

Neuroprotective, Behavioural and Biochemical Effects of Aqueous Crude Root Extract of *Salacia reticulata* on Aluminium Chloride Induced Damage in the Cerebral Cortex of Adult Male Wistar Rats

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Received: January 07, 2019; **Published:** March 13, 2019

DOI: 10.31080/ASCB.2019.03.0115

Abstract

Heavy metals like aluminium chloride has proven to have an effect with learning, understanding, memory, concentration and decision making, which affect everyday life of people in urban areas. The study was designed to ascertain if *Salacia reticulata* can protect the neurons of the cerebral cortex, alleviate anxiety, as well as protect the liver. Twenty-five (25) male wistar rats were randomly selected and divided into five groups of five (5) animals each as follows: Group A: Normal control; Group B: AlCl₃ control; Group C: AlCl₃ + 250mg/kgbw *salacia reticulata*; Group D: AlCl₃ + 300mg/kgbw *salacia reticulata*; Group E: AlCl₃ + Vitamin E. The following tests were carried out: Y-Maze test, Liver enzymes test and histopathological test. The results from the Y-maze test showed decrease in the meantime taken by the animals to make alternations in the aluminium chloride treated group compared to the normal control group, implying that AlCl₃ induced anxiety. The meantime increased in groups treated with *Salacia reticulata*. Histological examination showed full neuronal damage in the AlCl₃ treated group only, partial restoration of cellular and neutrophil profile was seen in the low dose groups treated with *Salacia reticulata*, restoration of cellular and neutrophil profile was seen in groups treated with high doses of *Salacia reticulata*. In AlCl₃ treated group, AST, ALT, ALP increased rapidly, while in *Salacia reticulata* and vitamin E treated groups, the level of these enzymes were reduced. From the results obtained, Aqueous Crude Root Extract of *Salacia reticulata* has Neuroprotective properties, it is capable of alleviating anxiety and stabilizing liver enzymes.

Keywords: Neuroprotective; *Salacia reticulata*; AlCl₃; Anxiety; Cerebral Cortex; Wistar Rats

Introduction

The cerebral cortex is a sheet of neural tissue that is outer-most to the cerebrum of the mammalian brain. The cerebral cortex has been reported to be the biggest part of the brain. This large and complicated neural circuit is involved in most of the brain's highest functions, such as memory, language and sight. In man and higher animals, modifications of behaviour are due to cortical activity [1]. It also integrates higher mental functions, general movement, visceral functions, and behavioural reactions. The cerebral cortex contains motor areas, which control complex motor skills and some involuntary movements; sensory areas, which receive information from the sense organs; and association areas, which are responsible for thought, learning language, and personality [2,3].

Heavy metals are potentially toxic to human and can lead to neural diseases [4]. Some researchers have reported that high concentrations of heavy metals (lead, arsenic) damages the nervous, endocrine, circulatory and excretory system [4].

Some authors have reported that drinking water with heavy metal toxicity has fatal effects which include reduced mental and central nervous function. They also cause irregularity in blood composition, badly effect vital organs such as kidneys and liver [5]. The long-term exposure of these metals result in physical, muscular, neurological degenerative processes that cause Alzheimer's disease (brain disorder), Parkinson's disease (degenerative disease of the brain), muscular dystrophy (progressive skeletal muscle weakness), multiple sclerosis (a nervous system disease that affects

brain and spinal cord), Also, Lead is one of the most common heavy metal in drinking water, if occurred more than its permissible limit shows general metabolic poison and enzyme inhibitor [6]. Heavy metals like aluminium chloride has proven to have an effect with learning, understanding, memory, concentration and decision making, which affect everyday life of people in urban areas.

Results from this study, can be used to improve cognitive impairment, neurobehavioral and memory burden in subjects that are been exposed to heavy metals in rural and urban areas in Nigeria. And Africa at large.

Materials and Methods

Reagent and Materials

The Reagents that were used are 10% formal calcium, 10% formal saline, formalin, methylated spirit, cotton wool, lab coat, sterile surgical blades, table top, hand gloves, face mask water bath, light microscope, memory card, digital camera, aqueous extract, Bouin's fluid, H&E stain, Bielschowsky stain.

