buffer (pH 8.0). The reaction was subsequently initiated by adding the AChE substrate (0.8 mM acetylthiocholine iodide). The absorbance of the mixture was thereafter monitored at 412 nm in spectrophotometer. Consequently, the activity of AChE was expressed as µmol AcSCh/h/mg protein.

Determination of Monoamine oxidase (MAO) activity: The MAO activity was measured according to a previously reported method (Green *et al*., 1961) with slight modifications. In brief, the reaction mixture contained 0.025 M phosphate buffer (pH 7.0), 0.0125 M semicarbazide, 10 mM benzylamine, 100 µl of brain tissue homogenate. After 30 min incubation, acetic acid was added and incubated for 3 min in boiling water bath followed by centrifugation. The resultant supernatant (1 ml) was mixed with equal volume of 2, 4-Dinitrophenylhydrazine, and 1.25 ml of benzene was added after 10 min incubation at room temperature. After separating the aqueous layer, the benzene layer was mixed with equal volume of 0.1 N NaOH. The resulting alkaline layer was decanted and incubated at 80 °C for 10 min. The orange–yellow color developed was measured at 450 nm in a spectrophotometer.

Determination of tissue malondialdehyde (MDA) content: This was carried out by the modified method of Ohkawa *et al*., (1979). In brief, 0.3 ml of tissue homogenate was added to 0.3 ml of 8.1% Sodium dodecyl sulfate (SDS), 0.5 ml HCl/ acetic acid (pH 3.4) and 0.5 ml of Thiobarbituric acid (TBA); the mixture was incubated at 100 ^{o}C for 1 hour. The resulting thiobarbituric acid reactive species (TBARS) was quantified at 532 nm in a spectrophotometer and calculated as MDA equivalent.

Determination of superoxide dismutase (SOD) activity: Superoxide dismutase (SOD) was determined by the method of Alia *et al*., (2003). An aliquot of 0.05 ml of tissue homogenate was treated with 1.0 ml of 50 mM carbonate buffer (pH 10.2) and 0.017 ml of adrenaline (0.06 mg/ml). The absorbance was read at 480 nm in spectrophotometer for 2 minutes at 15 seconds intervals. SOD activity was expressed as U/g protein.

Determination of tissue catalase (CAT) activity: Catalase activity in the brain homogenate samples was determined according to the method of Sinha, (1972). In brief, 0.1 ml of

each tissue homogenate sample was reacted with 0.4 ml of 2 M H_2O_2 in the presence of 1.0 ml of 0.01 M phosphate buffer (pH 7.0). The reaction was stopped by the addition of 2.0 ml dichromate acetic acid. The absorbance of the reaction mixture was taken at 620 nm in a spectrophotometer. A standard curve was prepared by reacting 0.4 ml of 2 M H_2O_2 with 2 ml dichromate acetic acid in the presence of 1.0 ml of 0.01 M sodium phosphate buffer (pH 7.0) to generate a straight line equation. Catalase activity was expressed as mole of H_2O_2 consumed/min/g protein.

Determination of tissue Glutathione-S-transferase (GST) activity: This assay was carried out according to the method of Habig *et al*., (1974). It involves the pre-incubation of reaction mixture containing 1.0 ml of 100 mM phosphate buffer (pH 6.5), 30 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 0.7 ml of distilled water for 5 mins at 37 ^{0}C. The reaction was started by the addition of 0.1 ml of the tissue homogenate and 0.1 ml 30 mM glutathione as substrate. The absorbance of the reaction mixture was monitored after 5 minutes at 340 nm in a spectrophotometer. Reaction mixture without enzyme was used as a blank. The activity of GST was expressed as mole of GSH-CDNB conjugate formed/min/g protein.

Determination of Total Thiol (TSH) content of tissue homogenate: TSH content was determined by modified method of Ellman (1959). Tissue homogenate (1 ml) was added to 0.5 ml of Ellman's reagent (19.8 mg of 5, 5' dithiobisnitrobenzoic acid in 100 ml of 0.1% sodium citrate) and 3.0 ml of 0.2 M phosphate buffer (pH 8.0). The absorbance was read at 412 nm in spectrophotometer.

