EFFECTS OF SUBACUTE ADMINISTRATION OF AQUEOUS EXTRACT OF WATER MELON(*CITRULLUS LANATUS*) SEED ON LEAD ACETATE INDUCED TOXICITY IN CEREBRUM OF ADULT WISTAR RATS

ABSTRACT

INTRODUCTION: As a reported biotoxic environmental and industrial pollutant, lead (Pb) do accumulate in almost all the body tissue such and cause health complications. The cerebrum is the superior-most(topmost) region of the central nervous system that is more affected by lead accumulation. However, watermelon (*Citruluslanatus*) is a flowering plants species of the Cucurbitaceae family which is associated with numerous medicinal properties. **AIM**: This study investigated the possible neuroprotective effect of aqueous extract of *citruluslanatus*seed on lead acetates-induced neurotoxicity in the Cerebral of adult Wistar rats. **PLACE OF STUDY:** Department of Anatomy, Faculty of Basic Medical Sciences Olabisi Onabanjo University, Ogun State.

METHOD: Twenty(20) healthy adult female wistar rats weighing 150±50g were assigned into 4 groups (n=5). The Control group received 2mls/kg of distilled water, Treatment group (T1) was administered 7.5 mg/kg of lead acetate, Treatment group (T2) was administered 200 mg/kg of aqueous extract of *citrulluslanatus* and Treatment group (T3) was administered 7.5 mg/kg of lead acetate and 200 mg/kg of aqueous extract of *citrulluslanatus*concomitantly orally for fourteen (14) days. H and E and cresyl violet staining Bicohemicalasays for SOD, CAT activities and MDA concentration was determined.

Data obtained from the study were analyzed using one way ANOVA and subjected to Turkeys post hoc test from the multiple comparison.

RESULT: Revealed no statistically significant differences ($p>0.05$) in body weight and brain weight changes across groups relatives to the control group, SOD and Catalase activity were increased significantly compared to

control. The histological finding revealed that the control group shows normal histoarchitectural appearance of the cerebral cortex with normal morphologically appearing neurons while the lead exposed group (T1) revealed features of neurodegenerative changes by nuclear shrinkage.

CONCLUSION: In summary, *citrulluslanatus* offer protection to the pyramidal neuronal cells against lead acetate induced neurotoxcity in Wistar Rats and also further affirms its antioxidative potential.

Key words: Cerebral cortex neuroprotective, lead acetate, *citrulluslanatus.*

1.0 INTRODUCTION

1.1 LEAD ACETATE

As a reported biotoxic environmental and industrial pollutant, lead (Pb) do accumulate in almost all the body tissue such as the liver, kidneys, lungs and immune system (1). It has been reported the physiological, biochemical, and behavioral effect of this toxic lead in disorder of central and peripheral nervous and cardiovascular systems (2), kidneys, liver and Reproductive system (3)

Lead acetate may induce oxidative stress leading to the generation of free radical and alterations in oxygen free radical scavenging enzymes system or antioxidant and damage membrane structure (4).

Lead acetate is a chemical compound that is soluble in water and glycerin, and more toxic than insoluble lead oxides or solid lead sheeting, Lead acetate in low concentration is the principal active ingredient in progressive type of hair coloring dyes. Lead acetate does not remain in tissue for long periods, except in bones when it is deposited in an inert form but from which it can be liberated at a later date in sufficient quantity to cause lead poisoning (5).

Lead (II) acetate paper is used to detect the poisonous gas hydrogen sulfide. This gas reacts with lead II) acetate on the moistened test paper to form a grey precipitate of lead (II) sulfide (6). An aqueous solution of lead (II) acetate is the byproduct of the 50/50 mixture of hydrogen peroxide and white

vinegar used in the cleaning and maintenance of stainless steel firearm suppressor (silencers) and compensator. The solution is agitated by the bubbling action of the hydrogen peroxide, and the main reaction is the dissolution of lead deposit within the suppressor by the acetic acid, which form lead acetate. Because of its high toxicity, this chemical solution must be appropriately disposed by a chemical processing facility or hazardous materials center (7).

Alternatively, the solution may be reacted with sulfuric acid to precipitate nearly insoluble lead (II) sulfate. The solid may then be removed by mechanical filtration and is safer to dispose of than aqueous lead acetate (8).It was also used in making of slow matches during the middle Age. It was made by mixing natural form of lead (II) oxide called litharge and vinegar. Sugar of lead was a recommended agent added to linseed oil during heating to produce "boiled" linseed oil, lead and heat acting to cause the oil to cure than raw linseed oil (9).

1.2 Water Melon (*Citrullus lanatus)*

Watermelon (*Citruluslanatus*) is a flowering plants species of the Cucurbitaceae family. It is associated with numerous medicinal properties. One of the most important ability of Citrullus lanatus is to prevent cancer.*Citrullus lanatus* a fruit crop, is a herbaceous creeping plant belonging to the family Cucurbitaceae. It is mainly propagated by seeds and thrives best in warm areas. It is a tropical plant and requires a lot of sunshine and high temperature of over 25°C for optimum growth. Watermelon thrives best in a drained fertile soil of fairly acidic nature. It can be grown along the coastal areas of Ghana, the forest zone and especially along river beds in the Northern Savannah areas (10). The sugar content and sweetness are the critical factors in determining the quality of many watermelon varieties. It is known to be low in calories but highly nutritious and thirst quenching.

The *Citrullus lanatus* seed has some heart healthy benefit such antioxidant called lycopene which gives red fruit and vegetables their vibrant pigment. The nutrients have been linked to tons of positive health benefits from cardiovascular health to cancer prevention.

Watermelon seed are known to be highly nutritional; they are rich source of protein, vitamin B, mineral such as (magnesium, potassium, phosphorus, sodium, iron, zinc, manganese and copper) are fat among other as well as photochemical. (10*)*

1.3 CEREBRUM

Thecerebrum is the part of the prosencephalon (forebrain) of the brain that contains the cerebral cortex as well as such subcortical structure as the hippocampus, amygdala, basal, ganglia, olfactory bulb, and corpus callosum (11). In humans, the cerebrum is the superior-most(topmost) region of the central nervous system (CNS). However, in nearly all vertebrates, the cerebrum is the anterior-most region of the CNS as most animals rarely assume as upright anatomical position (12).

