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Inhibitory effect of garlic (*Allium sativum)* on Fe^{2+} and Pb^{2+}- induced lipid peroxidation in rat brain and liver *in-vitro*

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ABSTRACT

High levels of iron [Fe^{2+}] and lead [Pb^{2+}] in the body have been linked to oxidative stress arising from peroxidation of membrane biomolecules and this usually results or manifests as neurologic, hepatic as well as cardiovascular disorders. Therefore, this study is sought to investigate the inhibitory effect and other antioxidant potentials of both aqueous and ethanolic extracts of garlic cloves (Allium sativum) against iron (ll) sulphate and lead acetate induced lipid peroxidation in rats' brain and liver tissues. From the results, incubation of the tissue homogenates with the pro-oxidants caused significant ($P<0.05$) increase in levels of thiobarbituric acid reactive substances (TBARS), while administration of the extracts provided a significant protection to TBARS levels in the tissues, suggesting their higher inhibitory potential on the pro-oxidants. Besides, the antioxidant potential of the extracts was determined via antioxidant activities that account for its Fe^{2+} chelating ability and reducing power, 1,1-diphenyl-2 -picrylhydrazyl [DPPH] as well as hydroxyl radicals[OH] scavenging ability. The extracts at different concentrations demonstrated significant antioxidant potentials, indicating their abilities to act as radical scavengers and chelators. Hence, oxidative stress in the brain and liver could be potentially prevented or managed by dietary intake of Allium sativum.*

Keywords: Peroxidation, oxidative stress, antioxidant, brain, liver, Fe^{2+}chelation

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INTRODUCTION

In recent years, it has become apparent that the oxidation of lipid is a crucial step in the pathogenesis of several disease-states most especially in adult and infant patients [1]. A variety of pro-oxidants especially heavy metals are known to cause oxidative damage to biomolecules such as proteins, lipids and DNA [2] via imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage [3]. Reactive oxygen species are chemically aggressive and their attack on the polyunsaturated fatty acids component of membrane lipids initiates lipid peroxidation, an auto-catalytic process that changes membrane structure and function [4]. However, measurement of thiobarbituric substances (TBARS) in the tissues is widely used as an index of lipid peroxidation [5]. Besides, several *in-vitro* and *in-vivo* studies reported that most organosulphur compounds protect against oxidative stress due to their high radical scavenging potentials [6]. Some of these medicinal plants have been investigated for their anti-oxidative properties and the treatment of such diseases [7]. This also has attracted increase attention as naturally occurring antioxidant compounds in plant sources are identified as free radicals or active oxygen scavengers which serve as an advantage over many synthetic chelators that have shown toxic or mutagenic effects [8]. The present study was planned to

evaluate the antioxidant potentials of *Allium sativum* and its inhibitory effect on some pro-oxidants (Fe^{2+} and Pb^{2+}) induced lipid peroxidation in rat's brain and liver tissues in-vitro.

MATERIALS AND METHODS

Preparation of aqueous extract

Crude aqueous extract of *Allium sativum* of a single clove variety was prepared from bulbs purchased from the market in Ado-Ekiti, Nigeria. The clove was sliced, ground into paste and then dissolved in distilled water 10mg/ml the sample concentration was later serially varied into 1.0ml, 2.0ml, 3.0ml, 4.0ml, and 5.0ml relative to the liver homogenate used for the in vitro experiment.

Preparation of ethanolic extract

120g of the powdered sample were extracted with solvent combination (via maceration) of 70% ethanol for 48hrs using the maceration method described by [9]. 1litre of 70% ethanol was used. The mixture was decanted and filtered using sterile whatman paper. The filterate measured up to 600ml and later evaporated to dryness using freeze dryer to obtain 9.92% yield.

