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NEUROPROTECTIVE AND ANTITOXIC POTENTIAL OF HYDROMETHANOLIC EXTRACT OF *ALLIUM CEPA* IN EXPERIMENTAL RATS

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ABSTRACT

This research studied the neuroprotective potential of *Allium cepa* hydromethanolic extract on neurotoxicity due to lead acetate treatment. 30 male wistar rats were used. The animals were grouped simple randomly into 6 separate compartments. The experimental period lasted for 42 days during which the animals were subjected to various treatments. From A the control administered normal saline, B administered *Allium cepa* extract 100mg/kg, C administered lead acetate 500mg/kg. D, E and F co-administered 100,150 and 200 mg/kg of *Allium cepa* extract and lead acetate 500mg/kg. The biomarkers assayed for include protein carbonyl (PC), malondialdehyde (MDA),isoprostanes (F_2IsoP) and reduced glutathione (GSH). With data significantly different at a confidence interval of 95%, there was a significant increase in GSH, but a decrease in PC, MDA and F_2IsoP in the groups treated with *Allium cepa* extract 100mgkg and *Allium cepa* 150 and 200mg/kg co-administered lead 500mg/kg.lead acetate treatment alone significantly increased PC, MDA and F_2IsoP but decreased GSH compared to control. *Allium cepa*, at the studied dose, can effectively be applied in neurotoxicity.

KEYWORDS: *Allium cepa* hydromethanolic, protein carbonyl (PC).

INTRODUCTION

Plants are major 'store-houses' for bioactive phytonutrients.[1] These diverse species have various health applications like in aromatherapy[2], physiotherapy[1], nutrition and dietics.[4] We ingest higher plants usually in form of vegetables and fruits[6]; our health and sustenance usually depends on our complex form of nutrition in form of a balanced diet.[2] The nervous system is one of the major control systems in the body[12], the other being the endocrine system. Our voluntary motor activity, learning ability, memory capacity and navigation, visceral and cognitive function is under the control of the nervous system[11], Maintaining the healthy functional and structural state of the nervous tissues is of utmost importance. *Allium cepa liliacae* (also called Onion) is of same family as ginger (*Allium sativum*) and shallot (*Allium aggregatum*).[2] The certainty of *Allium cepa* as effective in hyperglycaemic[3], hypertensive[6], inflammatory, sterility,pro-oxidative and hypercholestrolemic treatment is fairly established[5], but the usefulness of this vegetable in neurotoxic condition is clearly uncertain. This research is aimed at evaluating the potential for *Allium cepa* to be effective in management of neurotoxicity.

MATERIALS AND METHOD

Plant Collection

The *Allium cepa* used in this study was cultivated in a farm land in Amansi, Ifite-Nteje, Oyi Local Government Area, in Anambra state, South-Eastern Nigeria. The vegetable collected for this research was cultivated locally without the use of synthetic biochemical agents.

Plant Identification

Allium cepa identification and authentication was confirmed in the herbarium for plant science and biotechnology, Faculty of Natural and Applied Sciences, University of Port Harcourt.

Solvent Collection

Methanol and Lead Acetate were imported from an industrial chemicals store in Shenhua, China. Distilled water was prepared in Industrial Chemistry laboratory,

Industrial chemistry Department, Faculty of Natural Sciences, Madonna University.

Extract Preparation

Allium cepa was washed carefully with water and NaCl. The outer covering was removed and disposed properly. The fleshy part was allowed to dry in a laboratory oven at 70 o C. After 48 hours, the dried *Allium cepa* was crushed and made into a powdered form. Hydromethanol was prepared with distilled water and methanol in a ratio 4:1.[3] The solvent was used to dissolve 1000 grams of *Allium cepa* powder. After 72 hours, the mixture was filtered and the filtrate was collected into a test tube and stored in a refrigerator.

Phytochemical Analysis

Phytochemistry and phytochemical analysis was performed in the laboratory for Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, Madonna University.

Animal Collection

A total of thirty (30) male wistar rats were obtained from the experimental animals unit in Madonna University for the purpose of this research. The animals were confirmed to be physically and mentally healthy for the purpose of this research. The animals were allowed to acclimatize for two (2) weeks prior to the experimental period.