In mammals, cerebrum has been used synonymously with the term "telencephalon" or "end brain"; however, in other vertebrates, the term telencephalon is used to refer to the embryonic structure from which the mature cerebrum developed (13).

The surface of the cerebral cortex is highly convoluted in large mammals, with the folds and groove allowing a much greater surface area in a confined space as in the skulljia. In human, this highly folded nature with gyri and sulci is particularly pronounced, and allowing some15-33 billion neurons in the cerebral cortex, each connected by asynapses, to several thousand other neurons (14).

The cerebrum is also divided into approximately symmetric left and right cerebral hemisphere by a deep groove or fissure. Latin for "brain", the cerebrum is involved in speech and language, spatial recognition, leanings and memory, sensory processing and olfaction (15). With the assistance of the cerebellum, the cerebrum controls all voluntary actions on the body. Notably, while the two cerebral hemisphere of humans appear structurally similar, they differ in many functions. For example, in most people, the dominant hemisphere for language is the left hemisphere, where most of the neural processing takes place for speech comprehension, the formation of thought into speech, and the generation of motor output for language communication. In most people, the non-dominant hemisphere

is adopted at spatial reasoning and musical ability (16). Damage to specific areas can cause such condition as the inability to recall faces or have fluid and rapid speech but lacking meaning, with the words tossed together (17)

2.0 MATERIALS AND METHODS

2.1 LIST OF MATERIALS AND INSTRUMENTS

Aqueous extract of watermelon seed, Lead acetate, 20 Wistar rats, Standard rat feed, 2ml Syringes, Slap, measuring cylinder (100ml), Cotton wool, Cages, distilled water, Sensitive weighting balance, Scales, picking forceps, Bowl of varying sizes, Gloves, Camila, Water, Fixatives, Dissecting set, Water bath, Ice pad and freezer, Tissue processor, Mark, Microtome blade, Sample bottles, Opharyngeal cannula.

2.2 METHODOLOGY

2.2.1 SUBSTANCE PREPARATION

- Lead acetate of powdery form was procured from central drug house(P)LTD New Delhi (INDIA) Batch No: 330417

2.2.2 RAT MANAGEMENT

Twenty male and female Wistar albino rats (150±50g) were obtained and brought to the animal holding unit of the department of Anatomy Ladoke Akintola University of Technology, Ogbomoso for this study. They were kept in clean cages in a well- ventilated environment. The rats were allowed with free access to water and standard pelleted feed. Rat in all group were weighted thrice weekly and were handled per the guidelines of the internal animal care and life committee.

2.2.3 PLANT EXTRACTION:

Fresh *Citrullus Lanatus* was purchased from Odoba market, Ogbomoso. The seed was harvested 750g (0.0075g) of *Citrullus Lanatus* seed were sun dried and milled with grinding machine into rough and powdery form and it was weighted on a weighting balance. Then it was taken to the food science laboratory to be processed into an aqueous form.

2.2.4 EXPERIMENTAL DESIGN

Twenty rats were randomly assigned into four groups {n=5 each}. The control group {C} was administered distilled H2O only {2 ml/kg}.

Group T1 was administered Lead Acetate solution {7.5mg/kg}

Group T2 was administered Citrullus Lanatus only {200mg/kg}

Group T3 was concordantly administered lead acetate solution and Citrullus Lanatus

2.2.5 TISSUE EVISCERATION AND PRESERVATION:

The animals were sacrificed using the cervical dislocation method, the cervical region of Wistar rat were dislocated to elicit a fracture at the cervical region thereby rendering the animals temporarily conscious, and the organs was harvested and placed in fixative to prevent autolysis. The harvested brain and organ were fixed in 10% form calcium of whose constituent are: calcium chloride 10g, 40% formaldehyde 100mls and distilled water-900mls.

2.2.5.1 TISSUE PROCESSING

The tissue was allowed to fix in 10% formal-saline for 48 hours. The tissue was grossed and cut into smaller pieces, They were processed using automatic tissue processor passing through various reagent including alcohol (of various concentration starting from 70%, 80%, 90%, 95% and 100% or absolute

alcohol) for dehydration, two changes of xylene and three changes of molten paraffin wax set for 12 hours.

The tissue was embedded in paraffin wax by burying the tissue in a metal mold containing blocks, ready for sectioning. The tissue was sectioned at 4micron using rotary microtome and the section were floated on hot water bath to attach the section to relabeled slide. The section was dried on hot plate and ready for staining

2.2.6 MORPHOLOGICAL STUDIES FOR HAEMATOXYLIN AND EOSIN STAINING METHODS STAINING

The rats were weighed in grams using a weighing scale before the start of the experiment and before the sacrifice.

After careful removal of the tissue, they were trimmed of fats weighed and immediately fixed in 10% formal saline after fixing the tissue were put into ascending grades of alcohol and the cleared in xylene. They were embedded in the paraffin and serial sections of 3-5µm were obtained. Sections were stained with haematoxylin and eosin for histomorphological analysis and cresyl violet stain for Neurons

2.7 BIOCHEMICAL ASSAY

The brains were measured and cut to a constant 0.25mg, then homogenized and mixed with 2.5mls of the combination of phosphate buffered saline (7.4ph) and 1litres of distilled water.

After they are being mixed, it is placed inside a test tube under zero degree temperature then taken to laboratory for test of induced stress. The test is to target oxidative stress markers like SOD, CAT and MDA

2.7.1 BIOCHEMICAL PARAMETERS

- **Determination of Malondialdehyde concentration**

Normal saline (0.5ml) was pipette into test tube containing 05ml of the serum sample. About 2 ml of thiobarituric acid (TBA) mixture was added, allowed to boil for 1 hour, cooled to room temperature and centrifuged at 4000rpm for 5min. the clear supernatant was read at 535nm.