Determination of ferric reducing antioxidant power of Allium sativum extract [Frap assay]

Procedure: the reducing property of the garlic extract was determined using method described by [10]. 0.25ml of extract was mixed with 0.25ml of 200mM of sodium phosphate buffer at pH 6.6 and 0.25ml of KFC. The mixture was incubated for 20mins at 50^0C. 0.25ml of TCA (10%) was later added and centrifuged at 2000rpm for 10mins. 1ml of the supernatant was mixed with 1ml of distilled water and 0.2ml of $FeCl_3$ and were centrifuged at 650g for 10min. a 5ml of the supernatant were mixed with an equal volume of water and 1ml, 0.1% ferric chloride. The same treatment was used for ascorbic acid solution as standard and the absorbance was measured at 700nm. The reducing power was then calculated and expressed as ascorbic acid equivalent.

Determination of free radical scavenging potential of Allium sativum extract [DPPH]

Procedure: The free radical scavenging ability of the extract against DPPH (1,1-diphenyl-2-picryhydrazyl) using method by [11]. 1ml of the garlic extract was mixed with 1ml of the 0.4mM methanol solution of the DPPH and the mixture was left in the dark for 30mins before measuring the absorbance at 516nm. Also, a method based on the reduction of methanolic solution of colored DPPH radical. DPPH evidently offers a convenient and accurate method for titrating 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of the extract suspension in water at different concentrations (1, 2, 3, 4, and 5)mg and after 30min, absorbance was measured at 516nm. However, ascorbic acid was used as reference material while lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All the tests were performed in duplicate and the results averaged. The percentage reduction in absorbance was calculated from the initial and final absorbance of each solution [12] and percentage scavenging of DPPH radical was calculated using the formula;

Determination of iron chelation potential of Allium sativum extract

Procedure: The ability of the garlic extract to chelate pro-oxidants was determined using a modified method of [13] and [14]. 150mM $FesO_4$ was added to a reaction mixture containing 168ml of 0.1M Tris-HCl { pH 7.4}, 218ml saline and the extract while the volume is made up to 1ml with distilled water. The reaction mixture was incubated for 5mins and 13ml of 1, 10-phenantroline was added and the absorbance was read at 510nm.

Determination of antioxidant potential of Allium sativum extract on hydroxyl radical [OH*]

Procedure: The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of [15]. Briefly, freshly prepared 100µl of extract was added to a reaction mixture containing 120µl, 20mM deoxyribose, 400µl, 0.1M phosphate buffer {pH 7.4}, 40µl, 20mM hydrogen peroxide and 40µl, 500µM FeSO4**,** and the volume made up to 800µl with distilled water. The reaction mixture was then incubated at 37^Oc for 30min and the reaction was stopped by the addition of 0.5ml of 2.8**%** TCA, this was followed by the addition of 0.4ml of 0.6**%** TBA solution. The test-tubes were subsequently incubated in boiling water for 20min. The absorbance was then measured at 532nm in spectrophotometer.

Thiobarbituric acid reactive substances Assay (TBARS)

Procedure: The production of TBARS from animal tissues was determined using a modified method described by [16]. The rats were anesthetized with ether and sacrificed by decapitation. The tissue (liver, brain) were quickly

removed and placed on ice. 1g of tissues were homogenized in cold 20mm Tris-HCl buffer pH 7.4 (1:10w/v) with ten up and down strokes at appropriately 1200rev/min in a Teflon glass homogenizer. The homogenate were centrifuged for 10min at 1400g to yield a pellet that was discarded and a low-speed supernatant (SI) were used for the assay. The homogenates (100ML) were incubated with or without 50ml of the various freshly prepared oxidants (iron (ll) sulphate, lead acetate) and different concentrations of garlic extract together with an appropriate volume of deionized water to give a total volume of 300µl at 37^0C for 1hr. The color reaction was carried out by adding 200, 250, and 500µl each of the 8.1% sodium dodecylsulphate (SDS), acetic acid pH 3.4 and 0.6% TBA respectively were incubated at 97^0C for 1hr. The absorbance was read after cooling the tubes at 532nm wavelength in spectrophotometer.

