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Antidegenerative and Neurobehavioral Effects of Ethanolic Root Extract of *Salacia reticulata* on Mercury Chloride Induced Cellular Damage in the Hippocampus of Adult Male Mice

Godam Elv Tams*[1], Jibarang Nenwarngdung Kani[2], Didia Blessing C[1] and Akpulu Stephen Peter[1]

[1]*Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Rivers State Nigeria*

[2]*Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Bingham University, Karu Nasarawa State, Nigeria*

***Corresponding author:** Godam Elvis Tams, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Rivers State Nigeria, Tel: +2348063339602; E-mail: elvisgodam@gmail.com

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Abstract

Mercury is a very harmful heavy metal and it has been shown to affect the nervous system mainly, and has contributed a lot to neurodegenerative diseases and disorders. Through time, there has been an increase in the search for more knowledge on how to address the treatment of the diseases associated with Mercury Chloride exposure. Researchers have shown some protective effect of some medicinal plants, which is why this study is done to investigate *Salacia reticulata* for protective effects against nervous disorders. The study was aimed at evaluating the antidegenerative effects of *Salacia reticulata* on mercury chloride exposure on the cellular architecture and spatial learning and memory of adult male mice. Twenty-five male mice with an average weight of (14.5-26 g) were divided into five groups of five mice per group and treated as follows. Group A; control group=2 mls of Placebo, Group B ($HgCl_2$ at 7 mg/kgbw). Group C ($HgCl_2$ at (7 mg/kgbw with *Salacia reticulata* at (200 mg/kgbw), Group D (HgCl at 7 mg/kgbw) with *Salacia reticulata* at (250 mg/kgbw). Group E ($HgCl_2$; 7 mg/kgbw, with 0.2 ml pu of Vitamin E. $Hgcl_2$ was administered at a single dose orally while *S. reticulate* was administered orally daily for 14 days. The results from the Y-maze test showed an increase in the meantime taken by the animals to make alternations in the mercury treated group compared to the normal control group and *Salacia reticulata* treated groups, this is suggestive of neurological toxicity of mercury chloride to learning and memory loss. Histological results showed degeneration of the pyramidal cells of the hippocampus in mercury chloride treated group, while preservation of cellular profile and neuronal cells was seen in the *Salacia reticulata* treated group C and D as compared to group B ($HgCl_2$ group only) and Vitamin E treated groups. Therefore, *S. reticulata* ameliorated memory loss in the hippocampus caused by mercury toxicity in adult male mice and is neuroprotective as seen in the microarchitecture of Pyramidal cells, fibers and neuropil evidenced in the hippocampus of mice treated.

Keywords: Toxicity; Anthropogenic processes; Extraction of plant; Histological

Introduction

Human and animal populations interact with their environment on a daily basis and as such are exposed to a range of chemicals and heavy metals such as mercury, lead, thalium, aluminium and cadmium [1]. These interactions occur through food, air, and water in the environment [2], Mercury occur in the environment due to natural processes like degassing from earth crust, emissions from volcanoes and evaporation from water bodies and anthropogenic processes, particularly from coal-fires, power stations, residential heating systems and waste incinerators [1]. There is a growing appreciation of the effects that exposure to heavy metals such as mercury, lead, cadmium and aluminum may have on the nervous system. The toxicity of these compounds is variable and diffuses involving different parts of the nervous system [3].

Mercury has been a major nervous system problem over decades [4]; it is a potential factor in brain damage [5], mental impairment and behavioural anomalies [6], neuromuscular weaknesses, hearing problems, cognitive functions and coma [7-9]. This is because some of these heavy metals can cross the blood brain barrier and accumulate in brain tissues thus causing damage to these tissues [10]. Toxicity of mercury can result from inhalation, ingestions, and absorption through the skin.

The nervous, digestive and renal systems are most commonly affected in mercury exposure, while children and pregnant women are most vulnerable to mercury exposure [11].

Materials and Methods

Materials

The materials used in the study are; Cage, feeding trough, weighing balance, feed (vital feed), distil water, syringes and needles, oral cannula, gloves, glass slides (plain), dissecting set, dissecting board, plain bottles, specimen bottles, reagents bottles, beakers, test tubes, cotton wool, microscope, drinking bottles, stopwatch, EDTA bottles, and Y-maze test.