- **Determination of Catalase activity**

Distilled water (2.5ml) was pipetted into test tube containing 0.5ml H_2O_2 and about 40ul sample was added and mixed thoroughly. Rate of decomp[ostion of hydrogen peroxide was read at 240nm at 30 sec interval for 5 mins.

- **Determination of superoxide dismutase (SOD) activity**

Sample extract **(**20 ml) and 2.5 ml of 0.05 M carbonate buffer (pH 10.2) were mixed together and equilibrated in the spectrophotometer. In addition, 0.3 ml of 0.3mM freshly prepared adrenaline was added and mixed by inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 3 mins.

2.8 Photomicrography

Image acquisition and analysis: A bright light microscope (10 - 40x magnification objective) was used. Digital camera - OMAX Toup view 3.7 attached to P.C - HP was used. Java Application Software (image J Software) was used.

2.9 STATISTICAL ANALYSIS

The data obtained from this study were analyzed using ANOVA (Analysis of variance) comparison were made between control and experimental groups l using Tukey's multiple comparison test. All results were expressed as mean +-SEM. $P<0.05$ was taken as the statistical Significance level.

3.0 RESULTS

3.1 EFFECT OF LEAD ACETATE AND *CITRULLUS LANATUS* ON MEAN BODY WEIGHT

Assessment of the body weights (Fig1) of rats across groups following treatments with lead acetate, *Citrullus lanatus* showed no statistical difference (p= 0.7257, F= 0.4431) when the lead acetate only treated group T1 (198±3.80), *Citrullus lanatus* only T2 (193.6±10.19) and their co-treatment group T3 (189.2±3.20) was compared to the control (206.50±21.50). Also, no observable significant difference when T1 (198±3.80) was compared to the T2 (193.6±10.19) group, and also when the T2 (193.6±10.19) was compared to T3 (189.2±3.20).

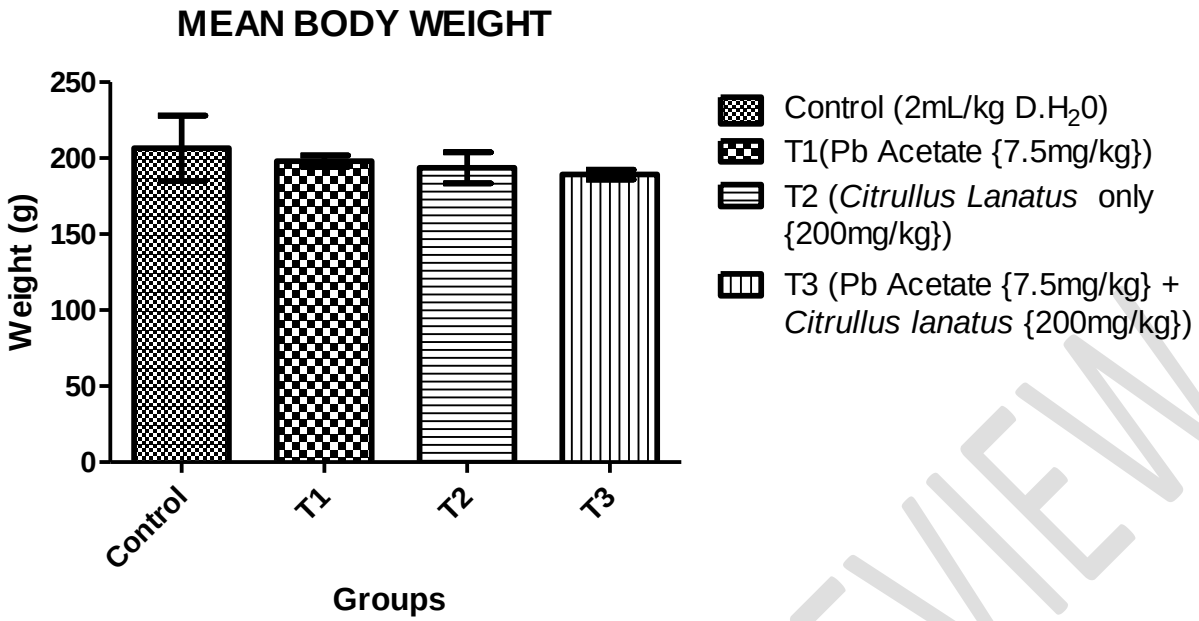

Fig 1: Bar chart represents the effect of Lead acetate, *Citrullus lanatus* and their co-treatments on the body weight of rats across the groups. Values are expressed as mean ± SEM (n= 5)Statistical significance ($p< 0.05$).

3.2 EFFECT OF LEAD ACETATE AND *CITRULLUS LANATUS* ON BRAIN WEIGHT

The effect of the administration of lead acetate and *Citrullus lanatus* on the brain weight is presented (Fig 2). In this study, lead acetate only treatment (1.64±0.07), *Citrullus lanatus* only (1.60±0.05) and their co-treatments (1.65±0.04) did not result in any significant changes ($p= 0.7488$, F= 0.4091) in the brain weight as compared to the control (1.69±0.07).