GC-MS Characterization: A qualitative characterization analysis of possible compounds present in Jimson weed leaf and fruit was carried out using GC-MS (using scan mode) as previously reported (Ademiluyi *et al*., 2016a) with slight modifications. Briefly, aliquot of samples (500 mg) were dissolved in 10ml of Methanol. Thereafter, the analysis was performed using 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness) (Agilent Technologies). The carrier gas was Helium used at constant flow of 1.6 ml/min at an initial nominal pressure of 19581.11 pascal and average velocity of 46 cm/sec. An aliquot of the samples (1µl) were injected in splitless mode at an injection temperature of 260 ^{0}C. Purge flow was 21.5 ml/min at 0.50 min with a total flow of 25.8 ml/min; gas saver mode was switched on. Oven was initially programmed at 60 ^{0}C (1 min) then ramped at 4 ^{0}C/min to 110 ^{0}C (3 min) then 8 ^{0}C/min to 260 ^{0}C (5 min) and 10 ^{0}C/min to 300 ^{0}C (12 min). Run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in electron ionization mode with Ionization energy of 70 eV with ion source temperature of 230 ^{0}C, quadrupole temperature of 150 ^{0}C and transfer line temperature of 280 ^{0}C.

Prior to analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of *m/z* 69, 219, 502 and other instrument optimal and sensitivity conditions.

Analysis validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, molecular weight (amu), Quality ion (Q-Ion) and %Total.

$$\%\text{Total} = \frac{\textit{Abundance of individual constituents}}{\textit{Total Abundance of all consituents in extract}} \times 100$$

Each compound identified via the NIST 11 Library Search report has a corresponding mass spectrum showing the abundance of the possible numerous *m/z* peaks per compound.

Data Analysis

Results were expressed as mean ± standard deviation (SD). Mean values were appropriately analyzed and compared using one way analysis of variance (ANOVA) followed by turkey post hoc test; significance was accepted at $P<0.05$. All statistical analysis was carried out using Graph Pad Prism version 5.00 for Windows.

RESULTS

Effect of Jimson weed Alkaloid Extracts on Behavioral Indices of Neurotoxicity in Rats: The effect of Jimson weed alkaloid extracts (leaf and fruit) on treated rats' spontaneous alternation (memory index) as a measure of their spatial working memory (Figure 1a) revealed that rats administered 100 and 200 mg/kg of extracts exhibited significantly ($P<0.05$) reduced memory indices compared to the control rat group.

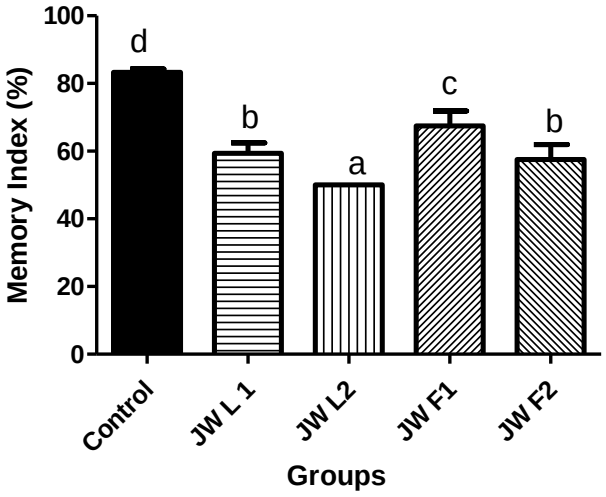

Figure 1a: Spontaneous Alternation (memory index) of Rats Administered Alkaloid Extracts from Leaf and Fruit of Jimson Weed (D. stramonium L). Values represent mean ± standard deviation (n = 6). Mean values with different letters are significantly different ($p < 0.05$).
Key: Control = control rat group; JW L1 = rat group administered Jimson Weed leaf alkaloid extract (100 mg/kg); JW L2 = rat group administered Jimson Weed leaf alkaloid extract (200 mg/kg); JW F1 = rat group administered Jimson Weed fruit alkaloid extract (100 mg/kg); JW F2 = rat group administered Jimson Weed fruit alkaloid extract (200 mg/kg)

