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Neurotoxicity and Oxidative Stress Prevention by Honey and Garlic in Adult Male Wistar Rats Exposed to Lead Chinyere Elizabeth Eze and Azuoma Lasbery Asomugha

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Abstract:- Honey and Garlic are natural products considered as possible pragmatic approaches to make null the ever growing pool of diseases brought about by lead toxicity. This research was designed to evaluate the neurotoxicity and oxidative stress prevention by honey and garlic in adult male wistar rats exposed to lead. Twenty five (25) male Wistar rats with body weights ranging between 150g – 180g were divided into 5 groups of 5 rats each. Group A was the control. Group B received lead acetate at 50mg/kg body weight for 4 weeks only. Group C first received honey 1000mg/kg body weight and garlic 300mg/kg body weight for 4 weeks; and then lead acetate at 50mg/kg body weight for the next 4 weeks; Group D first received honey 1000mg/kg body weight for 4 weeks; and then lead acetate at 50mg/kg body weight for the next 4 weeks. Group E first received garlic 300mg/kg body weight for 4 weeks; and then lead acetate at 50mg/kg body weight for the next 4 weeks. The administrations were given orally, once daily. The rats were starved for 24 hours after the last day of administration, and then sacrificed by decapitation after chloroform inhalation. Cerebral tissue samples were collected for analysis. Lead acetate caused a significant ($p<0.05$) decrease in glutathione, glutathione peroxidase, catalase and superoxide dismutase levels, while elevating the concentration of the oxidative stress marker (malondialdehyde). There was no significant change in the histology of the hippocampus. Pretreatment with honey and garlic, collectively and individually, increased the levels of the antioxidant enzyme activities and decreased malondialdehyde level. The significant toxicity brought about by lead acetate was abated by the pretreatment with honey and aqueous extract of garlic, with more amelioration seen in the combined treatment.

Keywords:- *Honey, Garlic, Lead, Neurotoxicity, Oxidative stress, Antioxidants.*

I. INTRODUCTION

The brain, part of the nervous system, is an organ that controls complex activities of the body which include memory, thought, emotion, motor skills, speech, vision, and all other processes that regulate the body. Low concentrations of inherent antioxidants as well as a rise of oxidative metabolic activity bring about brain oxidative insults (Chen *et al*., 2000). The nervous system is one of the major and critical targets for lead toxicity (Patrick, 2006; Sujatha *et al*., 2011). Even at low dose, lead is likely to penetrate into the brain by interfering and changing the architecture of the Blood-Brain Barrier (Sujatha *et al*., 2011).

Oxidative stress is one viable molecular mechanism by which lead enacts its toxicity (Gurer and Ercal, 2000; Halawa *et al*., 2009). Increased lead poisoning is a powerful element in brain damage, mental inefficiency and severe behavioral disturbances in the company of neuromuscular weakness, coma, as well as anemia (Surana and Jain, 2010).

Garlic and honey have been used in traditional medicines around the world. Honey serves as a natural food supplement, having great health benefits. It is known to be a therapeutic antioxidant agent in treating variety of diseases. Data report that it exhibits antiviral, antibacterial, antifungal, strong wound healing, anti-inflammatory and antidiabetic effects. It also has antimutagenic, immunomodulatory, anticancer, estrogenic regulatory, and numerous other robust effects (Ahmed *et al*., 2018). Data also show that oxidative stress and the many streams of diseases associated with it can be combatted by honey when used as a traditional therapy (Erejuwa *et al*., 2012; Ahmed *et al*., 2018). Garlic, on its part, has been studied and shown to prevent health problems including high blood pressure, bronchitis, gastrointestinal problems, colds, menstrual pain, flu, coughs and atherosclerosis (Kumar *et al*., 2010). It has also been shown to destroy a vast majority of intestinal parasites, viruses and bacteria. People use it mostly as a natural alternative for cardiovascular wellness (Kumar *et al*., 2010; Pakia *et al*., 2015).