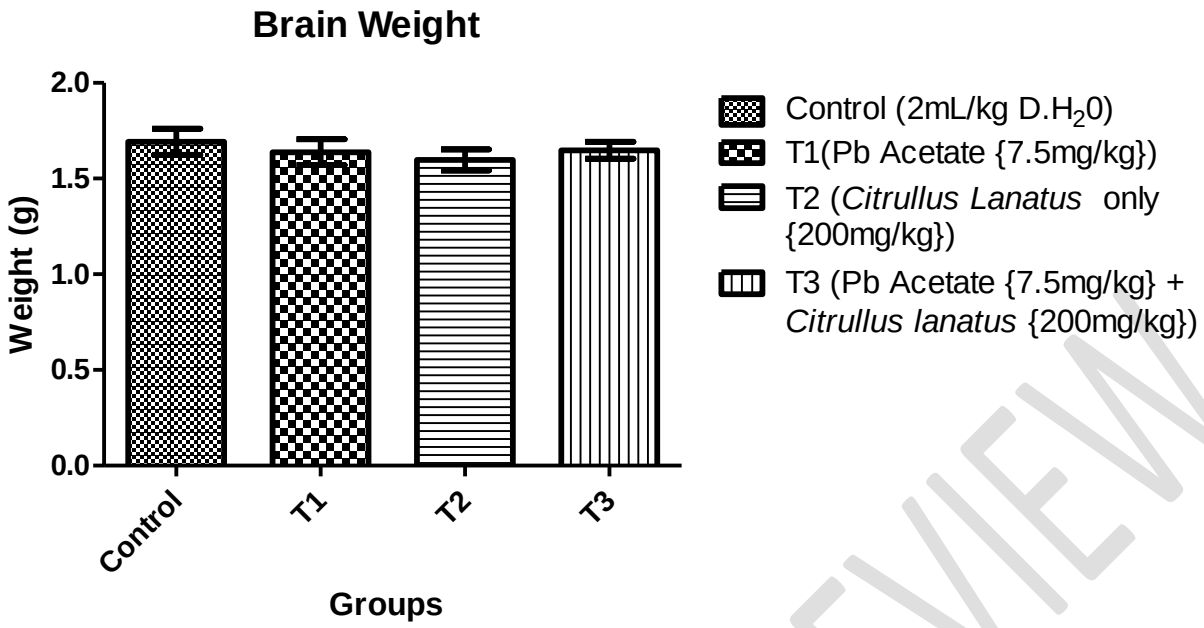

Fig 2: Bar chart represents the effect of Lead acetate, *Citrullus lanatus* and their co-treatments on the brain weights of rats across the groups. Values are expressed as mean ± SEM (n= 5). Statistical significance ($p< 0.05$).

3.3 EFFECT OF LEAD ACETATE AND *CITRULLUS LANATUS* ON THE RELATIVE BRAIN WEIGHT

The effect of the administration of lead acetate and *Citrullus lanatus* on the relative brain weight is shown in (Fig). Result obtained on the relative brain weight showed that lead acetate only treatment (T1) (0.83±0.02), *Citrullus lanatus* only (T2) (0.83±0.03) and their co-treatments (T3) (0.87±0.03) did not

cause any significant (p= 0.8521, F= 0.2614) changes in the relative brain weight as compared to the control (0.84±0.08).

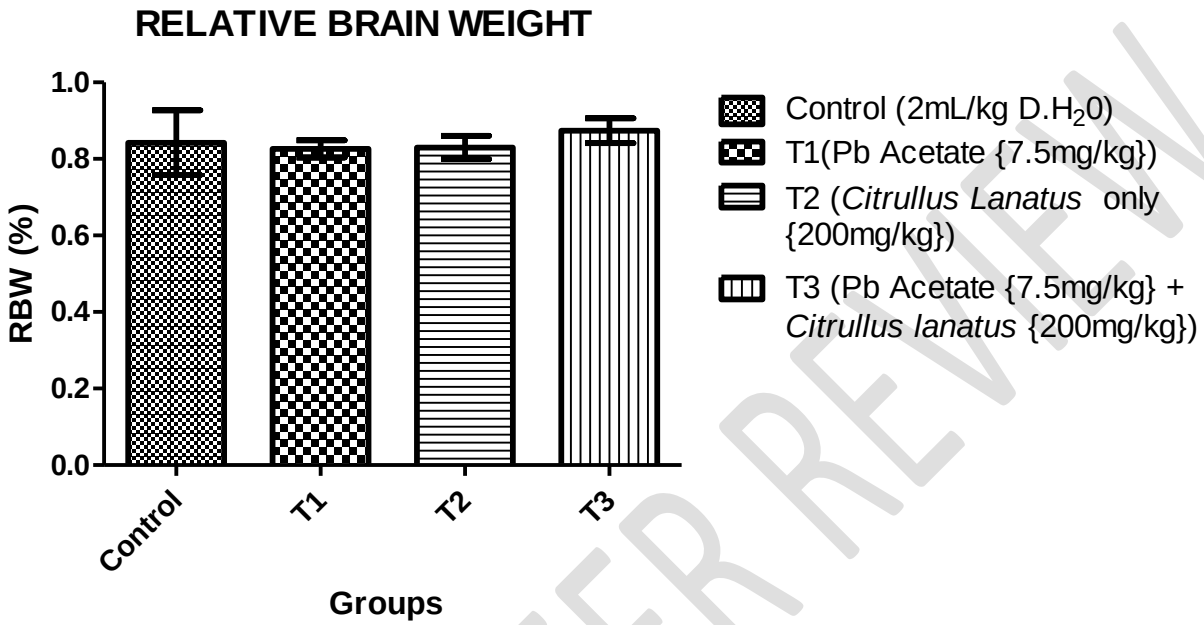

Fig 3: Bar chart represents the effect of Lead acetate, *Citrullus lanatus* and their co-treatments on the brain weights of rats across the groups. Values are expressed as mean ± SEM (n= 5). Statistical significance is indicated at (p< 0.05).

3.4 EFFECT OF LEAD ACETATE AND *CITRULLUS LANATUS* ON SOD ACTIVITY

As obtained in this study on the SOD activity (Fig 4) following administration of lead acetate and *Citrullus lanatus* extract treatments. There was statistically significant decrease (p = 0.021, F=12.70) in superoxide dismutase activity in the lead acetate only treated group (T1) (1.27 ± 0.01), *Citrullus lanatus*

only treated group (T2) (1.29 ± 0.01), and the co-treatment group (T3) (1.32± 0.02) as compared to the control (1.38 ± 0.02). However, there was no significantly different SOD activity when lead acetate only treated group (T1) was compared with *Citrullus lanatus* only treated group (T2) (1.29 ± 0.01) and when *Citrullus lanatus* only treated group (T2) (1.29 ± 0.01) was compared with Co-treatment group (T3) (1.32± 0.02).