Reagents

The reagents used in the study are; fixative (10% Formal Calcium solution), Normal saline, disinfectant (Hypo), methylated spirit, Vitamin E, Haematoxylin and Eosin (H&E) stain, Bieslschowsky stain

(Silver Stain), Mercury Chloride, 70% Ethanol, N-Hexane, ethyl acetate, and N-Butanol.

Collection and extraction of plant

Salacia reticulata root was sourced from Agbonchia farmland, Nchia, Eleme Local Government in Rivers state, Nigeria. The plant was verified by U.S Gallah of National Research institute of chemical technology, NARICT Zaria, Kaduna State, Nigeria. It was extracted with 70% ethanol and was fractionated with the following solvents; N-Hexane, ethyl acetate, and N-Butanol. The extract was kept in an air tight bottle, and was reconstituted in appropriate volume of distilled water and administered.

Phytochemical screening

Half of the crude extract was partitioned with petroleum, ether, benzene, chloroform, and ethyl acetate. The residue left was dissolved in methanol and the various fractions obtained were all subjected to Phytochemical screening employing the standard screening test [12]. The following tests were conducted.

Test for carbohydrates

Molisch test: To a small portion of the extract in a test tube, few drops of Molisch reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a layer, a reddish colored ring at the interphase indicates the presence of carbohydrate [12].

Test for free Anthracene derivatives (Bontrager's Test)

To a portion of the extract in a dry test tube, 5 ml of chloroform was added and was shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonia solution, bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones [12].

Test for unsaturated Steroid and Triterpenes

Liebermam Bucchard test: To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1 ml of concentrated sulphuric acid was added down the side of test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple color indicates the presence of triterpene [12].

Test for Cardiac glycosides

Keller-kiliani test: A portion of the extract was dissolved in 1 ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1 ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observe carefully at the interphase for purple-brown ring. This indicates the presence of desoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycoside [12].

Test for saponin glycoside

Frothing test: About 10 ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates presence of saponins [12].

Test for tannins

Ferric chloride test: To a portion of the extract, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish-blue precipitate [12].

Test for flavonoids

Shinoda test: A portion of the extract was dissolved 1-2 ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red colour indicates presence of flavonoids [12].

Test for alkaloids

Dragendoff's test: To a portion of the extract, few drops of dragendoff reagent were added. A rediish brown precipitate indicates presence of alkaloids [12].

Animal procurement

Thirty five adult male mice were sourced from National Veterinary Research Institute (NVRI) Vom in Jos-South Local Government area of Plateau State, Nigeria.

Animal care and conditioning (Handling)

The mice weighed between 14 g-26.5 g. They were kept in well ventilated cages cushioned with saw dust in the animal house of Bingham University, Karu, Nasarawa state, Nigeria. They were acclimatized for 2 weeks, and kept under standard conditions of room temperature and 12:12 hours light and dark cycle respectively. The Wistar rats were fed with standardized pellet (Vital Feed Limited Nyanya, Federal Capital Territory Abuja, Nigeria) and tap water ad libitum. The mice cages were regularly cleaned and saw dust changed every day.

Experimental grouping and treatment protocol

The animals were divided in five groups of five mice each and a pre-test using Y-maze to assess the animals' exhibit normal scores for memory and behaviour. Animals exhibiting abnormal test scores were excluded from the study (Table 1).

Serial number	Treatment/dosage/route of administration	Duration
A	Normal control+normal saline orally	14 days

B	$HgCl_2$ (7 mg/kg bw) orally	14 days
C	$HgCl_2$ (7 mg/kgbw single dose)+*S. reticulata* 200 mg/kgbw Orally.	14 days
D	$HgCl_2$ (7 mg/kg bw for 7 days) single dose orally+250 mg/kgbw orally for 7 days.	14 days
E	$HgCl_2$ (7 mg/kgbw orally)+vitamin E (0.2 ml pu) orally	14 days

Table 1: shows treatment and duration of groups.

The crude extract of *S. reticulata* and vitamin E were administered once daily for a period of 14 days respectively, and were all sacrificed on the 15th day. All groups were given treatment through oral intubation.

Administration of the neurotoxin

The neurotoxin $HgCl_2$ and distilled water was the vehicle used to induce the memory loss, it was given orally at a single dose of 7 mg/kg. The LD50 of Mercury chloride as adopted [13-15] was given as 166 mg/kg body weight.