Equipment

Equipment's used for this experiment are; cages, water rubbers, Ymaze, wistar rats, weighing scale, pencil, rotary microtome, slides, cover slips, Uranium (235U), light water, Beryllium, Nitric acid, gamma-ray data acquisition system, A Multi-Purpose Gamma-Ray, lab coat, water bath, light microscope, memory card, digital camera, Bouin's fluid, H&E stain, Bielschowsky stain.

Collection and extraction of plant

The plant (*Salacia reticulata*) was harvested from Agbonchia, Nchia, in Eleme local Government in Rivers state, Nigeria. The U.S. Gallah of institute of biochemical Research NARICT Zaria, Kaduna state, Nigeria, verified the plant. Extraction was carried out using water.

Procedure for phytochemical screening

50% of the crude extract was partitioned using petroleum, ether, benzene and ethyl acetate. And the remaining left was dissolved in methanol; all fractions obtained were subjected to phytochemical screening employing the standard screening test [7].

Experimental protocol

Twenty-five (25) male wistar rats were randomly selected and divided into five groups of five (5) animals each as follows:

Groups (n=5)	Duration	Treatment	Administration
A	14days	Normal saline	Oral
B	14days	AlCl ₃ (65mg/kg bw) orally	Oral
C	14days	AlCl ₃ (65mg/kg bw single dose) + <i>S. reticulata</i> 250mg/kgbw orally	Oral
D	14days	AlCl ₃ (65mg/kg bw for 7 days) single dose orally + <i>S. reticulata</i> 300mg/kgbw for 7 days	Oral
E	14days	AlCl ₃ (65mg/kg bw orally) + Vitamin E 250mg/kgbw orally	Oral

Table 1

Learning and memory (Y maze alternation Test)

Learning and memory test was carried out in a Y-maze with three [3] white opaque plastic arms at an angle of 120° from each other, labelled A, B, C respectively. This Y-maze test measures the ability of experimental animals to explore new environments; parts of the brain including the hippocampus, septum, basal forebrain and prefrontal cortex- are involved in this task [8]. Modified forced alternation test was used as protocol [8].

Learning and memory will have carried out three times for each group on the first day of administration, 7th day of administration and final day of administration. Learning and memory test using wistar rats was carried out three days of animal handling. The test involves two steps (sample trial and retrieval trial), each of these steps requires five minutes. For the aluminium experiment, wistar rats were doused with aluminium chloride before sample trial. In step one (sample trial), the wistar rats were placed at the end of the start arm, facing the wall and away from the Centre. And later allowed to explore two arms of the Y-maze, while entry into the third arm was blocked.

At the end of the first step, rats were returned to its home cage for a 30 min inter-trial interval. In the second step, the block in arm 3 was removed; the rats were again placed into the start arm, and then allowed to access all three arms of the maze. Any

rat that climbs on the maze wall was immediately returned into the abandoned maze arm. Inorder to prevent odour cues, between step one and step two, the maze was wiped with a Quatricide® dilution. When 85% of a rat’s body entered one arm, it is recorded as an arm entry. While the time spent in the novel arm divided by the time spent in all arms during the retrieval trial (second step) was recorded as Novel Arm. The number of rats in percentage entering first the novel arm during second step was recorded as Forced Alternation (%). Rats that were unable to explore three arm entries within the first sixty seconds of the second step of the test were excluded from the analysis [8].

Animal sacrifice

At the end of the experiment, body weight of the rats was obtained using a digital weighing balance; the animals were anaesthetize using chloroform and humanely sacrificed. Brain tissues were assessed by opening the sutures of the skull.

Biochemical analysis

The serum was centrifuged and analysed to quantify liver enzymes, (ALT, AST, and ALP). Protein, albumin and globulin presence were also analysed using an autoanalyzer machine.

Histological analysis

Harvested brain tissue was fixed in 10% formal calcium. Histological staining of samples was the carried out in the Department of Human Anatomy, ABU, Zaria. These tissues were stained using Hematoxylin and Eosin for general histological architecture. The special stain used for neurological cells and fibers was Bielchowsky’s silvers stain.