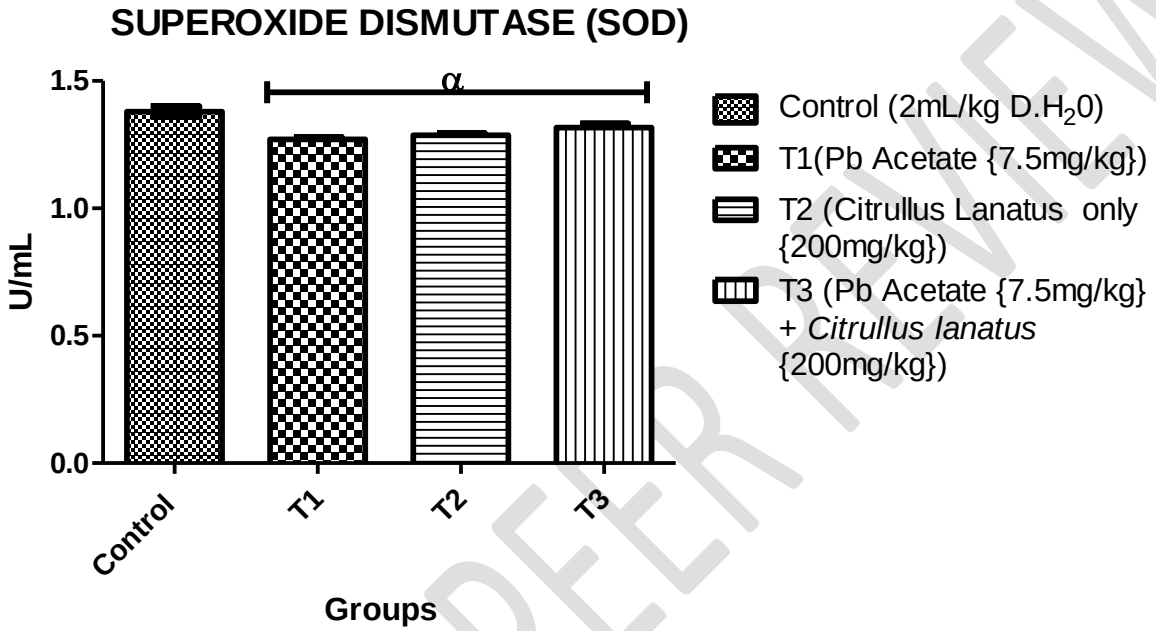

Fig 4: Bar chart represents the effect of Lead acetate, *Citrullus lanatus* and their co-treatments on SOD activity in the brain tissues of rats across the groups. Values are expressed as mean ± SEM (n= 5). Statistical significance ($p< 0.05$).

3.5 EFFECT OF LEAD ACETATE AND *CITRULLUS LANATUS* ON CATALASE ACTIVITY

The result obtained on this study on the CAT activity (Fig) following administration of lead acetate and *Citrullus lanatus* extract treatments showed no statistical significant difference (p = 0.7283, F=0.4438) when the lead acetate only treated group (T1) (16.05 ± 0.84), *Citrullus lanatus* only treated group (T2) (16.27 ± 2.10), and the co-treatment group (T3) (16.98 ± 1.49) were compared to the control group (18.30 ± 1.40). Likewise no statistically significant difference was observed when lead acetate only treated group (T1) (16.05 ± 0.84) was compared to *Citrullus lanatus* only treated group (T2) (16.27 ± 2.10) and the Co-treatment group (T3) (16.98 ± 1.49) and when *Citrullus lanatus* only treated group (T2) (16.27 ± 2.10) was compared to the Co-treatment group (T3) (16.98 ± 1.49).

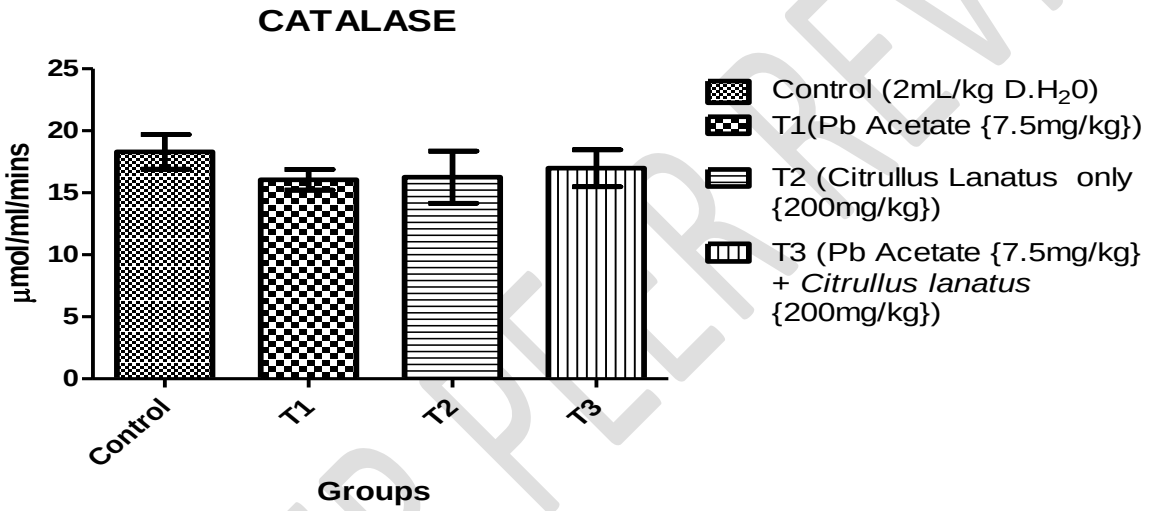

Fig 5: Bar chart represents the effect of Lead acetate, *Citrullus lanatus* and their co-treatments on catalase activity in the brain tissues of rats across the groups. Values are expressed as mean ± SEM(n= 5). Statistical significance (p< 0.05).