This research investigated the preventive role of honey and garlic, individually and collectively, against lead-induced toxicity in the brain. We also examined the degree of toxicity of lead on biochemical parameters (lipid peroxidation, glutathione, glutathione peroxidase, catalase and superoxide dismutase) of adult male Wistar rats.

II. MATERIALS AND METHODS

A. Place of the Study

This experiment was carried out in the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Anambra State.

B. Collection and Preparation of Garlic Extract

Fresh garlic (Allium sativum) was procured from Nkwo market, Nnewi, Anambra State. After which they were peeled, washed and then oven dried. The dried garlic was then grinded into powdered form. 100g of the fine powder was soaked in 50ml of water for 72 hours with intermittent

manual stirring. The mixture was then filtered with a sieve and further using No 1 Whitman filter paper. The extract was dried using water bath at a temperature of 40°C till it became concentrated and later kept in a refrigerator at 4°C until needed. This aqueous garlic extract method follows the method described by Senapati *et al*., (2000).

C. Collection of Honey

Honey was purchased from the Bee farm of the Department of Zoology, Nnamdi Azikiwe University, Awka.

D. Chemicals

All chemicals and reagents used were of analytical grade. Lead acetate was obtained from the Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi.

E. Ethical Consideration

Ethical approval was obtained from Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC).

F. Experimental Animals

Twenty-five (25) male Wistar rats weighing between 120 and 140g were obtained from an animal farm in the College of Health Sciences, Nnamdi Azikiwe University, Nnewi, and housed in the animal house of the College of Health Sciences, Nnamdi Azikiwe University. They were allowed to acclimatize to laboratory room conditions (12 hour dark/light periods) for two weeks before the onset of the experiment. The rats were fed with rat chow, and water ad libitum. All the animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Science and published by the National Institutes of Health (Garber *et al*., 2011).

G. Acute Toxicity (Ld50) for Lead Acetate

9 male wistar rats were used for the study. The rats were divided into 3 different groups of 3 animals each and they were administered 10mg/kg, 400mg/kg and 1000mg/kg of Lead Acetate respectively and observed for 24 hours.

H. Acute Toxicity (Ld50) for Honey

Phase One: 9 male wistar rats were used for the study. The rats were divided into 3 different groups of 3 animals each and they were administered 10mg/kg, 100mg/kg and 1000mg/kg of Honey respectively and observed for 24 hours.

Phase Two: 3 male wistar rats were used for the study. The rats were divided into 3 different groups of 1 animal each which received 1600, 2900 and 5000mg/kg of the honey respectively and observed for 24 hours.

I. Acute Toxicity (Ld50) for Garlic

Phase One: 9 male wistar rats were used for the study. The rats were divided into 3 different groups of 3 animals each and they were administered 100mg/kg, 200mg/kg and 1000mg/kg of the extract respectively and observed for 24 hours.

Phase Two: 3 male wistar rats were used for the study. The rats were divided into 3 different groups of 1 animal each which received 1600, 2900 and 5000mg/kg of the honey respectively and observed for 24 hours.

J. Experimental Design

The rats were randomly assigned into five (5) groups of five (5) rats each after a period of two (2) weeks of acclimatization.

Group A (control) received only water and rat chow.

Group B received lead acetate at 50mg/kg body weight for 4 weeks only.

Group C first received honey 1000mg/kg body weight and garlic 300mg/kg body weight for 4 weeks; and then lead acetate at 50mg/kg body weight for the next 4 weeks.

Group D first received honey 1000mg/kg body weight for 4 weeks; and then lead acetate at 50mg/kg body weight for the next 4 weeks.

Group E first received garlic 300mg/kg body weight for 4 weeks; and then lead acetate at 50mg/kg body weight for the next 4 weeks.

The administrations were done orally, once daily for 8 weeks, and we observed the animals every day.

K. Animal Sacrifice and Sample Collection

At the end of the experiment, the rats were starved for 24 hours and then sacrificed by decapitation after chloroform inhalation. The cerebral tissues were immediately removed and put in 10% formalsaline for histological examination. Some were kept in the refrigerator till analysis for biochemical parameters.