Statistical Analysis

Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by student t-test. The values ($P<0.05$) were regarded as significant.

$$\% \text{ Antioxidant Parameter} = \frac{(Abs._{control} - Abs._{testsample}) \times 100\%}{(Abs._{control})}$$

$$\% \text{ Inhibition} = \frac{(Abs._{control} - Abs._{testsample}) \times 100\%}{(Abs._{control})}$$

$IC_{50} = 11.32x - 17.28$
(Where x = %inhibition)

RESULTS

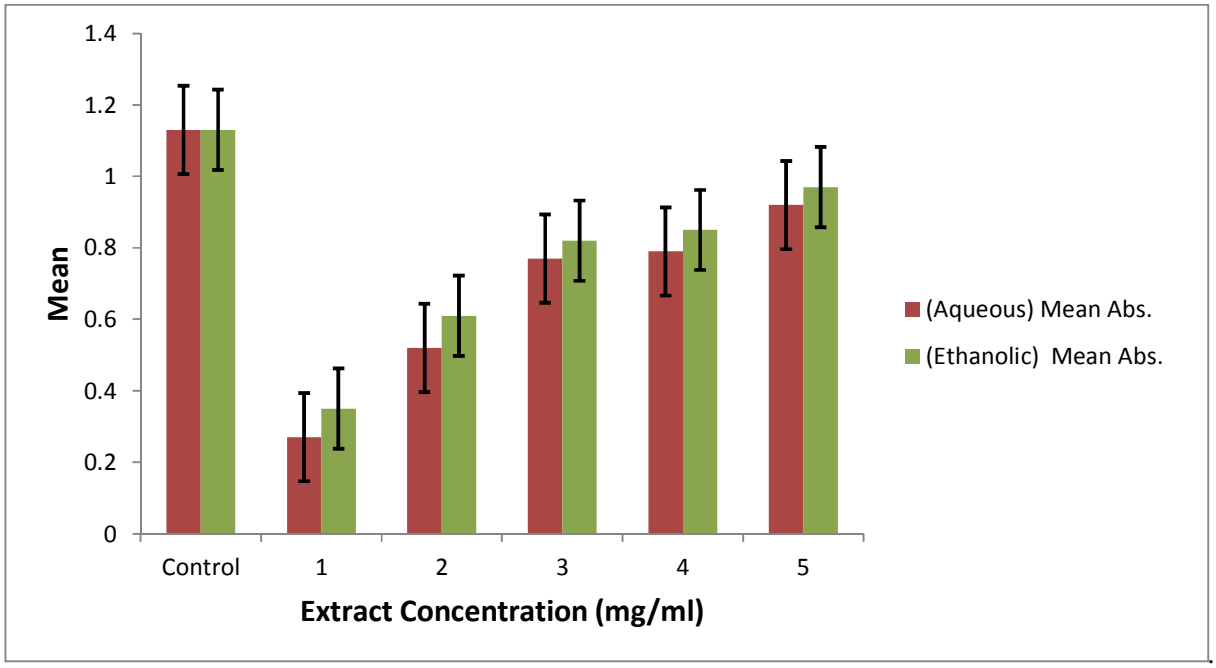

Figure 1: Ferric reducing antioxidant power of Allium sativum extract

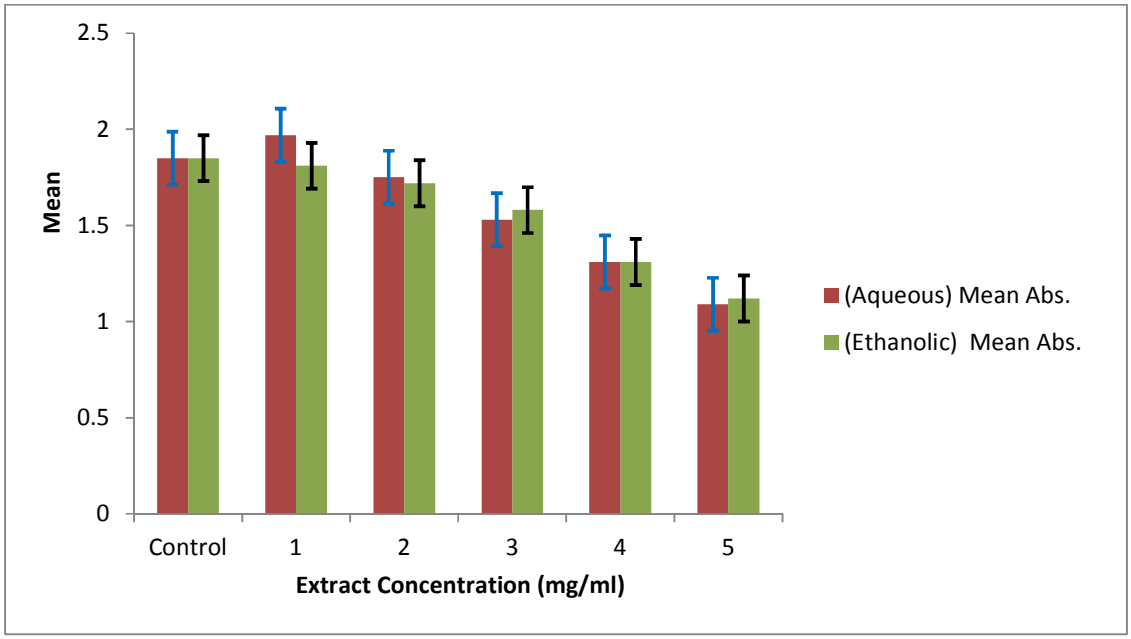

Figure 2: Free radical scavenging ability [DPPH] of Allium sativum extract

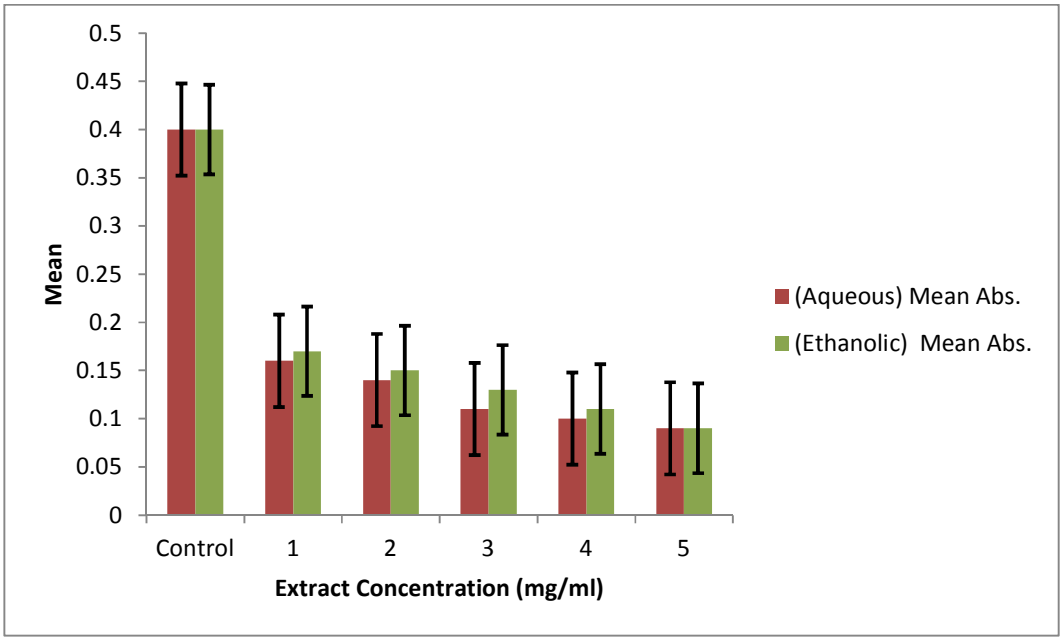

Figure 3: Iron chelation ability of *Allium sativum* extract [Fe^{2+}]