Cognitive and neurobehavioral test

Mice were assessed for cognitive and behavioural parameters in the Y-maze. The duration for the test was 5 minutes (300 seconds). The test was repeated for three days for evaluation of learning speed.

Learning and memory test (Y-maze alternation test)

Testing is carried out in a Y-maze with three (3) white opaque plastic arms at an angle of 120° from each other. The test is for measuring the willingness of rodents to explore new environments, many parts of the brain including the hippocampus, septum, basal forebrain and prefrontal cortex- are involved in this task [16].

The protocol for the forced alternation test was modified [16]. Mice were handled for three days before testing. The test consists of a 5 min sample trial (T1) followed by a 5 min retrieval trial (T2). For the mercury experiment, mice were doused with mercury chloride before T1. In T1, the mice were placed into the end of the start arm, facing the wall and away from the Centre. The mice were then allowed to explore two arms of the Y-maze, while entry into the third arm was blocked. After the sample trial, the mice were returned to its home cage for a 30 min inter-trial interval. In T2, the block in arm 3 was removed; the mice was again placed into the start arm, and then allowed to access all three arms of the maze. If a mouse climbs on the maze wall, it was immediately returned into the abandoned maze arm. After each animal and between T1 and T2, the maze was wiped with a Quatricide® dilution to prevent odour cues. An arm entry was recorded when 85% of a mouse's body entered the arm. Time in Novel Arm (%) will be defined as the time spent in the novel arm divided by the time spent in all arms during the first minute of the retrieval trial T2. Forced Alternation (%) was defined as the percent of mice entering first the novel arm during T2. Mice with less than three arm entries in the first minute of T2 were excluded from the analysis [16].

Animal sacrifice

The final body weight of the mice was obtained at the end of the treatment using a digital weighing balance; the animals were anaesthetize using chloroform and humanely sacrificed the brain tissues were removed by opening through the sutures of the skull.

Histological analysis

Tissue sample (brain) was harvested and fixed in 10% formol calcium. Samples were taken for histological staining in Department of Human Anatomy Faculty of Medicine, Ahmadu Bello University Samaru Zaria. The tissues (brain) were stained using H&E for general histological architecture and specifically for neurological cells and fibers using Bielschowskys Silver stain for neurons and neurofibrils.

The slides obtained were mounted on a microscope and studied using an Olympus microscope. Photomicrographs were taken using a digital camera (Amscope, MD 900) placed in an Olympus microscope eyepiece and image taken using application installed in Dell laptop latitude 2120.

Statistical analysis

All of the data are expressed as mean ± SD. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Dunnett's post hoc test as appropriate using a computer-based fitting program (SPSS/21). Differences were considered to be statistically significant when $p<0.05$.

Results

Phytochemical result

Serial number	constituents'	tests	inference
1	Carbohydrates	Molisch test	+
2	Anthraquinones	Bontrager's test	++
3	Saponins	Frothing test	+
4	Steroids and Triterpene	Liebermann Bucchard test	+++
5	Tannins	Ferric chloride test	+
6	Flavonoids	Shinoda test	+
7	Alkaloids	Dragendoff test	+
8	Cardiac glycoside	Kelle-kiliani test	+
Keys:+(present), - (absent), +++ (present abundantly)			

Table 2: showing chemicals present in Salacia reticulate.

Table 2 showing phytochemical substances present in *Salacia reticulata* and it shows all the chemicals present with steroids and triterpenes more abundant in concentration.

Morphological results

Table 3, demonstrated the changes in the body weight of mice after induction of mercury chloride and during the periods of treatment with *Salacia reticulata* and vitamin E. There was a significant difference at $p<0.05$ between the mercury chloride only treated group B, and the normal control group, which signifies that mercury chloride reduced the weight of the animals. The *Salacia reticulata* treated groups (C and D), regained their weights seen after 14 days of treatment, while the vitamin E treated group E also regained weighs during the course of treatment. The mean brain weight of the mercury chloride only treated group B, compared to normal control group A shows significant difference <0.05, and also compared to *Salacia reticulata* and vitamin E treated groups (C, D, and E), shows a difference, demonstrated in Table 3.