Treatment Doses

This research included three (3) doses of *Allium cepa* hydromethanolic extract, which include.

Low dose	**100mg/kg** of extract
Medium dose	**150mg/kg** of extract
High dose	**200mg/kg** of extract

Administration was via the orogastric route and lasted for 42 days.

Study Design

The animals were randomly grouped into six (6) separate compartments containing 5 rats each. The treatment for each group includes;

Groups	Treatments
A	Normal saline
B	*Allium cepa* **100mg/kg**
C	Lead Acetate **500mg/kg**
D	*Allium cepa* **100mg/kg** + Lead Acetate **500mg/kg**
E	*Allium cepa* **150mg/kg** + Lead Acetate **500mg/kg**
F	*Allium cepa* **200mg/kg** + Lead Acetate **500mg/kg**

Sacrifice and Sample Collection

At day 42 of the experimental period, the animals were anesthetized using Diethyl-ether and sacrificed. Each sample consists of 5 ml of blood collected in a heparinated bottle. The brains of the animals were collected after a suture was made in the cranial cavity.

Brain Tissue Homogenate

The brain tissue was cut into slices of appropriate sizes for analysis (100 to 300mg) and placed into a microcentrifuge tube. The typical sample size was 100mg. The tissue was washed properly with 1 ml PBS. Glass beads (0.5mm) equal to tissue mass followed by 0.1 to 0.6ml of buffer was added. The microcentrifuge tubes were placed into blender at speed 6 at time 3 to homogenize.

Brain Stress markers

Brain stress markers assayed for include protein carbonyl (PC), isoprostanes (F_2IsoP), Malondialdehyde (MDA) and reduced glutathione (GSH).

Protein carbonyl (PC) assay

IBL® assay kit was used for protein carbonyl assay. 2,4-Dinitrophenylhydrazine (DNPH)) reacts with protein carbonyl in plasma to form a Schiff base to produce a corresponding hydrazone which can be analyzed spectrophotometrically at an absorbance between 360-385mm.

Isoprostanes (F_2IsoP) assay

The Eagle® Biosciences 8-Isoprostane ELISA Assay kit was used for the quantitative determination of 8-isoprostane in biological samples by enzyme linked immunoassay (ELISA). 8-Isoprostane ELISA Assay kit is for research use only and not to be used in diagnostic procedures.

Malondialdehyde (MDA) assay

Malondialdehyde level of the plasma was measured by the following procedure.0.5 plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1ml of 0.6 % TBA was added to the mixture, shaken, and warmed for 30 min in a boiling water bathfollowed by rapid cooling. Then it was shaken into a 4 ml of nbutyl-alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 and 520nm by spectrophotometer against butanol. The standards of 5, 10, 20 nmol/ml TEP were used.

Reduced glutathione (GSH) assay

The Cayman Chemical Glutathione Reductase Assay Kit measures GR activity by measuring the rate of NADPH oxidation.

$$GSSG + NADPH + H^{+} \xrightarrow{GR} 2GSH + NADP^{+}$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm and is directly proportional to the GR activity in the sample. The Cayman GR Assay Kit can be used to measure GR activity in plasma, erythrocyte lysates, tissue homogenates, and cell lysates. Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidised to NADP+.[16] The decrease in absorbance at 340 nm is measured.

Statistical Analysis

Experimental data are presented in Mean ± Standard error of mean (SEM). Percentage (%) change was also calculated to make the data well translated. SPSS 20.0 was used for all calculations and statistical analysis such as One-way analysis of variance (ANOVA). Values are significant at $p \leq 0.05$ or at confidence interval of 95%.

Terpenoids	+
Phenols	+
Cardiac glycosides	+
Oils	+

+ = present - = Absent

RESULTS

Table 1: Phytochemical constituents of hydromethanolic extract of *Allium cepa.*

Phytochemicals	Indication
Saponins	–
Tannins	–
Flavonoids	++
Steroids	+
Alkaloids	++

Table 2: Effect of hydromethanolic extract of *Allium cepa* on brain stress markers.