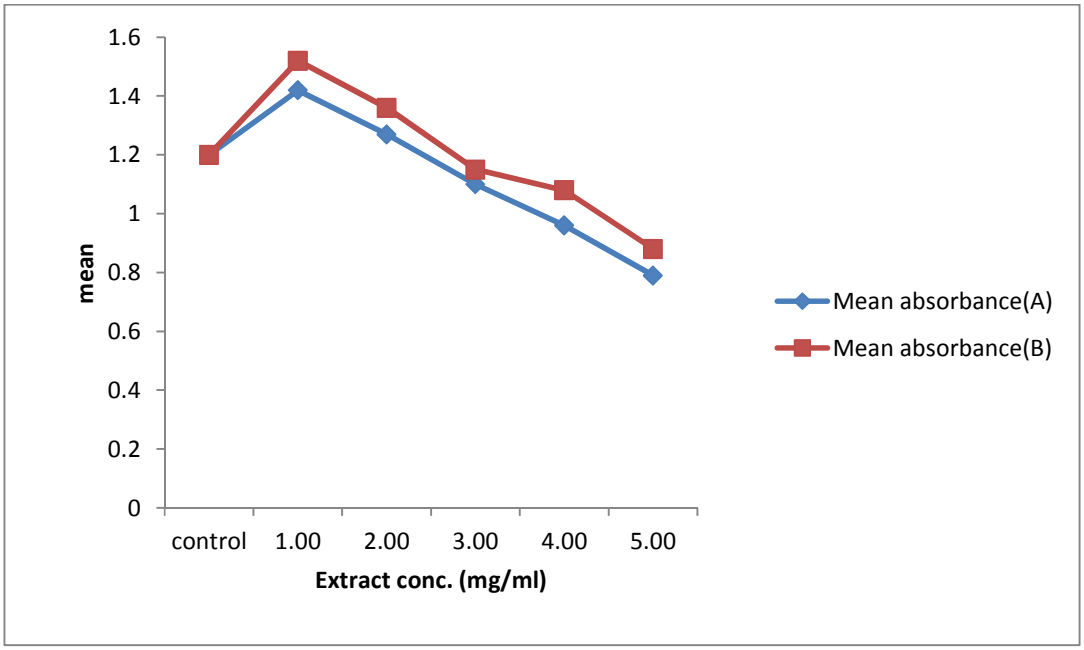

Figure 4: Graph of hydroxyl radical {OH*} scavenging ability of *Allium sativum* extract
A = aqueous extract
B = ethanolic extract

Table 1: Antioxidant activity of aqueous extract of Allium sativum (AS) on iron sulphate induced lipid peroxidation in liver homogenate in-vitro.

Treatments (Iron + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.08	-	-
Control (50µl Iron)	0.48	-	-
50µl Iron + 10mg/ml AS	0.33	30.90	1.20
50µl Iron + 20mg/ml AS	0.32	32.40	1.34
50µl Iron + 40mg/ml AS	0.21	55.90	3.41
50µl Iron + 80mg/ml AS	0.16	66.60	4.36
50µl Iron + 160mg/ml AS	0.14	70.40	4.69
*Results are expressed as mean of three experiments in duplicate			

Table 2: Antioxidant activity of ethanolic extract of Allium sativum (AS) on iron sulphate induced lipid peroxidation in liver homogenate in-vitro.