Groups N=5	Initial weight (g)	Final weight (g)
A	15.24 ± 0.77	20.80 ± 1.92
B	22.80 ± 1.48	18.68 ± 1.28*
C	21.86 ± 1.09	21.60 ± 5.81
D	25.32 ± 0.87	25.60 ± 2.40
E	16.74 ± 0.49	24.4 ± 3.43

Values expressed as mean ± standard deviation (SD) of mean body weight of mice during the experiment in grams. Values with superscript (*) in a column are significantly different (p<0.05). A: Normal Control; B: $HgCl_2$ only; C: $HgCl_2$+200 mg/kg/bw (14); D: $HgCl_2$+250 mg/kgbw [13]; E: $HgCl_2$+Vitamin E.

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

Groups N=5	Brain weight
A	0.36 ± 0.05
B	0.31 ± 0.04*
C	0.34 ± 0.05
D	0.36 ± 0.05
E	0.34 ± 0.05

Values expressed as mean ± standard deviation (SD) of mean brain weight of mice during the experiment in grams. Values with superscript (*) in a column are significantly different (p<0.05). A: Normal Control; B: $HgCl_2$ only; C: $HgCl_2$+200 mg/kg/bw (14); D: $HgCl_2$+250 mg/kgbw [13]; E: $HgCl_2$+Vitamin E.

Table 4: Mean brain weight of adult male mice.

Behavioural performance

The study showed significant increase (<0.05) 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 3 and 4, for spatial learning and memory during the period of mercury chloride administration especially in Group B; ($HgCl_2$ only) and also in group E, the vitamin E treated group. This can be said to be associated with memory loss which could be as a result of neuronal cell layer degeneration, and distortion of the general structure of the pyramidal cells of the hippocampus as observed from the study. The study also showed significant decrease ($p<0.05$) 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, for spatial learning and memory during the period of *Salacia reticulata* administration.

Groups N=5	SABPT%	SABT1%	SABT2%
A	64.70 ± 5.63	72.08 ± 9.16	81.22 ± 12.11
B	64.80 ± 8.4	60.47 ± 9.14	57.88 ± 9.28*
C	64.28 ± 13.06	56.96 ± 21.98*	71.42 ± 10.24
D	61.69 ± 14.82	69.94 ± 2.51	74.82 ± 18.36
E	59.68 ± 13.54	57.48 ± 3.22	63.60 ± 18.84*

Values expressed as mean ± standard deviation (SD) of spontaneous alternation behaviour of mice during the experiment in grams. Values with superscript (*) in a column are significantly different (p<0.05). A: Normal Control; B: $HgCl_2$ only; C: HgC_2+200 mg/kg/bw (14); D: $HgCl_2$+250 mg/kgbw [13]; E: $HgCl_2$+Vitamin E.

Table 5: Mean spontaneous alternation behaviour.

Groups N=5	NAEPT	NAET1	NAET2
A	22.00 ± 6.89	20.60 ± 9.55	17.60 ± 9.37
B	18.40 ± 5.41	17.60 ± 2.30	17.40 ± 5.17
C	17.80 ± 5.97	14.20 ± 7.46*	19.40 ± 6.80
D	19.80 ± 3.27	19.60 ± 6.98	22.00 ± 3.80*
E	19.80 ± 1.48	12.20 ± 4.26*	13.60 ± 8.35*

Values expressed as mean ± standard deviation (SD) of number of arm entries of mice during the experiment in grams. Values with superscript (*) in a column are significantly different (p<0.05). A: Normal Control; B: $HgCl_2$ only; C: $HgCl_2$+200 mg/kg/bw (14); D: $HgCl_2$+250 mg/kgbw [13]; E: $HgCl_2$+Vitamin E.

Table 6: Mean of number of arm entry.

Histological results

Hippocampus

Haematoxylin and Eosin: The normal control group A, (Figure 1) shows normal neuronal cells with distinctive cell layers. Mercury chloride only treated group B, (Figure 2) shows pericellular spaces signifying neuronal cells damage and neurodegeneration. *Salacia reticulata* treated group C (14 days), (Figure 3) shows renewal of neuronal cells with distinctive cell layer. *Salacia reticulata* treated group D (14 days), (Figure 4) shows restoration of neuronal cells and more distinctive cell layer. Vitamin E treated group E, (Figure 5) shows less restoration of neuronal cells and less distinctive cell layer.