Treatments	PC(µg/ml)	%→A	F_2IsoP(µg/ml)	%→A	MDA(µg/ml)	%→A	GSH(µg/ml)	%→A
A	19.3±0.23	0	41.2±0.14	0	98.4±4.24	0	14.2±2.14	0
B	7.0±0.11[a]	-64	23.1±1.20[a]	-44	40.3±9.23[a]	-59	23.1±6.34[a]	63
C	37.2±0.42[a]	93	74.1±2.10[a]	80	140.2±8.34[a]	42	4.3±1.22[a]	-70
D	30.2±0.4[a]	56	47.0±3.0	14	120.2±2.33[a]	22	13.1±7.44	-8
E	18.4±0.31	-5	35.2±1.13[a]	-15	96.4±2.43	-2	17.3±7.13[a]	22
F	11.2±1.4[a]	-42	27.4±4.0[a]	-33	57.3±18.2[a]	-42	22.3±1.40[a]	57

Key:A= Normal saline **B**= *Allium cepa* **100mg/kg C**= Lead Acetate **500mg/kg D**= *Allium cepa* **100mg/kg** + Lead Acetate **500mg/kg E**= *Allium cepa* **150mg/kg** + Lead Acetate **500mg/kg F**= *Allium cepa* **200mg/kg** + Lead Acetate **500mg/kg,** [a] $p<0.05$was considered significant compared with control.

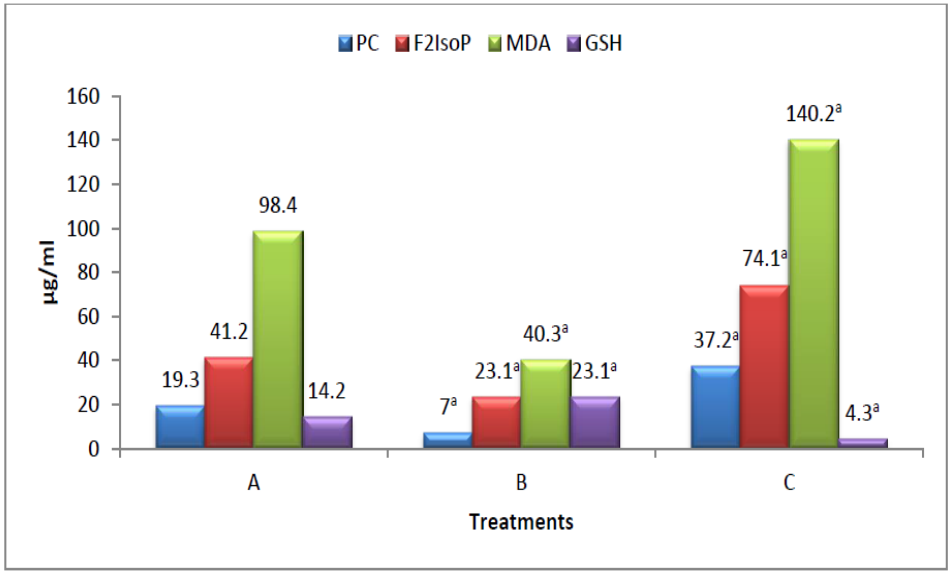

Figure 1; Effect of treatments on brain stress markers.

A=Normal saline **B**=*Allium cepa* extract 100mg/kg **C**=Lead acetate 500mg/kg.