Treatments (Iron + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.08	-	-
Control (50µl Iron)	0.48	-	-
50µl Iron + 10mg/ml AS	0.34	29.60	1.09
50µl Iron + 20mg/ml AS	0.33	31.50	1.26
50µl Iron + 40mg/ml AS	0.21	55.00	3.33
50µl Iron + 80mg/ml AS	0.17	65.31	4.24
50µl Iron + 160mg/ml AS	0.14	70.60	4.71
*Results are expressed as mean of three experiments in duplicate			

Table 3: Antioxidant activity of aqueous extract of Allium sativum (AS) on iron sulphate induced lipid peroxidation in brain homogenate in-vitro.

Treatments (Iron + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.09	-	-
Control (50µl Iron)	0.48	-	-
50µl Iron + 10mg/ml AS	0.24	49.30	-0.55
50µl Iron + 20mg/ml AS	0.13	72.60	3.49
50µl Iron + 40mg/ml AS	0.12	74.70	3.86
50µl Iron + 80mg/ml AS	0.12	75.60	4.01
50µl Iron + 160mg/ml AS	0.11	76.60	4.19
*Results are expressed as mean of three experiments in duplicate			

Table 4: Antioxidant activity of ethanolic extract of Allium sativum (AS) on iron sulphate induced lipid peroxidation in brain homogenate in-vitro

Treatments (Iron + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.11	-	-
Control (50µl Iron)	0.49	-	-
50µl Iron + 10mg/ml AS	0.18	64.30	4.15
50µl Iron + 20mg/ml AS	0.15	69.41	4.60
50µl Iron + 40mg/ml AS	0.14	72.42	4.87
50µl Iron + 80mg/ml AS	0.13	73.50	4.97
50µl Iron + 160mg/ml AS	0.13	74.50	5.05

*Results are expressed as mean of three experiments in duplicate

Table 5: Antioxidant activity of aqueous extract of Allium sativum (AS) on lead acetate induced lipid peroxidation in liver homogenate in-vitro

Treatments (Lead + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.12	-	-
Control (50µl Lead)	0.47	-	-
50µl Lead + 10mg/ml AS	0.24	49.20	1.18
50µl Lead + 20mg/ml AS	0.21	54.80	2.05
50µl Lead + 40mg/ml AS	0.21	55.70	2.19
50µl Lead + 80mg/ml AS	0.13	72.00	4.75
50µl Lead + 160mg/ml AS	0.13	72.50	4.83

*Results are expressed as mean of three experiments in duplicate

Table 6: Antioxidant activity of ethanolic extract of Allium sativum (AS) on lead acetate induced lipid peroxidation in liver homogenate in-vitro

Treatments (Lead + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.08	-	-
Control (50µl Lead)	0.50	-	-
50µl Lead + 10mg/ml AS	0.33	34.31	1.50
50µl Lead + 20mg/ml AS	0.32	36.70	1.72
50µl Lead + 40mg/ml AS	0.21	57.40	3.54
50µl Lead + 80mg/ml AS	0.21	58.80	3.67
50µl Lead + 160mg/ml AS	0.13	73.90	5.00

*Results are expressed as mean of three experiments in duplicate

Table 7: Antioxidant activity of aqueous extract of Allium sativum (AS) on lead acetate induced lipid peroxidation in brain homogenate in-vitro

Treatments (Lead + AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.09	-	-
Control (50µl Lead)	0.48	-	-
50µ Lead + 10mg/ml AS	0.24	50.70	-0.03
50µl Lead + 20mg/ml AS	0.16	67.40	3.06
50µl Lead + 40mg/ml AS	0.15	69.30	3.41
50µl Lead + 80mg/ml AS	0.13	73.10	4.11
50µl Lead + 160mg/ml AS	0.12	74.90	4.45

*Results are expressed as mean of three experiments in duplicate

Table 8: Antioxidant activity of ethanolic extract of Allium sativum (AS) on lead acetate induced lipid peroxidation in brain homogenate in-vitro