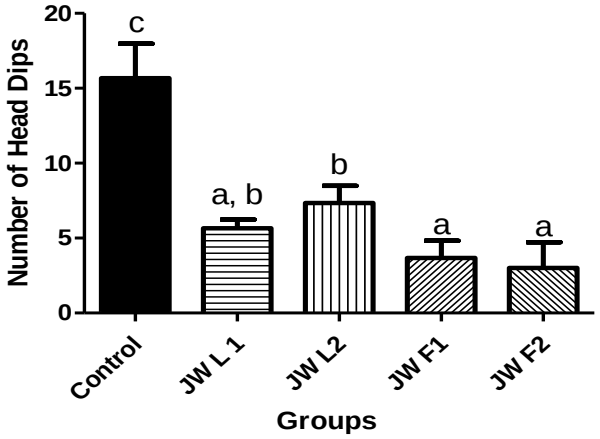

Figure 1b:
Anxiety-Like Behaviors (hole-board/nose dipping test) of Rats Administered Alkaloid Extract for Leaf and Fruit of Jimson Weed (D. stramonium L).
Values represent mean ± standard deviation (n = 6). Mean values with different letters are significantly different ($p < 0.05$).
Key: Control = control rat group; JW L1 = rat group administered Jimson Weed leaf alkaloid extract (100 mg/kg); JW L2 = rat group administered Jimson Weed leaf alkaloid extract (200 mg/kg); JW F1 = rat group administered Jimson Weed fruit alkaloid extract (100 mg/kg); JW F2 = rat group administered Jimson Weed fruit alkaloid extract (200 mg/kg).

The lowest memory index was observed in rats administered 200 mg/kg leaf extract. Also, the anxiety-like behavior of treated rats as monitored from the hole-board behavioral test (Figure 1b) revealed that rats administered extracts (100 and 200 mg/kg) exhibited significant lower ($P<0.05$) amounts of head dips, and thus increased level of anxiety-like behaviors compared to the control groups. However, rats administered with the fruit extract (100 and 200 mg/kg) exhibited significantly higher anxiety-like behavior compared to rats administered the leaf extract.

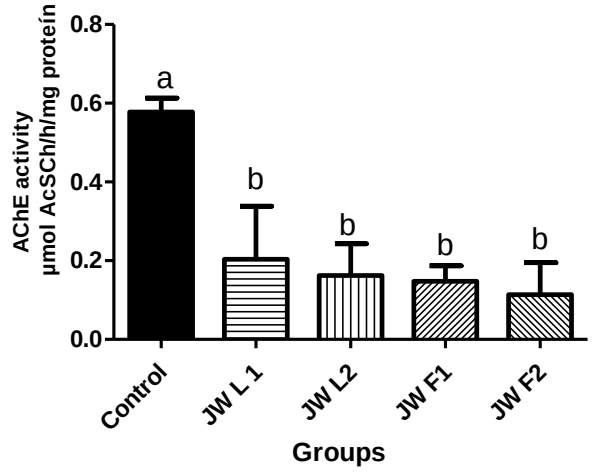

Figure 2:
AChE activity on Brain Tissue of Rat Administered Alkaloid Extracts from the Leaf and Fruit of Jimson Weed (D. stramonium L.). Values represent mean ± standard deviation (n = 6). Mean values with different letters are significantly different ($p < 0.05$).
Key: Control = control rat group;; JW L1 = rat group administered Jimson Weed leaf alkaloid extract (100 mg/kg); JW L2 = rat group administered Jimson Weed leaf alkaloid extract (200 mg/kg); JW F1 = rat group administered Jimson Weed fruit alkaloid extract (100 mg/kg); JW F2 = rat group administered Jimson Weed fruit alkaloid extract (200 mg/kg).

Effect of Jimson weed Alkaloid Extracts on Brain Acetylcholinesterase Activity: Brain acetylcholinesterase (AChE) activity in treated rats was measured and presented in figure 2. According to this result, rats administered the extracts (100 and 200 mg/kg) exhibited significantly ($P<0.05$) reduced acetylcholinesterase activity compared to the control rat group. There was however, no significant difference in AChE activity among rats groups administered the leaf and fruit extracts.