The slides were mounted and studied using an Olympus microscope. And photomicrographs were taken with the aid of a digital camera (Amscope, MD 900). Digital camera was placed in the eyepiece of an Olympus microscope, image was taken using application installed in a Dell laptop Latitude 2120.

Statistical analysis

Data was expressed as mean ± SD. One-way ANOVA was used to test for the statistical difference between more than two groups. Dunnett’s post hoc test was used to test were the significance lies. SPSS version 21 was used. p<0.05 was considered as statistically significant

Results

Table 2 shows the presences of phytochemical substances in *Salacia reticulata*. It also shows that Saponins are more abundant in concentration than other chemicals.

Serial number	Constituents	Tests	Inference
1	Carbohydrate	Molisch test	+
2	Anthraquinones	Bontragers test	+
3	Cardiac glycosides	Kelle killani test	+
4	Saponins	Frothing test	+++
5	Steroids and Triterpene	Liberman Buchard test	+
6	Tannins	Fervic Chloride test	+
7	Flavonoids	Shinodes test	+
8	Alkaloids	Dragondorff Test	+
I. Key: + = present; - = absent; +++ abundance			

Table 2: Phytochemical result.

Morphological result [body weight]

Table 3, administration of aluminum chloride, or *Salacia reticulata* or Vitamin E affected the weight of the animal. There was a significant decrease in weight of animals in group B, C and D, while Group A and E weight increased. There was a significant difference at p<0.05 between the Aluminum chloride and *Salacia reticulata* treated groups (C, and D) and the normal control group, which implies that aluminum chloride reduced the weight of the rats. It was observed that animals in Group C and D (*Salacia reticulata* treated groups), lost their weights after 14 days of treatment, while animals in Group E (vitamin E treated group) gained weight in course of treatment. (Table 4)

Groups (n=5)	Initial weight	Final weight
A	73.60 ± 2.50	81.60 ± 2.70
B	79.00 ± 2.64	77.20 ± 1.92
C	74.80 ± 3.56	71.20 ± 4.32*
D	76.80 ± 4.43	72.80 ± 4.76*
E	78.40 ± 2.70	81.60 ± 2.30

The values of the mean body of wistar rats in grams were expressed as mean ± standard deviation (SD). * = p<0.05 . Group A: Normal control; Group B: Alcl₃ control; Group C: Alcl₃+ 250mg/kgbw *salacia reticulata*; Group D: Alcl₃+ 300mg/kgbw *salacia reticulate*; Group E: shows a level of significance.

Table 3: Mean initial and final body weight of adult male wistar rat.

Groups (n=5)	Brain
A	1.48 ± 0.04
B	1.44 ± 0.08
C	1.48 ± 0.04
D	1.50 ± 0.00
E	1.50 ± 0.14

Table 4: Mean brain weight of adult male wistar rat.

Behavioural performance

Tables 5 and 6, showed a decrease in the meantime taken by the experimental animals to make alternations and to explore new arms in the y-maze test especially in Group B (AlCl₃ only) and also in group C (AlCl₃ + 250mg/kgbw *salacia reticulata*).

Groups (n=5)	SABPT (%)	SABPT (1 %)	SABPT (2 %)
A	69.00 ± 4.84	54.80 ± 8.54	56.20 ± 8.37
B	61.00 ± 4.71	49.00 ± 6.51*	45.80 ± 6.97*
C	66.80 ± 7.70	54.00 ± 11.40	57.80 ± 5.40
D	70.00 ± 3.53	52.00 ± 6.12	54.00 ± 6.51
E	68.80 ± 5.61	50.00 ± 8.36*	53.36 ± 8.36*
The values of the mean body of wistar rats in grams were expressed as mean ± standard deviation (SD). * = p<0.05 . Group A: Normal control; Group B: AlCl ₃ control; Group C: AlCl ₃ + 250mg/kgbw <i>salacia reticulata</i> ; Group D: AlCl ₃ + 300mg/kgbw <i>salacia reticulata</i> ; Group E: shows a level of significance. SABPT % = Spontaneous alternation behaviour test			

Table 5: Spontaneous alternation test.