Bielschowsky's silver stain: The normal control group (Figure 6) shows normal pyramidal cells and cellular profile. Mercury chloride only treated group (Figure 7), shows distorted cell layers with pyramidal cells degeneration. *Salacia reticulata* treated group C (Figure 8), shows preservation of the hippocampus cell layers and pyramidal cells. *Salacia reticulata* treated group D (Figure 9) shows more preservation of pyramidal cells. Vitamin E treated group (Figure 10)

shows distortion and degeneration of cell layers and pyramidal cells with mild preservation of the hippocampus.

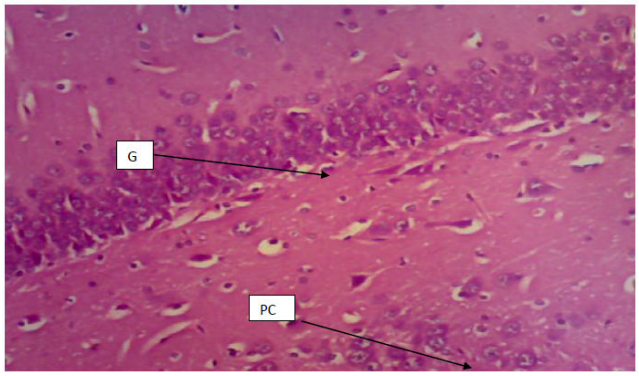

Figure 1: Photomicrograph of the hippocampus of Group A normal control, granular cell layer (GL) and normal pyramidal cell (PC) (H&E) X100.

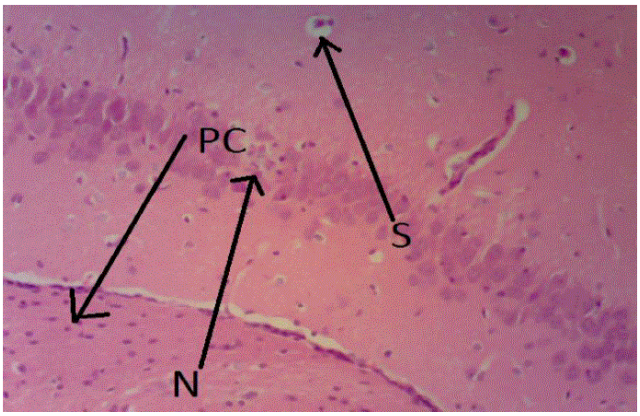

Figure 2: Photomicrograph of the hippocampus of Group B $HgCl_2$, degenerated pyramidal cell (PC), more Peri-cellular spaces (S) and reduced neuronal cell layer (N). (H&E) ×100.

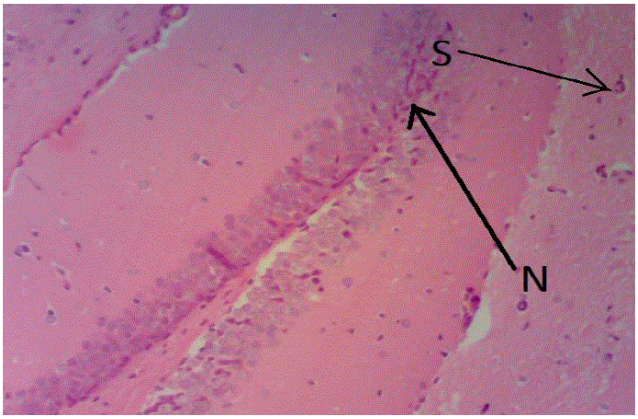

Figure 3: Photomicrograph of the hippocampus of Group C *S. reticulata* 200 mg/kgbw, preserved neuronal cell layer (N), and less pericellular spaces. (H&E) ×100.

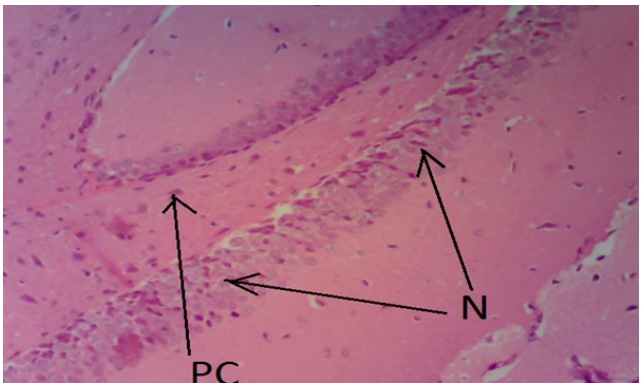

Figure 4: Photomicrograph of the hippocampus of Group D *S. reticulata* 250 mg/kgbw, preserved neuronal cell layer (N). H&E ×100.