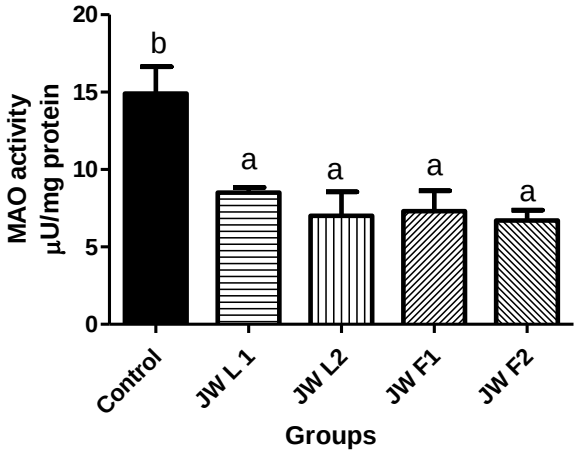

Figure 3:
Monoamine oxidase (MAO) activity of Brain Tissue of Rats Administered Alkaloid Extracts from the Leaf and Fruit of Jimson Weed (D. stramonium L). Values represent mean ± standard deviation (n = 6). Mean values with different letters are significantly different ($p < 0.05$).
Key: Control = control rat group; JW L1 = rat group administered Jimson Weed leaf alkaloid extract (100 mg/kg); JW L2 = rat group administered Jimson Weed leaf alkaloid extract (200 mg/kg); JW F1 = rat group administered Jimson Weed fruit alkaloid extract (100 mg/kg); JW F2 = rat group administered Jimson Weed fruit alkaloid extract (200 mg/kg).

Effect of Jimson weed alkaloid Extracts on Brain Monoamine oxidase Activity: Also monitored was the brain monoamine oxidase (MAO) activity in treated rats. This showed (Figure 3) that rats administered the leaf and fruit extracts (100 and 200 mg/kg) exhibited significantly ($P<0.05$) reduced MAO activity compared to the control rat group. Nevertheless, no significant difference was observed in the MAO activities among rats administered the leaf and fruit extracts.

Effect of Jimson weed alkaloid Extracts on Brain Antioxidant Status: Furthermore, the brain malondialdehyde (MDA) content in treated rats was also measured (Figure 4). This showed that rats administered the leaf and fruit extracts (100 and 200 mg/kg) exhibited significantly ($P<0.05$) increased MDA content compared to the control rat group. In addition, presented in Table 1 were the results of effect of the alkaloid extracts on brain superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities of treated rats. Table 1 also includes the result of the effect of the alkaloid extracts on brain TSH contents of treated rats. These showed that rats administered the leaf and fruit extracts (100 and 200 mg/kg) exhibited significantly ($P<0.05$) reduced SOD, CAT, and GST activities, as well as TSH contents compared to the control rat group.

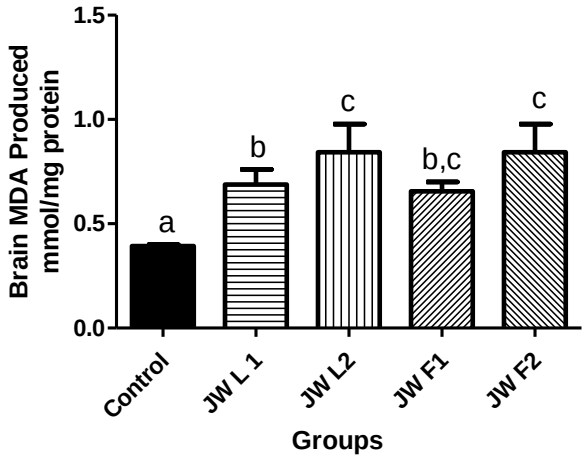

Figure 4:
Malondialdehyde (MDA) content of Brain Tissue of Rats Administered Alkaloid Extracts from the Leaf and Fruit of Jimson Weed (D. stramonium L).
Values represent mean ± standard deviation (n = 6). Mean values with different letters are significantly different ($p < 0.05$).
Key: Control = control rat group; JW L1 = rat group administered Jimson Weed leaf alkaloid extract (100 mg/kg); JW L2 = rat group administered Jimson Weed leaf alkaloid extract (200 mg/kg); JW F1 = rat group administered Jimson Weed fruit alkaloid extract (100 mg/kg); JW F2 = rat group administered Jimson Weed fruit alkaloid extract (200 mg/kg).

Table 1:
Superoxide dismutase (SOD), Catalase (CAT), Glutathione-S-transferase (GST) activities, as well as Total Thiol (TSH) Content of Brain Tissue of Rats Administered Alkaloid Extracts from the Leaf and Fruit of Jimson Weed (D. stramonium L).