Groups (n=5)	NAEPT	NAET1	NAET2
A	8.40 ± 2.88	10.40 ± 3.20	12.60 ± 2.60
B	11.00 ± 5.78	8.80 ± 2.48	5.80 ± 1.30*
C	7.40 ± 1.51	6.20 ± 3.96	6.40 ± 3.75*
D	9.00 ± 3.46	5.60 ± 0.54	7.40 ± 2.50
E	11.20 ± 2.38	9.00 ± 1.87	9.40 ± 2.28
The values of the mean body of wistar rats in grams were expressed as mean ± standard deviation (SD). * = p<0.05 . Group A; Normal control; Group B: AlCl ₃ control; Group C: AlCl ₃ + 250mg/kgbw <i>salacia reticulata</i> ; Group D: AlCl ₃ + 300mg/kgbw <i>salacia reticulata</i> ; Group E: shows a level of significance. NAEPT = Number of arm entering test			

Table 6: Number of arm entering.

Biochemical Results

AST, ALT, ALP levels were elevated significantly (p < 0.05) in AlCl₃ when compared with normal control (group A). *salacia reticulata* and vitamin E treated group expressed significantly (p < 0.05) reduced level of ALT and AST when compared to normal group. *Salacia reticulata* and Vitamin E stabilizes liver enzyme level in Group C, D and E, while Group B showed high level. A significant difference was observed between Group A and Group B. (Table 7)

Values expressed as mea enzyme ± SD of the serum liver (U/L) of wistar rats during the experiment. Values with superscript in a column are significantly different (p < 0.05). A; Normal Control; B AlCl₃; Group C AlCl₃+ 250mg/kgbw *salacia* extract; Group D AlCl₃+ 300mg/kgbw *salacia reticulata*; Group E AlCl₃+ Vitamin E. T-protein; Albumin; Globulin.(Table 8)

Groups (n=5)	AST (U/L)	ALT (U/L)	ALP (U/L)
A	67.60 ± 3.61	45.80 ± 5.31	105.8 ± 4.43
B	79.90 ± 1.00	62.80 ± 2.86	125.2 ± 2.4
C	66.20 ± 1.64	46.00 ± 5.14	89.00 ± 1.41
D	69.60 ± 5.54	48.80 ± 1.78	93.60 ± 2.50*
e	69.20 ± 1.64	46.80 ± 0.83	88.60 ± 1.34*
The values of the mean body of wistar rats in grams were expressed as mean ± standard deviation (SD). * = p<0.05 . Group A: Normal control; Group B: AlCl ₃ control; Group C: AlCl ₃ + 250mg/kgbw <i>salacia reticulata</i> ; Group D: AlCl ₃ + 300mg/kgbw <i>salacia reticulata</i> ; Group E: shows a level of significance. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase.			

Table 7: Mean of liver serum enzyme test.

Groups (n=5)	T-protein (g/dl)	Albumin (g/l)	Globulin (g/l)
A	5.66 ± 0.05	3.82 ± 0.83	105.8 ± 4.43
B	5.22 ± 34.2	3.94 ± 0.19	125.2 ± 2.4*
C	5.70 ± 0.10	4.20 ± 0.10	89.00 ± 1.41
D	5.76 ± 0.05	0.39 ± 0.54	93.60 ± 2.50
E	5.64 ± 0.54	0.42 ± 0.10	88.60 ± 1.34

Table 8: Mean value of T-protein, Albumin and Globulin.

Histological Results

Heamatoxylin and Eosin Stain [9].

The normal group (Plate 1) shows normal cellular profile and neutrophil there was no observable neuronal damage. The AlCl₃ control group B (Plate 2) shows full neuronal damage to the brain cells and neutrophil (14 days). Group C (Plate 3) shows partial restoration of cellular and neutrophil profile with *Salacia reticulata* treated for (14days). Group D (Plate 4) shows full recovery of cellular and neutrophil profile (14 days). Group E (Plate 5) shows partial recovery of neural cells and cerebral cortex profile.

Plate 1: Photomicrograph of the cerebral cortex Group A, normal Cellular profile(PC) (H&E) x100.