L. Biochemical Parameters

The cerebral tissues were homogenized (10%,w/v) in ice-cold phosphate buffer (0.01M, pH 7.4) containing 1.15% KCl in a Potter-Elvehjem type homogenizer. The resulting homogenates were centrifuged at 4°C at 1500 rmp for 10 minutes and the supernatant was used for biochemical evaluation. The following oxidative stress marker and antioxidants were assayed using spectrophotometer; lipid peroxidation (LPO) in line with the method described by Varshney and Kale (1990); reduced glutathione (GSH) in line with the method used by Beutler *et al*., (1963); catalase (CAT) in accordance with Claiborne (1985); superoxide dismutase (SOD) activity following Misra and Fridovich's (1972) method, and glutathione peroxidase (GPx) activity by the method of Rotruck *et al*., (1973).

M. Histological Examination

Portions of the cerebrum were fixed in 10% formalsaline for 24 hours at room temperature. They were dehydrated, cleared in xylene, sectioned into a thickness of 4-5 µm and embedded in paraffin. Staining was done using hematoxylin and eosin and they were then imaged on a compound light microscope (Zeedo microscope) with 5MP internal camera, and e-PLAN objectives. The thickness in the CA1 area was taken digitally with accompanying software of the microscope, USB2.0 Camera viewer. Labelling was done with Photoscape v3.7.

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N. Statistical Analysis

Statistical Package for the Social Sciences (SPSS; Version 20) was used for data analysis, and the results expressed as mean ± SEM. One way analysis of variance (ANOVA) was applied for comparative analysis followed by post Hoc LSD multiple comparison. Values were considered significant at $P < 0.05$.

III. RESULTS

A. Acute Toxicity (Ld 50)

The LD50 of Lead Acetate was found to be 632.45mg/kg. The median lethal dose of Honey and aqueous extract of Garlic were both greater than 5000mg/kg.

B. Biochemical Parameters

		MEAN	±SEM	P-value	F-value
Malondialdehyde (nmol/ml)	Group A (Control)	4.89	±0.60	0.02*	
	Group B (50mg/kg of Pb-A)	6.57	±0.29		
	Group C (1000mg/kg Honey + 300mg/kg Garlic 4-wks + 50mg/kg of Pb-A 4-wks)	3.70	±0.09	0.01*	8.42
	Group D (1000mg/kg Honey 4-wks + 50mg/kg of Pb-A 4-wks)	3.05	±0.24	0.00*	
	Group E (300mg/kg Garlic 4-wks + 50mg/kg of Pb-A 4-wks)	4.31	±0.74	0.01*	

Table 1: Pre-treatment with honey and garlic on MDA levels of lead acetate treated Wistar Rats

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05.*

Pb-A: Lead acetate, SEM: standard error of mean, **[a] (not significant), * (significant)**

Table 1 result shows a significant (*p<0.05*) increase in MDA level in group B compared to group A. Groups C, D, and E had a significant (*p<0.05*) decrease compared to group B.

		MEAN	**±SEM**	**P-value**	**F-value**
Glutathione (U/ml)	Group A (Control)	55.13	±2.58	0.00*	
	Group B (50mg/kg of PbA)	32.34	±0.78		
	Group C (1000mg/kg Honey + 300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	65.38	±5.80	0.01*	16.38
	Group D (1000mg/kg Honey 4-wks + 50mg/kg of PbA 4-wks)	56.22	±1.92	0.00*	
	Group E (300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	60.23	±2.09	0.02*	

Table 2: Pre-treatment with honey and garlic on GSH levels of lead acetate treated Wistar Rats

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05.*

Pb-A: Lead acetate, SEM: standard error of mean, **[a] (not significant), * (significant)**

Table 2 result reveals a significant (*p<0.05*) decrease in GSH level in group B compared to group A. Groups C, D, and E had a significant (*p<0.05*) increase in GSH level compared to group B.