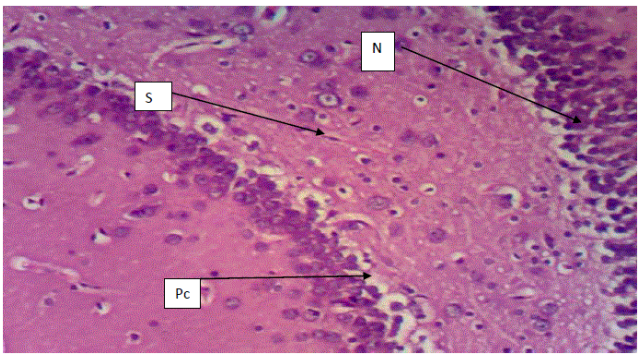

Figure 5: Photomicrograph of the hippocampus of Group E vitamin E, showing less recovery of neuronal cells (N) layer, pericellular spaces (S), and less preserved pyramidal cells (H&E) ×100.

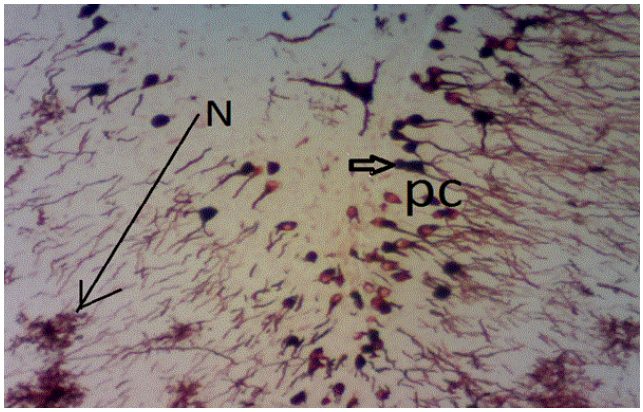

Figure 6: Photomicrograph of the hippocampus of Group A normal control, showing pyramidal cells (PC), and normal cellular profile and neuropils (N). Bielschowsky's Silver stain ×100.

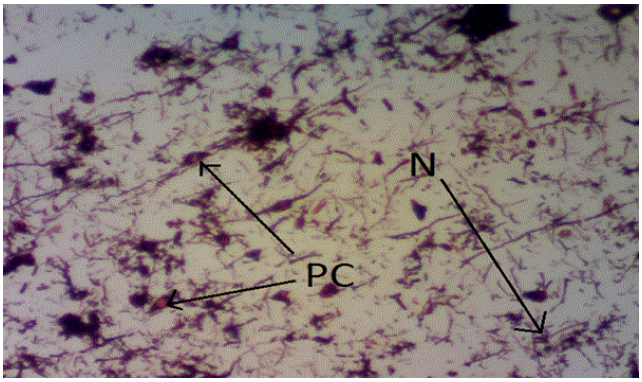

Figure 8: Photomicrograph of the hippocampus of Group C *S. reticulata* 200 mg/kgbw, showing preserved pyramidal cells (PC), recovery of cellular profile architecture and neuropils (N). Bielschowsky's Silver stain ×100.

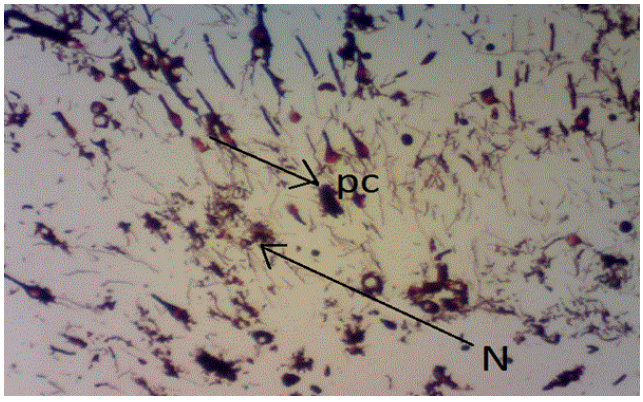

Figure 7: Photomicrograph of the hippocampus of Group B $HgCl_2$, showing degenerated pyramidal cells (PC), distorted cellular profile and neuropils (N). Bielschowsky's Silver stain ×100.