	SOD	CAT	GST	TSH
Groups	Protein	U/g	U/mg Protein	mg/g Protein
Control	62.04 ±8.00$_b$	1.49 ±0.15$_b$	75.50 ±5.45$_c$	21.49 ±4.48$_b$
JW L1	35.05 ±2.00$_a$	0.80 ±0.09$_a$	41.45 ±1.00$_b$	15.10 ±2.50$_a$
JW L2	32.05 ±1.50$_a$	0.75 ±0.08$_a$	40.45 ±2.50$_b$	13.50 ±1.45$_a$
JW F1	31.96 ±0.50$_a$	0.72 ±0.10$_a$	40.00 ±2.96$_b$	11.45 ±0.50$_a$
JW F2	30.48 ±1.00$_a$	0.66 ±0.01$_a$	18.50 ±4.50$_a$	12.00 ±4.01$_a$

Values represent mean ± standard deviation (n = 6). Mean values with different letters are significantly different ($p < 0.05$).
Key: Control = control rat group; JW L1 = rat group administered Jimson Weed leaf alkaloid extract (100 mg/kg); JW L2 = rat group administered Jimson Weed leaf alkaloid extract (200 mg/kg); JW F1 = rat group administered Jimson Weed fruit alkaloid extract (100 mg/kg); JW F2 = rat group administered Jimson Weed fruit alkaloid extract (200 mg/kg).

Table 2.
GC-MS characterization of the alkaloid extract from leaf of Jimson weed

S/N	RT(min)*	Compound Name	MW(amu)**	%Total#	CAS Number	EN##
1	7.3544	Amphetamine	135.105	0.9002	000300-62-9	15558
2	10.4229	1-Methyl-2-piperidinone	113.084	0.8592	000931-20-4	6941
3	20.3942	5-Methyl-2-heptanamine	129.152	0.9085	053907-81-6	13050
4	22.0977	2,4-Dimethylamphetamine	163.136	0.3977	075659-61-9	32904
5	24.0207	p-Hydroxynorephedrine	167.095	0.0652	000552-85-2	36231
6	25.184	4-Methoxy-3-hydroxyamphetamine	181.11	0.3369	1000378-88-7	46321
7	28.1161	3-Piperidinol	101.084	0.2821	006859-99-0	4066
8	28.211	Norpseudoephedrine	151.1	0.1123	036393-56-3	24943
9	30.7988	Hyoscyamine (Atropine),	289.168	1.5018	000051-55-8	135262
10	31.5348	Cathine	151.1	0.1092	000492-39-7	24936
11	35.1078	p-Hydroxyamphetamine	151.1	0.3659	000103-86-6	24953
12	35.6005	Phenylephirine	167.095	0.2475	000059-42-7	36222
13	37.2386	3-Methylamphetamine	149.12	0.2747	000588-06-7	23338
14	43.1086	N-Vaneryl-pentadecyl sarcosine ester	383.34	0.4054	1000321-56-8	204024
15	43.4766	n-Hexanoylpentadecyl sarcosine ester	397.356	0.7867	1000321-13-0	210813

**RT (min) = Retention time (min); **MW (amu) = Molecular Weight (atomic mass unit); #% Total= Percentage Total of all compounds; ##Entry Number in NIST11.1 Library*

Table 3.
GC-MS characterization of the alkaloid extract from seed of Jimson weed

S/N	RT(min)*	Compound Name	MW(amu)**	%Total#	CAS Number	EN##
1	9.9658	Histamine-2-carboxylic acid	155.069	0.1713	1000128-89-6	27917
2	12.1262	5-Methyl-2-heptamine	129.152	0.3538	053907-81-6	13050
3	22.0797	Pterin-6-carboxylic acid	207.039	0.5019	000948-60-7	67060
4	22.5605	1-Methyl-2-piperidinone	113.084	1.5631	000931-20-4	6945
5	25.9258	4-Fluorohistamine	129.07	0.5082	049872-60-8	12838
6	28.0269	Tropine trifluoroacetate	237.098	0.3707	1000375-73-8	91172
7	30.7809	Hyoscyamine (Atropine)	289.168	2.9268	000051-55-8	135264
8	31.867	N-(2-aminoethyl)-1,3-propanediamine	117.127	0.1622	013531-52-7	8532
9	32.1935	Scopolamine	303.147	7.0923	000051-34-3	147098
10	33.2737	2,4-Dimethylamphetamine	163.136	0.1513	075659-61-9	32904

**RT (min) = Retention time (min); **MW (amu) = Molecular Weight (atomic mass unit); #% Total= Percentage Total of all compounds; ##Entry Number in NIST11.1 Library*