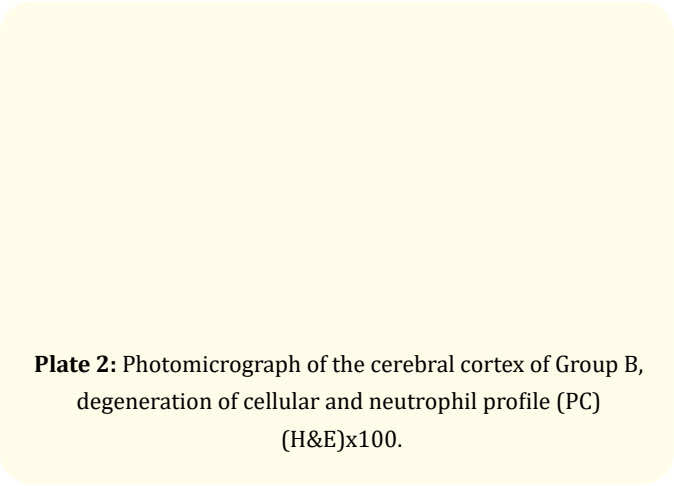


Plate 2: Photomicrograph of the cerebral cortex of Group B, degeneration of cellular and neutrophil profile (PC) (H&E)x100.

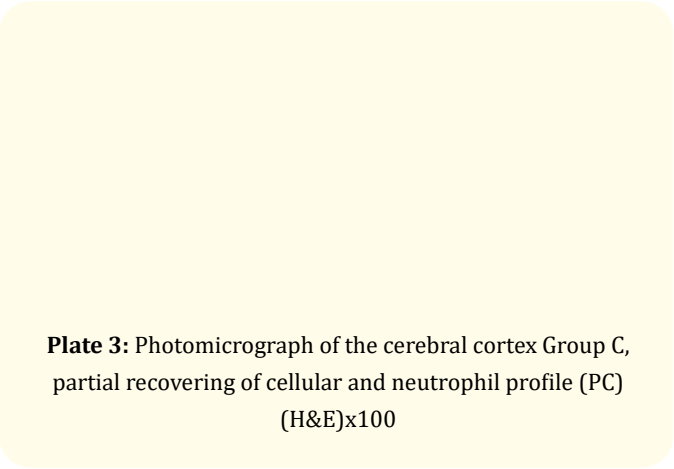


Plate 3: Photomicrograph of the cerebral cortex Group C, partial recovering of cellular and neutrophil profile (PC) (H&E)x100

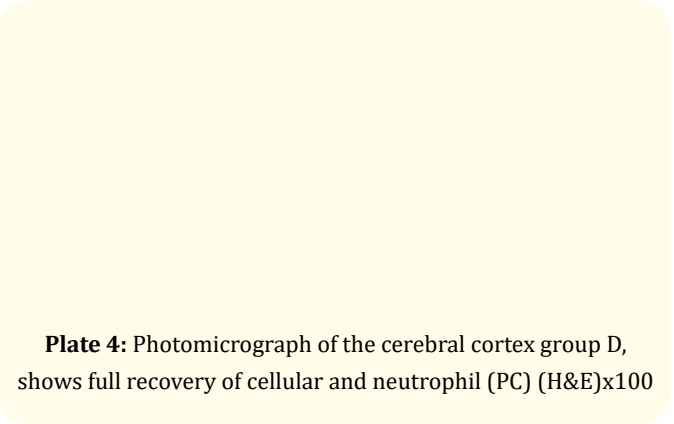


Plate 4: Photomicrograph of the cerebral cortex group D, shows full recovery of cellular and neutrophil (PC) (H&E)x100

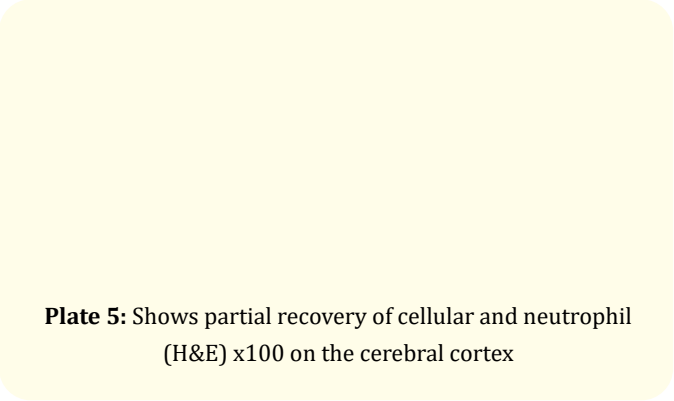


Plate 5: Shows partial recovery of cellular and neutrophil (H&E) x100 on the cerebral cortex

Silver stain [10].