Treatments (Lead +AS)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Basal	0.09	-	-
Control (50µl Lead)	0.49	-	-
50µl Lead + 10mg/ml AS	0.30	40.22	2.02
50µl Lead + 20mg/ml AS	0.29	41.23	2.11
50µl Lead + 40mg/ml AS	0.19	61.50	3.91
50µ Lead + 80mg/ml AS	0.15	69.80	4.64
50µl Lead + 160mg/ml AS	0.14	72.43	4.87

*Results are expressed as mean of three experiments in duplicate

DISCUSSION

Generally plants containing phenolic compounds have very high antioxidative potentials [17] and their antioxidant activity has been reported to be concomitant with the development of reducing power [18]. The reducing power of the extracts might be due to its hydrogen donating ability [19]. From the experiment above as depicted by Figure 1, the reducing power of the extracts shows that aqueous extract has higher reducing potential than ethanolic extract where highest percentage obtained was 76.11% while that obtained for ethanolic extract was 69.03% at same extract concentration. Also, DPPH scavenging ability of the extracts was higher in aqueous extract than ethanolic extract as 41.08% was obtained for aqueous extract while 39.46% was obtained for ethanolic extract at same concentration as depicted by Figure 2. However, results from hydroxyl radical scavenging and iron chelation abilities of the extracts show a different pattern from this where the ethanolic extract has higher percentage hydroxyl radical scavenging potential than aqueous extract. 29.17% was obtained for ethanolic extract while 26.67% was obtained for aqueous extract at same extract concentration. Besides, there was no marked difference in the values obtained for both extracts while determining their iron chelation potentials as both extracts had highest iron chelation of 77.50% at same extract concentration. It is however worthy to note that only iron chelation ability of the extracts required higher extract concentration for effective chelation and this was attained at highest extract concentration (5.0ml) while others had their highest percentages attained at the least extract concentration (1.0ml). Results from the lipid peroxidation experiment show a significant increase in the levels of thiobarbituric acid reactive substances (TBARS) on iron and lead administrations only as indicated by the control in table 1-8 above. This increase in TBARS may be attributed to oxidative deterioration of membrane polyunsaturated fatty acids which damage membrane integrity which are characteristics of pro-oxidants such as iron and lead. Also it has been reported that metal ions play important role in the acceleration of oxidation of biological molecules which are critical to life where they initiate the propagation of radical chain reaction in lipid peroxidation [20]. However, the results further show marked decrease in TBARS levels on administration of the extracts where the inhibitory potential of the extract was exerted on the pro-oxidants. From the results, ethanolic extract had highest percentage inhibition of both lead (75.90%) and iron (70.60%) in the liver tissue while aqueous extract had the highest percentage inhibition of lead (74.90%) and iron (76.60%) in the brain tissue respectively. The increase in lipid peroxidation in the presence of pro-oxidants like Fe^{2+} and Pb^{2+} could be due to their roles in electron transfer reaction that normally generates reactive oxygen species [21] as well as their ability to decompose lipid peroxides, thus generating peroxyl and alkoxyl radicals which promotes the propagation of lipid oxidation [22]. Elevated metal ions levels in the brain and liver has been linked to neurodegenerative diseases like Parkinson's disease and liver diseases like cirrhosis respectively. However, the extractable phytochemicals from the extracts exhibited a concentration dependent (10-160mg/ml) significant decrease ($P<0.05$) in lipid peroxidation induced by Fe^{2+} and Pb^{2+} in the rat's liver and brain homogenates while the higher ability of the extract either in aqueous or ethanolic form may be attributed to its higher extractable phytochemical content.

CONCLUSION

The aqueous and ethanolic extracts of *Allium sativum* were able to protect the brain and liver from Fe^{2+} and Pb^{2+} induced lipid peroxidation due to presence of high antioxidant phytochemicals and properties. This further suggests the involvement of reactive oxygen species in oxidative stress and also reveals the beneficial role of garlic therapeutic efficacy, thus supporting the hypothesis that cellular redox status may be significantly reversed by the use of plant containing -thiol or phenolic antioxidant compounds.

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