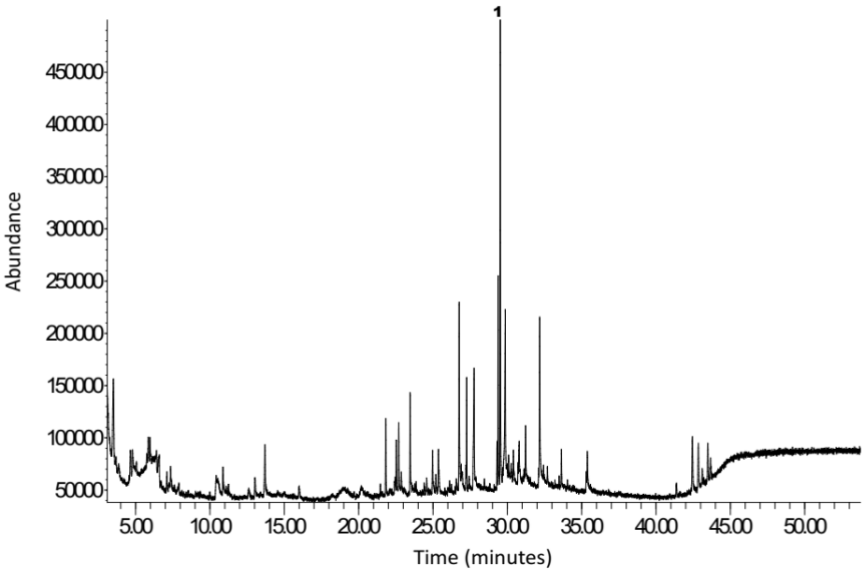

Figure 5a
Total ion chromatogram (TIC) for GC-MS Characterization of the alkaloid extract from leaf of Jimson weed (D. stramonium L). Based on Retention time, peak 1 is suspected to be hyoscyamine (atropine).

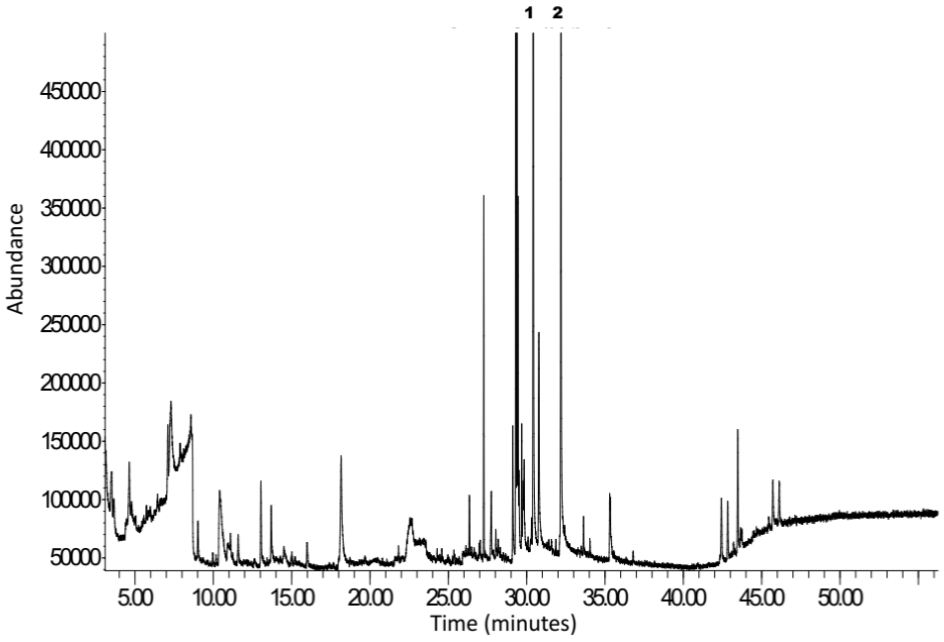

Figure 5b
Total ion chromatogram (TIC) for GC-MS Characterization of the alkaloid extract from seed of Jimson weed (D. stramonium L). Based on retention time, peaks 1 and 2 are suspected to be hyoscyamine (atropine) and scopolamine respectively.

GC-MS Characterization of Jimson weed alkaloid Extracts: The GC-MS characterization of the leaf and fruit alkaloid extracts were presented in Tables 2 and 3, with the chromatographic representations shown in Figures 5a-b respectively.