The normal group A (Plate 6) shows normal pyramidal cells and neutrophil there was no observable neuronal damage. The AlCl_3 control group B (Plate 7) shows full degeneration of pyramidal cells and neutrophil (14 days). Group C (Plate 8) shows partial restoration of pyramidal cells and neutrophil profile with *salacia reticulata* treated for (14 days). Group D (Plate 9) shows full recovery of pyramidal cell and neutrophil profile. Group E shows partial recovery using vitamin for administration for 14days. (Plate 10)

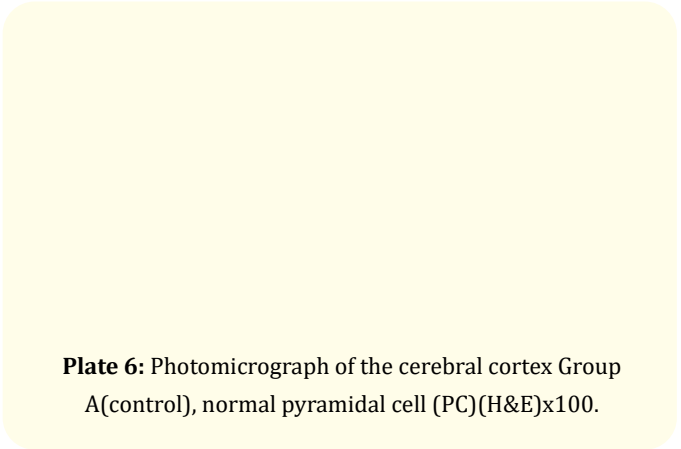


Plate 6: Photomicrograph of the cerebral cortex Group A(control), normal pyramidal cell (PC)(H&E)x100.

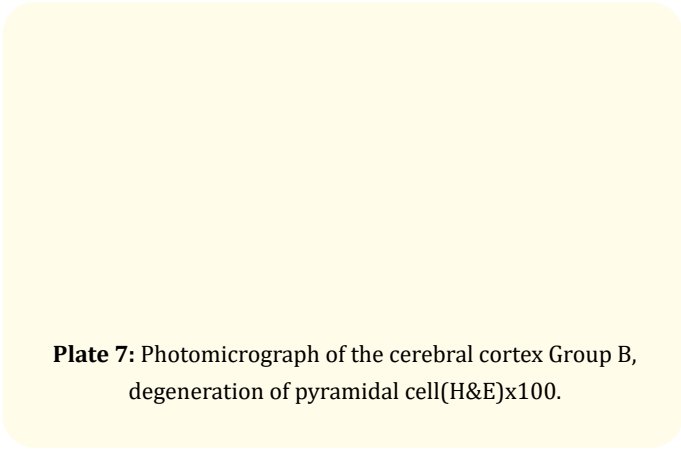


Plate 7: Photomicrograph of the cerebral cortex Group B, degeneration of pyramidal cell(H&E)x100.

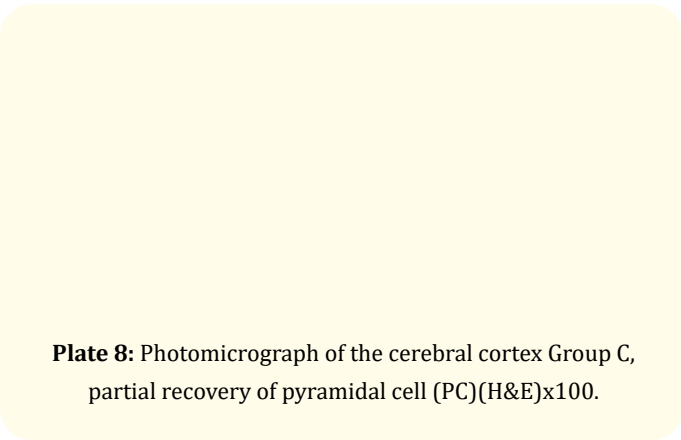


Plate 8: Photomicrograph of the cerebral cortex Group C, partial recovery of pyramidal cell (PC)(H&E)x100.