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		MEAN	±SEM	P-value	F-value
Glutathione Peroxidase (U/ml)	Group A (Control)	0.78	±0.29	0.03*	
	Group B (50mg/kg of PbA)	0.44	±0.03		
	Group C (1000mg/kg Honey + 300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	0.81	±0.05	0.02*	2.13
	Group D (1000mg/kg Honey 4-wks + 50mg/kg of PbA 4-wks)	0.68	±0.17	0.12[a]	
	Group E (300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	0.62	±0.06	0.23[a]	

Table 3. Pre-treatment with honey and garlic on GPx levels of lead acetate treated Wistar Rats

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.

Pb-A: Lead acetate, SEM: standard error of mean, **[a] (not significant), * (significant)**

Table 3: The GPx result revealed a significant ($p<0.05$) decrease in group B compared to group A. Group C had a significant ($p<0.05$) increase, and groups D and E had an insignificant ($p>0.05$) increase compared to group B.

		MEAN	±SEM	P-value	F-value
Catalase (U/ml)	Group A (Control)	35.18	±0.80	0.00*	
	Group B (50mg/kg of PbA)	25.57	±1.15		
	Group C (1000mg/kg Honey + 300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	33.64	±1.76	0.02*	13.07
	Group D (1000mg/kg Honey 4-wks + 50mg/kg of PbA 4-wks)	34.77	±0.76	0.01*	
	Group E (300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	34.16	±0.69	0.00*	

Table 4. Pre-treatment with honey and garlic on Catalase levels of lead acetate treated Wistar Rats

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.

Pb-A: Lead acetate, SEM: standard error of mean, **[a] (not significant), * (significant)**

Table 4 result shows a significant ($p<0.05$) decrease in catalase level in group B compared to group A. Groups C, D, and group E had significant ($p<0.05$) increase compared to group B.

		MEAN	±SEM	P-value	F-value
Superoxide-dismutase (U/ml)	Group A (Control)	19.22	±0.67	0.04*	
	Group B (50mg/kg of PbA)	14.50	±1.34		
	Group C (1000mg/kg Honey + 300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	20.67	±2.04	0.01*	4.23
	Group D (1000mg/kg Honey 4-wks + 50mg/kg of PbA 4-wks)	22.04	±1.13	0.01*	
	Group E (300mg/kg Garlic 4-wks + 50mg/kg of PbA 4-wks)	21.39	±1.75	0.01*	

Table 5. Pre-treatment with honey and garlic on SOD levels of lead acetate treated Wistar Rats

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Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$.

Pb-A: Lead acetate, SEM: standard error of mean, [a] **(not significant), * (significant)**

Table 5: The SOD result shows a significant ($p<0.05$) decrease in group B compared to group A. Groups C, D, and group E had significant ($p<0.05$) increase compared to group B.

C. Histopathological Findings

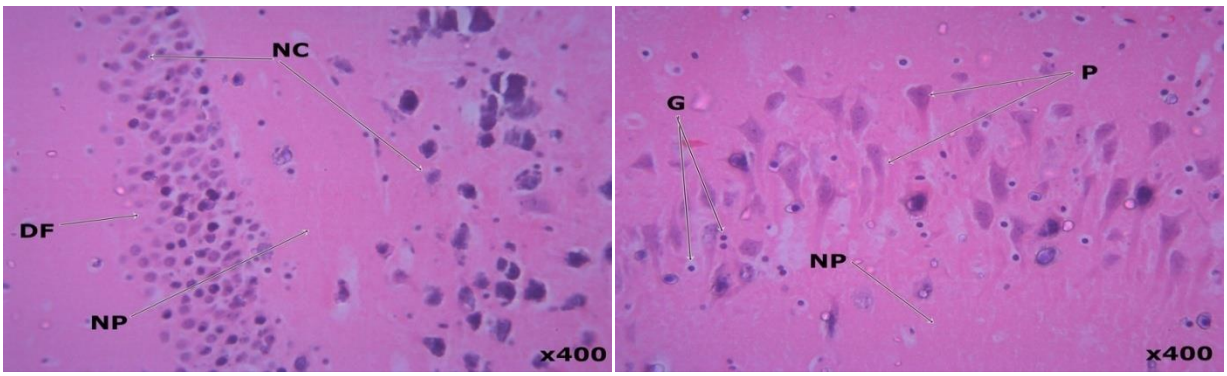

Plate 1: Histopathological section of the hippocampus of group A (control). NC – Neuron cell, DF – Dentate fascia, NP – Neuropil, G – Glial cells, P – Pyramidal cells