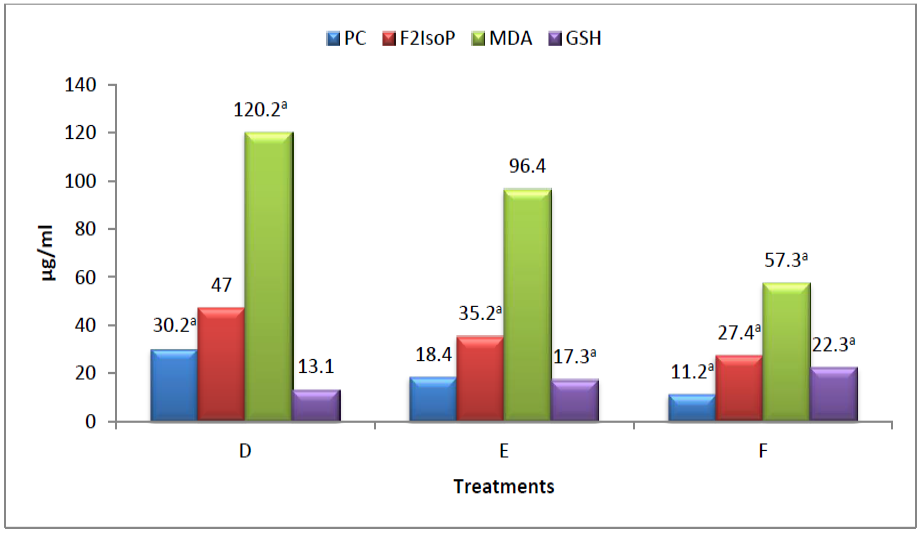

Figure 2: Effect of treatments on brain stress markers.

D= *Allium cepa* extract 100mg/kg + Lead acetate **E**=*Allium cepa* extract 150mg/kg + Lead acetate. **F**= *Allium cepa* extract 200mg/kg+ Lead acetate.

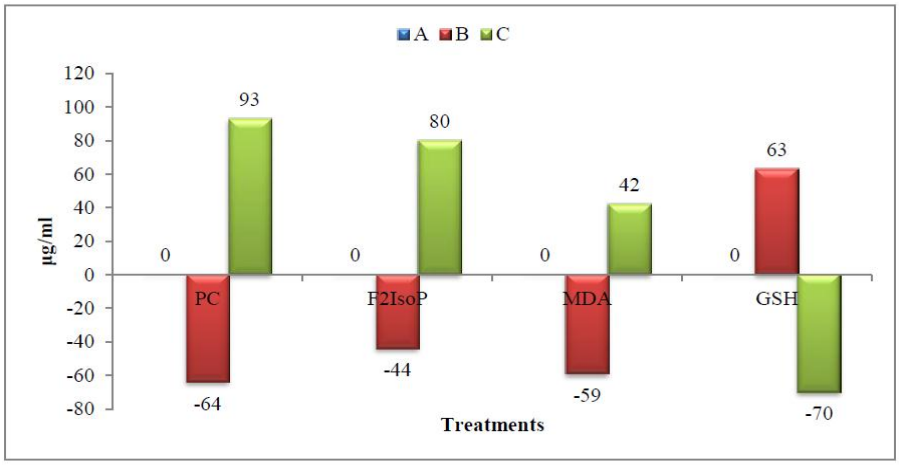

Figure 3: Effect of treatments on brain stress markers using percentage (%) change.
A=Normal saline **B**=*Allium cepa* extract 100mg/kg **C**=Lead acetate 500mg/kg.

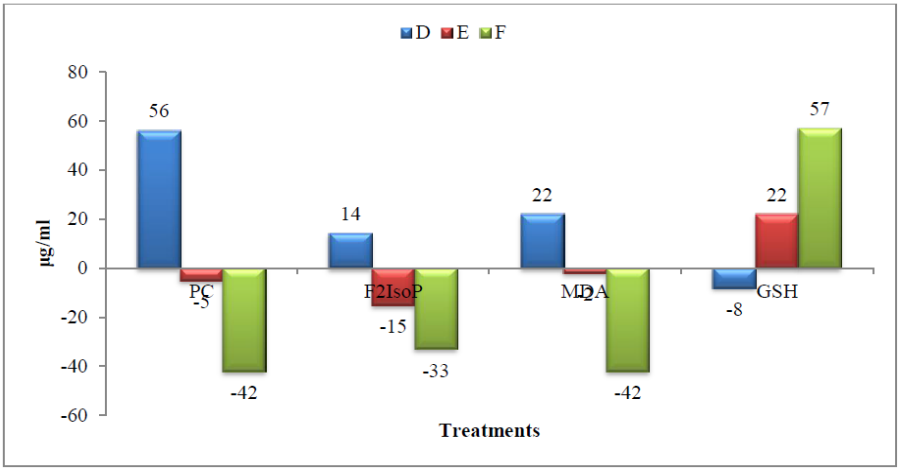

Figure 4; Effect of treatments on brain stress markers using percentage (%) change.