DISCUSSION

It can be opined that Jimson weed alkaloid extracts can induce impairment in learning and memory function in rats; this could be associated with some of the reported symptoms of Jimson weed poisoning which includes impaired short-term memory, disorientation, confusion and hallucinations (Hasegawa *et al*., 2016). Interactions among the various constituent alkaloid compounds could have resulted in the observed significant reduction in memory index in Jimson weed administered rats. The mechanisms by which these extracts induced impairment in cognitive functions could be through oxidative stress and/or impaired neuronal functions.

Reduction in acetylcholinesterase activity has been reported to be one of the toxicological mechanisms of many environmental toxicants and organopesticides. The ability of Jimson weed leaf and fruit alkaloid extracts to significantly

reduce the activity of AChE (*in vitro*) has been previously reported (Ademiluyi *et al*., 2016a). In addition, Namdeo *et al*., (2013) have earlier reported and associated the neurotoxicity of Jimson weed extract to its anticholinesterase effect in brain of catla fish (*Catla catla*); in this study the leaf extract was reported to induce higher level of neurotoxicity compared to chlorpyriphos. Excessive reduction in brain acetylcholinesterase activity could result into accumulation of acetylcholine at synaptic cleft leading to overstimulation of postsynaptic neurons (Rodrigues *et al*., 2011; McDonald *et al*., 1988), causing neuronal death. Interestingly, prolong impairment of acetylcholinesterase activity in experimental rats exposed to chlorpyriphos and other organophosphate pesticides have been reported to exhibit reduced learning and memory functions (Cañadas *et al*., 2005; Lee *et al*., 2014). Therefore, the observed significant reduced brain acetylcholinesterase activity in rats administered Jimson weed (leaf and fruit) alkaloid extracts, compared to control rats could contribute to the observed reduction in their memory index as observed from the spontaneous alternation (Y maze) behavioral test.

Monoamine oxidase (MAO) catalyzes the oxidative deamination of biogenic amines including dopamine, serotonin, norepinephrine and tyramine (Bouziri *et al*., 2011). Although MAO inhibitors are used as antidepressants (Herraiz *et al*., 2005; Tabakman *et al*., 2003), several toxicological issues are associated with excessive inhibition of monoamines which could be as a result of excessive intake of MAO inhibitors, adverse food-drug reactions or MAO inhibitors from plant sources (Ademiluyi *et al*., 2016a). An example is toxicity resulting from excessive intra-synaptic serotonin which is referred to as serotonin toxicity; this usually results from excessive inhibition of MAO. Serotonin toxicity has been clearly characterized as a triad of neuro-excitatory features which are neuromuscular hyperactivity, autonomic hyperactivity, and altered mental status, agitation, excitement and confusion (Ben-Shlomo *et al*., 2004). Therefore, as observed in this study, the increased anxiety-like behavior in Jimson weed treated rats could be associated with their observed MAO inhibition. Furthermore, previous findings have indicates that under conditions of compromised defense systems or impaired regulatory mechanisms in the brain, accumulation of dopamine accelerates neurodegeneration in rats (Stafford *et al*., 2007). Therefore, the observed reduction of MAO activity in Jimson weed extracts administered rats could lead to increase in brain dopamine level and thus, initiate and accelerate neurodegeneration. Several studies have equally reported the MAO inhibitory properties of plant alkaloid extracts (Ademiluyi *et al*., 2016a; Chen *et al*., 2006; Stafford *et al*., 2007).

Free radicals such as reactive oxygen species (ROS)-induced lipid peroxidation have been connected in the origin and development of various diseases. Lipid peroxidation is assessed indirectly by the measurement of the secondary products, such as malondialdehyde (MDA) (Anoopkumar-Dukie *et al*., 2001). MDA is a three-carbon low molecular weight aldehyde and spontaneous breakdown product of peroxides that can be produced from free radical attack on poly unsaturated fatty acids (Pilz *et al*., 2000; Slatter *et al*., 2000) which makes up the cell membrane. Free radical attack on neural cells contributes calamitous role to neurodegeneration. Toxicity of ROS contributes to protein misfolding, glia cell activation, mitochondrial dysfunction and subsequent cellular apoptosis (Fulda *et al*., 2010). From the result gotten from this study, rats administered Jimson leaf and fruit extracts have high levels of brain MDA content compared to control rats which signifies high level of lipid peroxidation; this could be as a result of excessive free radicals generation and/or impaired antioxidant system. Membrane lipids are an integral structural part of neuronal cell membrane. Therefore, high level of lipid peroxidation in neuronal cells could disrupt the cell membrane, which could compromise the cells structural and functional integrity and may result in loss of intracellular contents.