Photomicrograph of hippocampal formation show the cornuAmmnonis (CA) with variable cellularity in the constituent layers (CA1-4). The average thickness in the CA1 is 78.01µm. The CA1 is shown in the image and shows rich neuronal cell density within the dentate fascia and adjacent areas. Other regions show variable cellularity with evenly distributed glial cells and pyramidal cells, all set in a meshwork of neuropils.

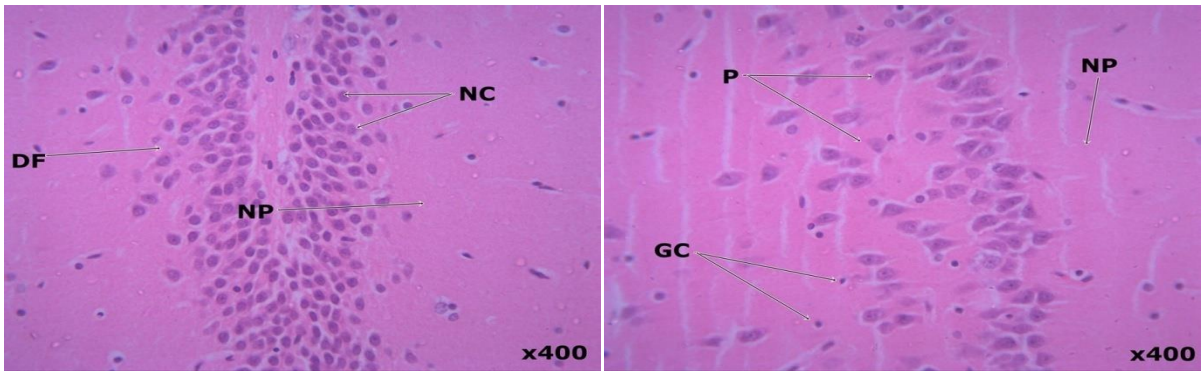

Plate 2: Histopathological section of the hippocampus of group B. NC – Neuron cell, DF – Dentate fascia, NP – Neuropil, GC – Glial cells, P – Pyramidal cells

Compared to CONTROL, there is no significant change in cell density with average thickness in the CA1 area reaching 76.50µm. Other areas show no significant thinning or loss of cellular density.

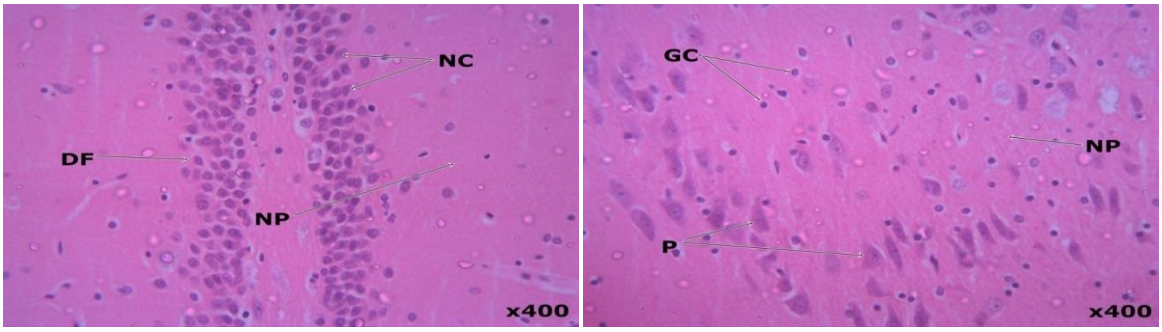

Plate 3: Histopathological section of the hippocampus of group C. NC – Neuron cell, DF – Dentate fascia, NP – Neuropil, GC – Glial cells, P – Pyramidal cells

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Compared to CONTROL, there is no significant change in cell density with average thickness in the CA1 area reaching 78.31µm. Other areas show no significant thinning or loss of cellular density.