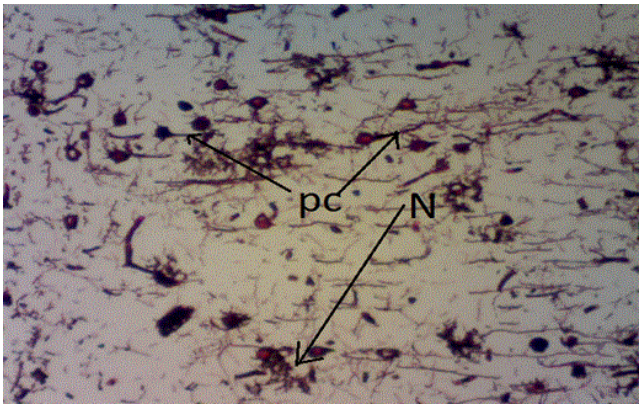

Figure 9: Photomicrograph of the hippocampus of Group D *S. reticulata* 250 mg/kgbw, showing more preserved pyramidal cells (PC), more recovered neuropils and cellular profile. Bielschowsky's Silver stain ×100.

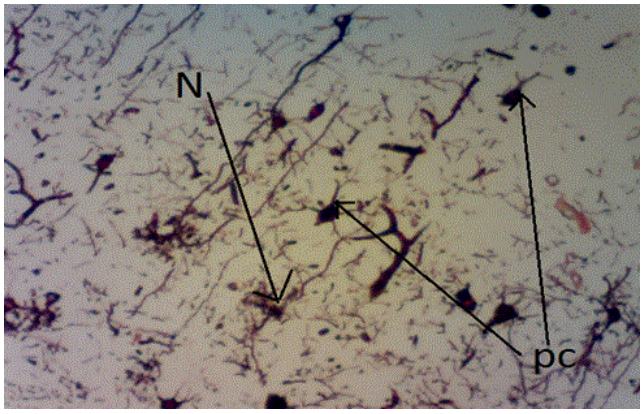

Figure 10: Photomicrograph of the hippocampus of Group E Vitamin E, less preserved pyramidal cells (PC), much less preserved cellular profile and neuropils (N). Bielschowsky's Silver stain ×100.

Discussion

Physical observation of the animals

On physical observation of the animals, the control group A animals were observed to be very active, while after the administration of mercury chloride, the animals were observed to be using their forelimbs to scratch their mouth, restlessness and had watery faeces, this observation was seen mostly in the mercury chloride control group B, the animals got weakened progressively and it can be related to reduction in their physical activities. The *Salacia reticulata* treated groups (C and D), showed improvement in physical activities during the period of treatment. The vitamin E treated group E, also showed improvement in physical activities during the period of administration, this agrees with the findings [17] that mercury chloride decreases physical activities.

Morphological result

Table 2, demonstrated the changes in the body weight of mice after induction of mercury chloride and during the periods of treatment with *Salacia reticulata* and vitamin E. There was a significant difference at $p<0.05$ between the mercury chloride only treated group B, and the normal control group, which signifies that mercury chloride reduced the weight of the animals. The *Salacia reticulata* treated groups (C and D), regained their weights seen after 14 days of treatment, while the vitamin E treated group E also regained weighs during the course of treatment. The mean brain weight of the mercury chloride only treated group B, compared to normal control group A shows significant difference <0.05, and also compared to *Salacia reticulata* and vitamin E treated groups (C, D, and E), shows a difference, demonstrated in Table 3. This agrees with the findings [17] that say that antioxidants such as ascorbic acids are helpful in reversing mercury toxicity and maintenance of body weight.