3.6 EFFECT OF LEAD ACETATE AND *CITRULLUS LANATUS* ON MDA CONCENTRATION

MDA concentration (Fig 6) in the brain tissues was evaluated across the groups following administration of lead acetate and *Citrullus lanatus* extract treatments. In the study, there was no significant difference (p = 0.1089, F=2.796) in MDA concentration in the brain tissues with the lead acetate only treated group (T1) (11.09 ± 2.65), *Citrullus lanatus* only treated group (T2) (6.44± 2.44), and the Co-treatment group (T3) (11.78 ± 0.57) were compared to the control group (5.88 ± 0.29). therewas also no statistically significant difference when lead acetate only treated group (T1) (11.09 ± 2.65) was compared to *Citrullus lanatus* only treated group (T2) (6.44± 2.44) and the co-treatment group (T3) (11.78 ± 0.57) and when *Citrullus lanatus* only treated group (T2) (6.44± 2.44) was compared to the co-treatment group (T3) (11.78 ± 0.57).

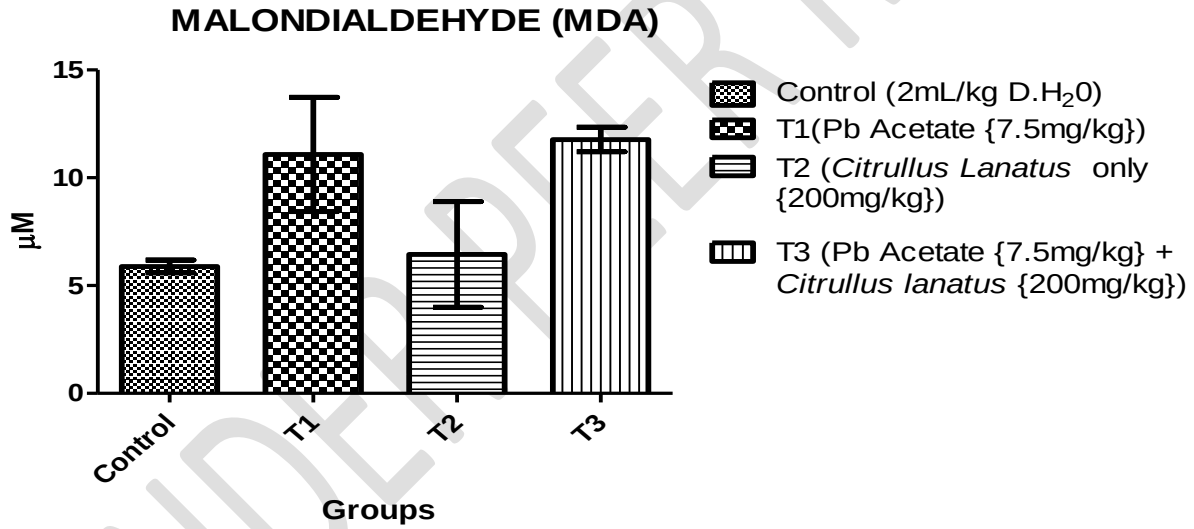

Fig6: Bar chart represents the effect of Lead acetate, *Citrullus lanatus* and their co-treatments on MDA concentration in the brain tissues of rats across the groups. Values are expressed as mean ± SEM (n= 5). Statistical significance (p< 0.05).

HISTOLOGY OF THE CEREBRUM

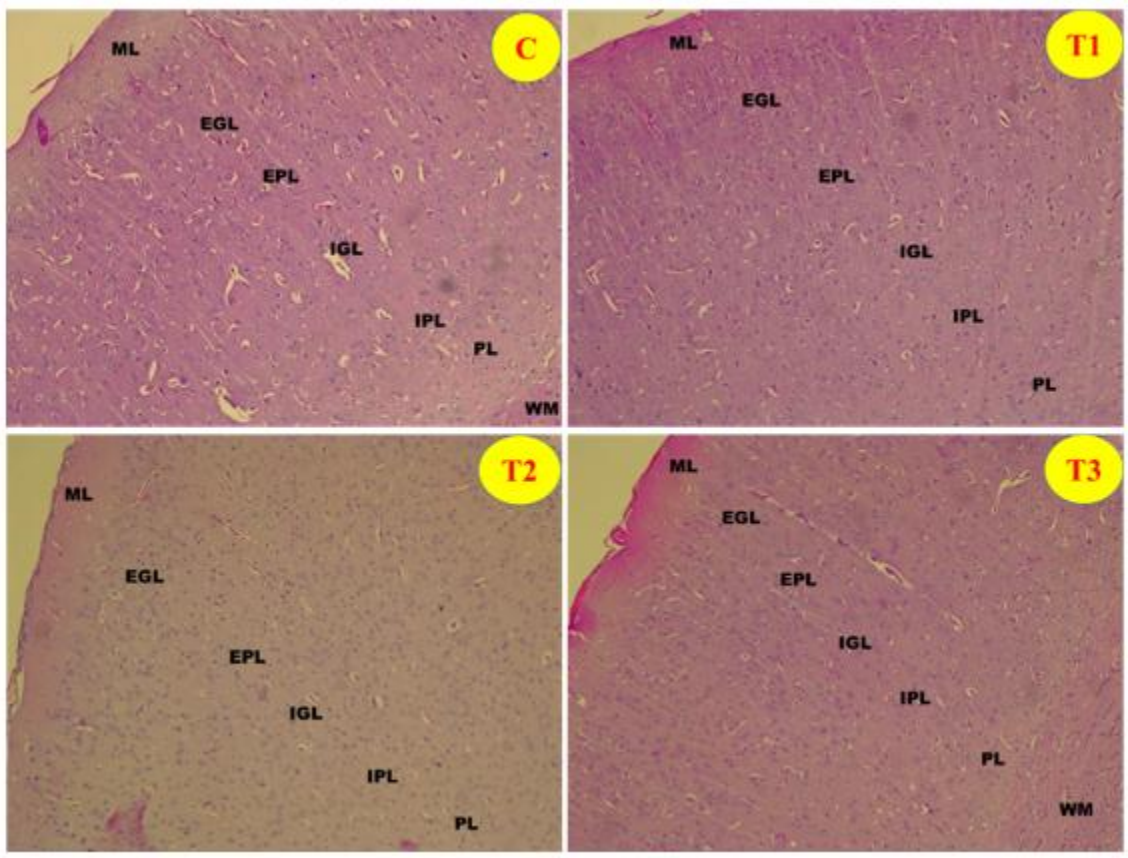

PLATE 1: Representative light photomicrographs showing the cortical layers stained cortex of the control, T1 (lead acetate only), T2 (*Citrullus lanatus* only) and co-treatment groups T3 (lead acetate + *Citrullus lanatus*) groups. **Stain**: H and E; **Magnification**: x100.