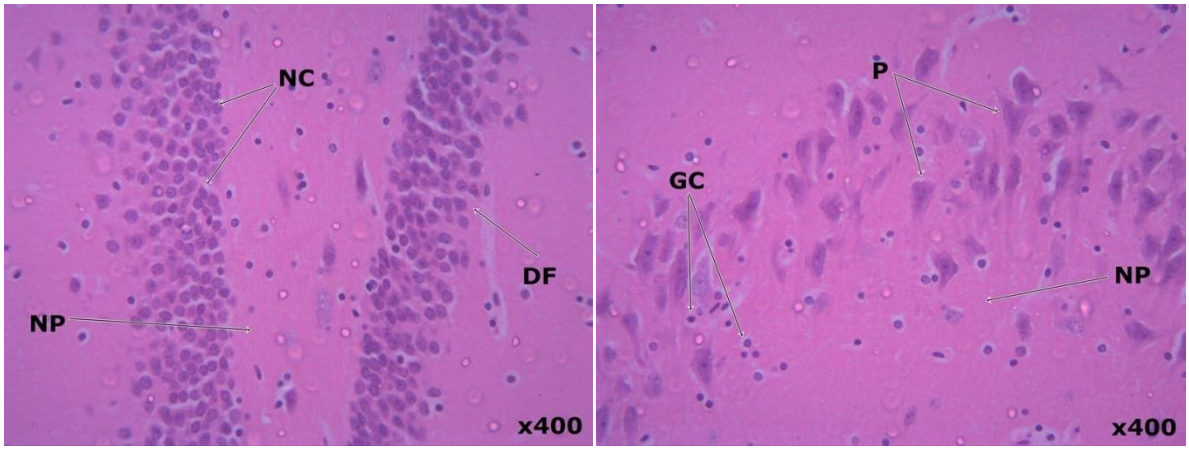

Plate 4: Histopathological section of the hippocampus of group D. NC – Neuron cell, DF – Dentate fascia, NP – Neuropil, GC – Glial cells, P – Pyramidal cells

Compared to CONTROL, there is no significant change in cell density with average thickness in the CA1 area reaching 79.66µm. Other areas show no significant thinning or loss of cellular density.