D= *Allium cepa* extract 100mg/kg + Lead acetate **E**=*Allium cepa* extract 150mg/kg + Lead acetate **F**= *Allium cepa* extract 200mg/kg+ Lead acetate.

From table 1.
The phytoconstituents of *Allium cepa* hydromethanolic extract includes alkaloids, flavonoids, terpenoids, phenols, steroids, cardiac glycosides and oils.

From table 2.
With values statistically significant at P≤0.05, *Allium cepa* hydromethanolic extract (100mg/kg) significantly reduced PC (7.0±0.11[a]), F_2IsoP (23.1±1.20[a]), and MDA (40.3±9.23[a]), but significantly increased GSH (23.1±6.34[a]) compared to control administered normal saline. Lead acetate (500mg/kg) significantly increased PC (37.2±0.42[a]), F_2IsoP (74.1±2.10[a]), MDA (140.2±8.34[a]) and significantly decreased GSH (4.3±1.22[a]) compared to control administered normal saline. At low dose treatment *D*, there was a significant increase in PC (30.2±0.4[a]) andMDA (120.2±2.33[a]), at medium dose treatment *E*, there was a significant decrease in F_2IsoP (35.2±1.13[a]) and a significant increase in GSH (17.3±7.13[a]) compared to control. At high dose treatment *F*, there was a significant decrease in PC (11.2±1.4[a]), F_2IsoP (27.4±4.0[a]) and MDA (57.3±18.2[a]) and a significant increase in GSH (22.3±1.40[a]) compared to control.

DISCUSSION
From the outcome of this research, lead acetate exhibited neurotoxic manifestations. The neurotoxicity of lead acetate in this study is clearly seen in group *B* and to a lesser extent; in group *D*. Lead acetate depletes reduced glutathione (GSH) due to its enhancing and promoting effect on production of pro-oxidants.[15] Lead acetate may have probably reduced the amount of free protons needed to neutralize reactive oxygen species and peroxides or prevent their formation. The significant increase in protein carbonyl (PC), malondialdehyde (MDA) and isoprostanes (F_2IsoP) due to increased oxidation of proteins (carbonylation) and lipids accordingly, reflects the pro-oxidant nature of lead acetate. Lead acetate may have depleted the level of GSH in the test sample. This GSH depletion may be responsible for the increase in PC, MDA and F_2IsoP[9][10], Earlier studies have reported that lead acetate increases the formation and subsequent liberation of reactive oxygen species.[14] Lead acetate does not suppresses peroxidation.[16] *Allium cepa* may be said to possess anti-peroxidative or brain cell membrane- protecting agents.[13] *Allium cepa* hydromethanolic extract due to its bioactive phytoconstituents may have the intrinsic biologic effect of preventing oxidative stress.[7] Its bioactive components especially flavonoids are antitoxic.[8] *Allium cepa* is the most abundant source of quercetin[5], which is a flavonol. Quercetin is neuroprotective.[6] *Allium cepa* also has antioxidant phytoconstituents like anthocyanin and kaempherol.[4][7] These neuroprotective phytoconstituents may be the reason for the antagonism exhibited in high dose treatment *F*. Lead acetate may be a neurotoxic agent[11], but this neurotoxicity is fairly or significantly suppressed when it is co-administered medium (150mg/kg) and high dose (200mg/kg) hydromethanolic extract of *Allium cepa*. The neuroprotective effect of *Allium cepa* treatment at 100mg /kg may be suppressed by the toxicity of lead acetate at 500mg/kg. From this study, *Allium cepa* neuroprotective effect can suppress the neurotoxic effect of lead acetate at a particular dose.

CONCLUSION
Allium cepa hydromethanolic extract contains neuroprotective components capable of preventing neurotoxicity induced by lead acetate. The phytoconstituents of *Allium cepa* are suspected to be responsible for the neuroprotective effect.

CONFLICT OF INTEREST
The author declares no conflict of interest.

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