Abbreviation: ML-Molecular layer; EGL-External granular layer; EPL- External pyramidal layer; IGL-Internal granular layer; IPL- Internal pyramidal layer; PL (Polymorphic layer or Multiform); WM- white matter (Subcortical)

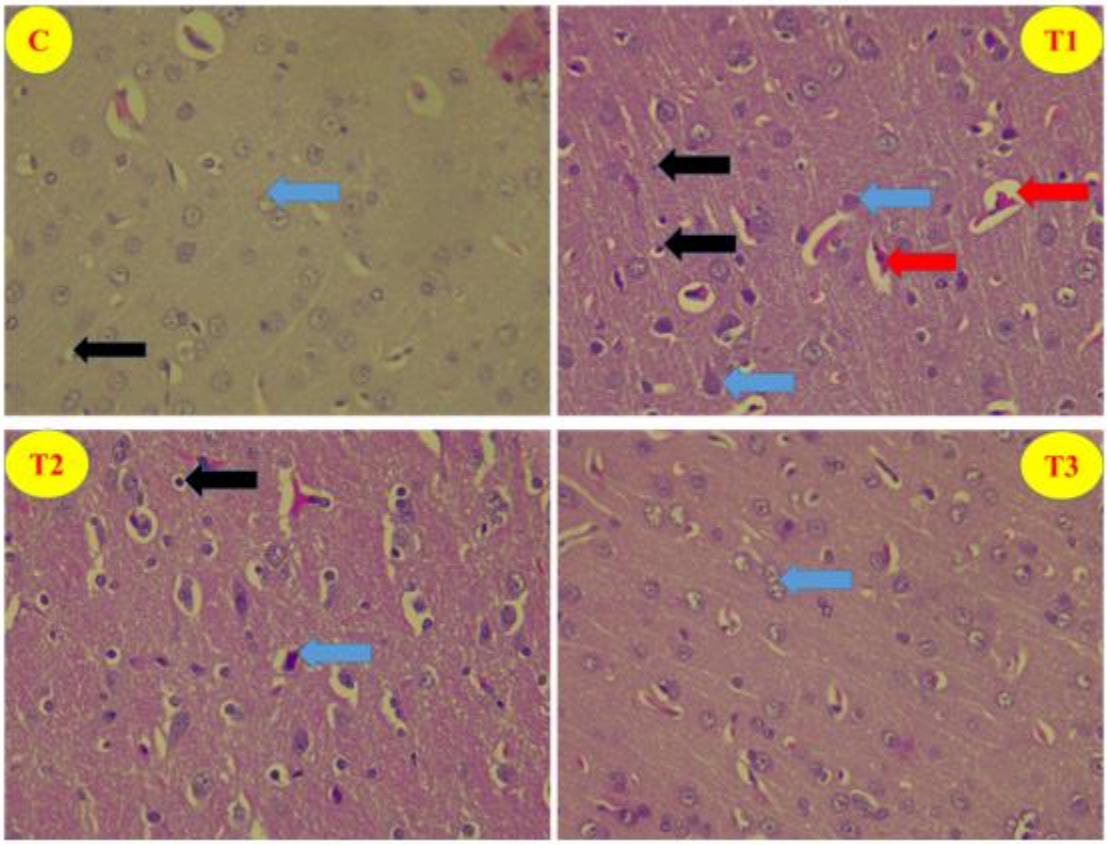

PLATE 2: Representative light photomicrographs showing frontal cortex of the control, T1 (lead acetate only), T2 (*Citrullus lanatus* only) and co-treatment groups T3 (lead acetate +*Ciyrulluslanatus*) groups. The control group showed normal histoarchitectural appearance of the frontal cortex as evident by intact Pyramidal neurons (green arrow), oligodendrocytes (black) are also remarkable. Observed in T1 are neuronal vacuolations and pyknotic neurons (red arrow), few oligodendrocytes are also remarkable. T2 and T3 showed frontal cortical sections with the normal neuronal morphology. **Stain**: H and E; **Magnification**: x400