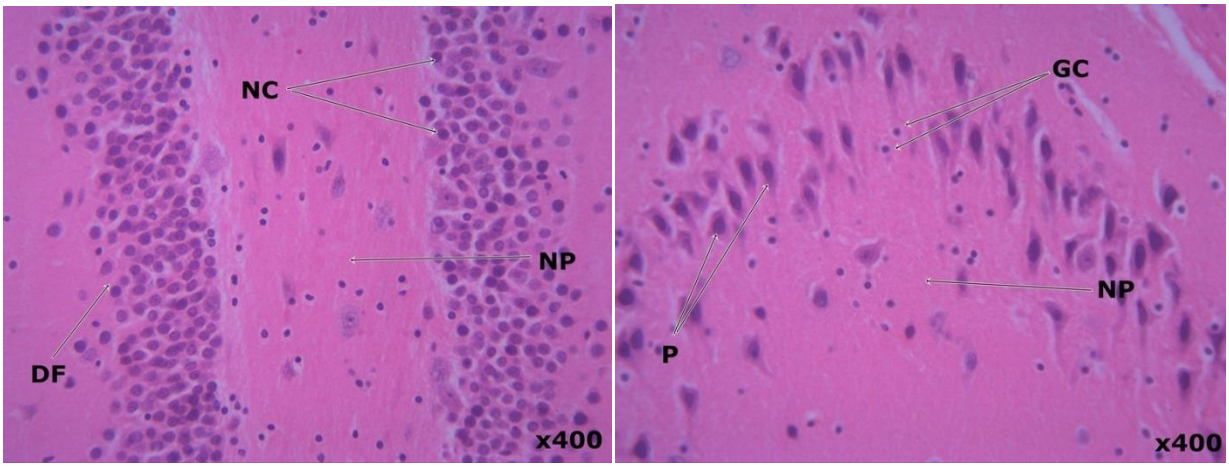

Plate 5: Histopathological section of the hippocampus of group E. NC – Neuron cell, DF – Dentate fascia, NP – Neuropil, GC – Glial cells, P – Pyramidal cells

Compared to CONTROL, there is no significant change in cell density with average thickness in the CA1 area reaching 80.23µm. Other areas show no significant thinning or loss of cellular density.

IV. DISCUSSION

Over the years, natural products have been investigated and their health benefits harnessed. Garlic and honey have proven to be among these natural remedies with great medicinal benefits. On the other hand, researchers have made the many chronic diseases brought about by increased levels of oxidative stress evident (Aminjan *et al*., 2019; García-Sánchez *et al*., 2020; Padureanu *et al*., 2019; Sharifi-Rad *et al*., 2020). Heavy metals, including lead induce oxidative stress by generation of free radicals. The measures of oxidative stress markers serve as a principal index in knowing the physiological condition of an animal. (Morsy *et al*., 2021).

Our research showed that exposure to lead acetate caused a significant increase in the MDA levels of group B animals. This significant increase in the MDA level in the lead treated rat groups implies that there is an amplification of lipid peroxidation (Morsy *et al*., 2021). This occurrence is of great value in hunting the free radicals generated by lead acetate (Wichai *et al*., 2019). These findings are in parallel to (Waheeb and Ali, 2020; Halawa *et al*., 2009; Hatice *et al*., 2015) that reported increased levels of MDA in animals treated with heavy metals. Administration of honey and garlic ameliorated the toxic effect of this heavy metal as the pretreated animals showed a significantly lower level of MDA as compared to the control. Aqueous extract of garlic was seen to have a more ameliorative effect than honey in reducing the MDA level. A study by Wichai *et al*., (2019) reported the ameliorative effect of aged garlic extract (AGE) by the reduction of MDA levels in animals pretreated by AGE.

Additionally, our findings revealed the high antioxidative activities of honey and garlic by the significant increase observed in the levels of GSH, GPx, CAT and SOD in the pretreated groups. These findings are in corroboration with the study carried out by Wichai *et al*., (2019) that reported that pretreatment of AGE caused significant increases of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, but the activity of catalase (CAT) had no significant change. We observed that the synergistic effect of both honey and garlic was greater in increasing the levels of GSH and GPx than when they acted singly. Our findings also revealed a significant decrease in the levels of these antioxidants in the lead-intoxicated animals. The reduction of the antioxidant enzyme activities shows the presence of oxidative stress caused by lead. Hatice *et al*., (2015) reported significant inhibition of SOD, CAT, GPx and glutathione-S-transferase activities and increased malondialdehyde (MDA) level in rat brain tissues intoxicated with lead nitrate and mercury chloride.

Histological analysis showed a reduction in the average thickness in the CA1 regions of hippocampus of animals exposed to only lead. Although not significant, an improvement was observed in the average thickness in the CA1 regions in groups pretreated with honey and garlic as there was a reversal of cellular density loss in groups C, D and E when compared to the control. This agrees with the findings of Wichai *et al*., (2019) that reported that aged garlic extract reversed the loss of neurons that occurred due to amyloid-β toxicity in the hippocampus.

V. CONCLUSION

Lead ingestion is very much capable of causing oxidative stress and neurotoxicity. Interestingly, this adverse effect of lead can be prevented by treatment with honey and garlic. Our study revealed that pretreatment with honey and aqueous extract of garlic cushioned the harmful effects of lead acetate. It also revealed the greater ameliorative effect of honey and garlic when they act in combination than when they function individually.

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