Plate 9: Photomicrograph of the cerebral cortex Group D, showing recovery of the Pyramidal cells (PC), (H&E)x100.

Plate 10: Photomicrograph of the cerebral cortex Group E, partial regeneration of pyramidal cells (PC), neuropin(N), radial fibers (RF), (H&E) x100.

Discussion

Morphological result

Table 3, Administration of aluminium chloride and treatment with *Salacia reticulata* and Vitamin E affected the body weight of experimental animals. There was a significant decrease in weight of animals in group B, C and D, while in Group A and E weight increased. The findings of [11] were in agreement with this study.

Behavioural performance

The study showed a decrease in the meantime taken by the experimental animals to make alternations and to explore new arms in the y-maze test as shown in Tables 5 and 6. The decrease in time taken for animals in group B to explore arms when compared with animals in group A could be as a result of anxiety which is associated with aluminium treatment [12]. *Salacia reticulata* administration was able to increase the time even better than vitamin E, implying that *Salacia reticulata* is able to alleviate anxiety.

Histological Results

The normal group (plate1) shows normal cellular profile and neutrophil there was no observable neuronal damage. The

AlCl₃ control group B (plate2) shows full neuronal damage to the brain cells and neutrophil (14days). This supports a hypothetical statement by [13]. that Aluminium exposure has neuro-degenerating effect resulting in learning deficits. Group C (plate 3) shows partial restoration of cellular and neutrophil profile with *Salacia reticulata* treated for (14days). Group D (Plate 4) shows full recovery of cellular and neutrophil profile (14days). Group E (plate 5) shows partial recovery of neural cells and cerebral cortex profile. *Salacia reticulata* treated groups (Plate 3 and 4), showed restoration of cellular and neutrophil profile when compared with Plate 2 (AlCl₃ treated groups) where the cells are damaged; this confirms that *Salacia reticulata* has neuroprotective effects on brain cells. Plate 5 (vitamin E treated group), showed partial recovery when compared with Plate 4 (*Salacia reticulata* treated groups), which showed full recovery of cellular profile and preservation of neuronal cells, implying that more antioxidants properties found in *Salacia reticulata* than in vitamin E. The result from this study is in agreement with the findings of [14].

The normal group A (plate 6) shows normal pyramidal cells and neutrophil there was no observable neuronal damage. The AlCl₃ control group B (plate 7) shows full degeneration of pyramidal cells and neutrophil (14 days). Group C (plate 8) shows partial restoration of pyramidal cells and neutrophil profile with *Salacia reticulata* treated for (14 days). Group D (Plate 9) shows full recovery of pyramidal cell and neutrophil profile. Group E shows partial recovery using vitamin E for administration for 14days. The results collaborate with [15]. They reported that ethanolic root extract of *Salacia reticulata* improves memory and cognition in Mercury Chloride induced cellular damage of the hippocampus adult male mice.

Biochemical results

In group B (AlCl₃), AST, ALT, ALP increased rapidly, which implies that aluminium chloride treatment has damaged the liver. This result is in agreement with [11], they reported congested central vein and distorted sinusoids in the liver of wistar rats due to aluminium chloride exposure. *Salacia reticulata* and vitamin E treated group expressed significantly (p < 0.05) reduced level of ALT and AST when compared to normal group, which implies that *Salacia reticulata* and Vitamin E are capable of stabilizing liver enzyme. Total protein, Albumin and globulin shows that the Aluminium chloride treatment affected the biochemical parameters negatively while *Salacia reticulata* treatment attenuates these parameters resulting in the positive improvement in the animal behaviour decreasing anxiety.

Conclusion

From the results obtained, Aqueous Crude Root Extract of *Salacia reticulata* at the above recommended dosage has

Neuroprotective properties and is capable of alleviating anxiety. Salacia reticula also stabilizes liver enzymes and attenuates total protein, globulin and albumin levels in aluminium chloride (AlCl₃) induced cellular damage the cerebral cortex in Wistar rats.

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Volume 3 Issue 4 April 2019
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