The impairment of brain antioxidant systems could be another indicator of neurotoxic potentials of Jimson weed leaf and fruit alkaloid extracts. As observed from this study, both leaf and fruit extracts reduced the activities of the antioxidant enzymes (catalase GST and SOD) and also reduced the level of the non-enzymatic antioxidant molecule (thiols) in brain of treated rats. These impaired antioxidant systems could cause a shift in the delicate balance between antioxidants and free radicals in favor of the latter, resulting in oxidative stress. Oxidative stress in body tissues leads to cellular damage and this can be related to low level of antioxidant defense systems such as catalase, glutathione, superoxide dismutase (SOD) and glutathione-S-tranferase (GST) (Elkashef *et al*., 1999). The brain has been reported to be particularly sensitive to oxidative damage caused by high level of lipid content and high metabolic rate (Oboh *et al*., 2016). The brain is extremely susceptible to highly reactive free radicals (Oboh *et al*., 2016); these free radicals cause varying damages leading to cascade of neurochemical events culminating into neurodegeneration and cell death (Vesna *et al*., 2003). Superoxide dismutase (SOD) is an antioxidant enzyme which neutralizes superoxide anion to a less reactive hydrogen peroxide (H_2O_2). This means a reduced level of SOD signifies an increased level of ROS or free radicals. The action of SOD therefore is to protect the biological integrity of the cells and tissues against harmful effects of superoxide free radical by inactivating superoxide free radicals. The H_2O_2 produced by the action of SOD is converted to water and molecular oxygen by catalase. Catalase is a ubiquitous antioxidant enzyme found in all known organisms and it provides protection against oxidative stress (Bemtssen *et al*., 2003).

Previous studies have reported an association between oxidative stress and impaired cognitive function. Oxidative stress has been implicated in mitochondrial dysfunction and neuronal cell death which are often precursors to many neurodegenerative diseases (Wang *et al*., 2014). Also, previous studies have shown that oxidative stress can induce anxiety-like behaviors and cognitive impairments in experimental animals (Allam *et al*., 2013). Therefore, the observed anxiety-like behavior and impaired memory index observed in Jimson weed administered rats could also be associated with the reduction in their brain antioxidant status which could induce oxidative stress. Furthermore, in this study, the mechanism(s) behind the observed significant reduction in brain acetylcholinesterase activity in Jimson weed administered might not be fully understood.

Nevertheless, there are hypothesis implicating oxidative stress in reduced acetylcholinesterase activity (Liu *et al*., 2017). In addition, it has been reported that oxidative stress could trigger reduction in acetylcholinesterase mRNA expression (Isik *et al*., 2017).

The GC-MS characterization study shows the presence of tropane alkaloids (hyoscyamine [atropine] and scopolamine) as the predominant constituent alkaloids, as well as compounds such as amphetamine and its derivatives (2, 4-dimethylamphetamine, p-hydroxyamphetamine, 4-methoxy-3-hydroxyamphetamine and 3-methylamphetamine), cathine, tropine trifluoroacetate and phenylephrine among others; this is an improvement on our previous report (Ademiluyi *et al*., 2016b). The tropane alkaloids (hyoscyamine (atropine) and scopolamine) are well established central nervous system modulators which elicit neurological effects such as hallucination, depression and memory impairment (Ademosun *et al*., 2014). Other identified compounds such as amphetamine and cathine have also been reported to have several neuromodulatory effects (Funada *et al*., 2014; Santillo *et al*., 2014; Gashawa *et al*., 2015). Hence, the significant impairment in the neuronal enzyme systems understudied in rats administered Jimson weed alkaloid extracts could be linked to the presence of these neuromodulatory compounds.

In conclusion, this study has revealed that rat administered alkaloid extracts from leaf and fruit of Jimson weed exhibited impaired activity of brain AChE, MAO, and antioxidant enzymes as well as reduced levels of their TSH content and elevated brain MDA content. These impairments could be significantly correlated to the observed reduced memory indices and elevated anxiety-like behavior in Jimson weed administered rats compared to the control rats. This study which has hence, provided correlation between some behavioral indices of Jimson weed-induced neurotoxicity and some possible underlying biochemical mechanisms could further improve understanding on its neurotoxicity and possibly improve diagnosis and treatment of Jimson weed-induced neurotoxicity.

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