Histology studies

Hippocampus: The hippocampus is a part of the limbic system and functions basically in long-term memory and spatial navigation, damage to any cell in the hippocampus will result to serious effects on learning processes and retaining information of an individual. Astrocytes are the most abundant Glial cells in the brain, and they help in filling up spaces caused by neuronal damage forming Glial scars. Another function is that they help in repairing damaged cells that cannot be regenerated. Therefore any damage that will cause a reduction in these Glial cells that connects other cells will result to a deficit in cell to cell connection. Figure 1 shows the normal neuronal cell layer of the hippocampus without injury. Mercury chloride control shows a profile of damaged neuronal cell layer which is prominent in the granular layer, Figure 2, this neurodegeneration is evident in the behavioural test as the group B, had low performance. *Salacia reticulata* treated groups (Figures 3 and 4), shows recovery and preservation of neuronal cells with more preservation observed in Figure 4 as compared to Plate 2 where the cells are damaged; this confirms that *Salacia reticulata* has neuroprotective effects on brain cells. The vitamin E treated group, Figure 5 showed mild recoveries as compared to Figure 4, which indicates presence of more antioxidants properties found in *Salacia reticulata* than in vitamin E. Figure 6, the normal control group shows the normal profile of pyramidal cells. Figure 7 mercury chloride treated group shows neurodegeneration of pyramidal cells as a result of the damage of mercury chloride on the brain as demonstrated by a study [18] which observed that increased levels of free radicals reduces antioxidants and furthermore leads to brain cells degeneration.

The findings from this study agrees with the work of other researchers who reported that heavy metals such as cadmium, thallium, manganese, mercury, drugs, and solvents [19] and other organic compounds have the ability to cause damage to the nervous [20] and this is because these metals can cross the blood brain barrier to accumulate in brain tissues [10]. The brain uptake of mercury in rats is transported to the central nervous system from the blood across the blood-brain barrier by the L-type neutral amino acid carrier transport (LAT) system [21]. Glutamate dyshomeostasis in the central nervous system represents a critical target in mercury induced neurotoxicity [22]. Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system responsible for development, learning, memory and response to injury [23]. However, at high concentrations glutamate at the synaptic cleft begins to act as a toxin, inducing neuronal injury and death [24]. Glutamate-mediated neurotoxicity has been conferred to as 'excitotoxicity', this refers to the consequence of the over activation of the N-methyl D-aspartate (NMDA) type glutamate receptors, which leads to increased Na+ and Ca^{2}+ influx into neurons [25]. Hence, increased intracellular Ca^{2}+ levels are associated with the generation of oxidative stress and neurotoxicity [10].

Salacia reticulata treated groups (Figures 8 and 9), show preservation and renewal of the pyramidal cells which indicating neuroprotective effects of antioxidants present in *Salacia reticulata*. Vitamin "E" Figure 10 shows poor recovery of pyramidal cells as compared to *Salacia reticulata* groups.

Behavioural performance

The present study showed significant increase (<0.05) 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 3 and 4, for spatial learning and memory during the period of mercury chloride administration especially in Group B; ($HgCl_2$ only) and also in group E, the vitamin E treated group. This can be said to be associated with memory loss which could be as a result of neuronal cell layer

degeneration, and distortion of the general structure of the pyramidal cells of the hippocampus as observed from the present study. These distortions mean shows that activity such as memory and learning abilities from the brain region projecting into the pyramidal layer of the hippocampus will be lost [20] and these could always impair the activities of the hippocampus in learning, memory formation, storage and retrieval of information.

The present study showed significant decrease ($p<0.05$) 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 3 and 4, for spatial learning and memory during the period of *Salacia reticulata* administration. Thus, this study has shown the ameliorative effects of *Salacia reticulata* on spatial learning and memory in the experimental animals with mercury chloride. The administration of *Salacia reticulata* has shown some improvement in the hippocampus of animals when compared to the animals given to mercury chloride only. It has been shown that heavy metals such as mercury, lead, and thallium have the capability to induce oxidative stress via reduction of antioxidative enzymes such as SOD, CAT, GLU, and proliferation of lipid peroxidation levels. The decrease in the activity of antioxidative enzymes such as superoxide dismutase level and the elevation of lipid peroxidation levels, suggests the formation of free radical induced oxidative cell injury in mediating the toxic effect of mercury [19].

However, *Salacia reticulata* serves as antioxidant which plays significant role in the reversion of the toxicity of mercury by forming inert complexes and inhibiting their toxicity [2].

Conclusion

From this study, *S. reticulata* has potentiating effects in ameliorating the harmful effects of Mercury Chloride on the neural cells and cellular architecture of the hippocampus by preserving the cellular architecture, reversing the effects of free radicals and showed improved learning and memory skills in adult male mice as compared to the Vitamin E and $HgCl_2$ treated groups respectively.

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