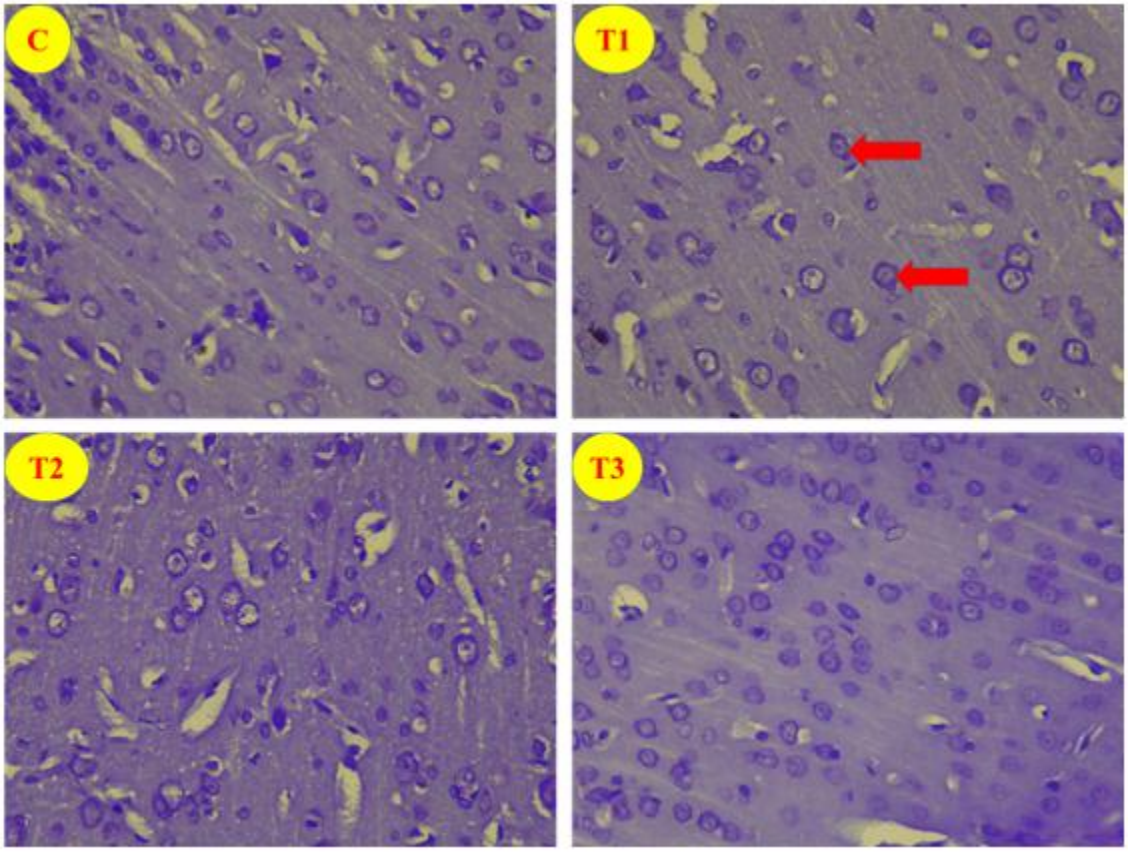

PLATE 3: Representative light photomicrographs showing cortex of the control, T1 (lead acetate only), T2 (*Citrullus lanatus* only) and co-treatment groups T3 (lead acetate + *Citrullus lanatud*) groups. The control group showed normal histoarchitectural appearance of the frontal cortex as evident by intact Pyramidal neurons, while in T1, neuronal cytoplasm appears intensely stained and also there is loss of cytoplasmic Nissl substances. T2 and T3 showed frontal cortical sections with the normal neuronal morphology. **Stain**: Cresyl violet; **Magnification**: x400

4.0 DISCUSSION

This study investigated the potential effect of subacute administration of aqueous extract of water melon(*citrulluslanatus)* seed on lead acetate induced toxicity in the cerebrum which is one of the system vulnerable to the adverse effect of administration of lead acetate is the central nervous system (18). Lead is a heavy metal that has also been regarded as a neurotoxicant. It is known to accumulate in soft tissues. Lead acetate possess the ability to penetrate the blood brain barrier thereby exerting its deleterious effects. Different routes of exposure to lead toxicity has been documented, which may include food, water, air, or occupational exposure (19).

The result obtained from this study (fig 1) revealed significant decrease in the body weight of T1 administered lead acetate only when compared to the control. This is in tandem with the report of (20) also reported decrease in body weight following lead acetate administration.

The reduction in the body weight could be attributed to increased metabolism or propable suppression in the appetite as a consequence of lead treatment.

The brain weight (Fig 2) of the rats across the groups were not significantly different from the control group. Although, there was neurodegenerative change in the brain of group T1which could lead to decrease in the cortical volume or thickness and as a result causing reduction or decline in the brain weight. The possible explanation why there was no effect lead on the brain weight in this group and other experimental groups relative to the control could based on the frequency, time and route of exposure. Increasing the frequency, time all the exposure could impact negatively on the brain weight. However, this subject to further studies

The relatively brain weight is the ratio of brain to the body weight express in percentage. The relatively brain weight (Fig 3), show no significance different across all experimental groups when compare to the control changes in the brain weight relative to the body weight is a possible event as observed in the reduction of the relative weight of T1, albeit non-significant to control. Lead is highly toxicity metal that persist in the environment and has been known to disrupt neurological and normal biological function (21). Chronic poisoning by lead is one the major public concerns in developing countries (22). Several mechanisms have been proposed by which lead induced neurotoxicity which includes oxidative stress, inflammation, disruption in the neurotransmitter systems e.t.c. Lead acetate exposure results in the generation of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide and lipid peroxide there increasing oxidative damage to cellular materials (23). The neurotoxic effect of lead acetate could also be interference with the cholinergic system and also alteration in the intercellular communication.

The histological assessment of both haematoxylin andcresyl violet stained cerebral cortical section of T1group shows distortion in the histoarchitectural organization of the cerebral cortex evident by distortion in the pyramidal neuronal cell, with wide perivascular space, broken axon with scantily distributed glial cells, in GroupT2 concomitantly administered lead acetate and citrulluslanatus extract showed a preserved cerebral cortex histoarchitecture showing the neuroprotective ability of aqueous extract of citrulluslanatus which could be due to the phytochemical constituent present in citrulluslanatuswhich has been previously documented by (24).

Our study shows that administration of lead significantly reduced the superoxide dismutase (SOD) and catalase (CAT) level while there was a significant increasedmalondialdehyde(MDA) Antioxidants which exerts a protective activity on neurons against a variety of experimental neurodegenerative conditions, (25). Citrullus lanatus has antioxidants that have been used to reserve lead mediated toxicity by ameliorating oxidative stress status (26).

5.0 CONCLUSION

In conclusion, *citrulluslanatus* extract shows a promising mitigating and ameliorative property against lead induced neurotoxicity, offering a potential therapeutic intervention.

6.0 RECOMMENDATION

Further research should explore the underlying molecular and immunological application of *citrulluslanatus*in mitigating and addressing neurotoxicity caused by heavy metals. There is a need to further understand the long term effect and optimum dosage and potential side effects

CONSENT

Not applicable

ETHICAL APPROVAL

All animal experiments and protocols adhered to the guidelines and regulations set forth by the National Research Council in regards to laboratory animal care and utilization(2011)

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