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Full Length Research Paper

## Combined aqueous extracts of garlic and ginger reduces neuronal damage by gfap and p53 protein alterations in rats exposed to lead acetate

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#### ABSTRACT

**Background:** The etiology of neurodegeneration involves both environmental and genetic predisposition with redox metal abuse occupying a central role as most of the symptoms stems from abnormal metal metabolism.

Aim: The study was aimed at evaluating the neuroprotective potentials of combined aqueous extracts of ginger and garlic in lead-induced neuronal loss.

**Methodology:** Thirty (30) *Rattus norvegicus* of average weight (149-179 g) were allowed to acclimatize for 14 days. The animals were divided into 5 groups, n=6.Group A received 2 ml/kg body weight of water and normal feed, Group B (disease control), C, D and E all received 2 mls of 5000 mg/kg body weight of lead acetate once after 24 hours of starvation to induce neurodegeneration. Group C, D and E were treated (post 72 hrs) with 2 ml of 1000 mg/kg body weight of aqueous extract of garlic, ginger, and in combination for 4weeks respectively. The brain tissue tissues was removed and fixed immediately in 20% phosphate buffered formal saline for 48 hrs and some frozen. The tissues were further processed and sectioned using rotary microtome and stained with Heamatoxylin and eosin. Glial fibrillary acidic protein (gfap) and tumor suppressor gene (p53) immunohistochemistry antibodies were used for detection and localization of the specific tissue antigens. The frozen tissues were used for lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) antioxidant activity. Semi-quantitative method and histomorphology changes were used to assess the extent of neurodegeneration in the cerebellar Purkinje neuronal population.

**Results:** Histomorphology revealed that combined extracts exhibited neuronal protection by inhibiting apoptosis and cellular changes. There was a statistically significant increase in mean value of GSH in the group treated with combined extracts compared with lead induced group. Also, there was a decrease in MDA level in the group treated with combined extracts compared with lead induced group, statistically significant at P=0.01. The aqueous extract of garlic and ginger combined showed decrease in gfap expression and p53 tumor suppressor gene expression.

**Conclusion:** The synergistic effect of aqueous garlic and ginger extracts promoted increase in GSH antioxidant activity and decreases level of MDA with corresponding inhibition of p53 and gfap activity, thereby reducing neuronal damage in rats exposed to lead. This combined extracts is a good pointer in the management of neuronal disorders.

Keywords: gfap, p53, neurodegeneration, antioxidant.

#### INRODUCTION

The etiology of neurodegeneration interplay a number of factors including environmental and genetic predisposition with redox metal abuse occupying central role as most of the symptoms stems from abnormal metal metabolism [1]. Previous study suggested that cognitive, motor, and behavioral changes are often caused by exposure of developing central nervous system to lead poisoning (Stretesky PB, 2004). Although the underlying mechanism of neurotoxicity is complex, lead appears to alter the release of neurotransmitter leading to excitotoxicity and apoptotic changes (Toscano C, 2005). Lead is able to penetrate the endothelial cells at the blood brain barrier and substitute for calcium ions and be taken up by calcium-ATPase pumps, thus interfering with the release of neurotransmitters (Toro RP, 2008; Stewart WF, 2006).

Wilson et al. (Wilson MA, 2000) previously reported the effects of lead on second messengers and how it alters normal neuronal development and causes volumetric changes in the developing hippocampus and morphological changes in the developing cortex. Leadexposed rats had shown altered dendritic branching of cerebellar Purkinje cells in postnatal exposed kittens (Patrick GW, 2000). Other studies have also shown destruction of the myelin sheaths in lead-exposed rats and could be secondary damage to oligodendrocytes. Also, acute lead exposure causes a decrease in the activity of CNPase located in the myelin sheath, an integral protein for myelin synthesis during neuronal development (Dabrowska-Bouta B, 2000). There are also converging lines of evidence suggesting that cognitive, motor, and behavioral changes results from exposure of the developing central nervous system to lead poisoning (Lidsky TI, 2003). Animal model have also shown garlic and ginger to possess many known therapeutic and health benefits, such as fighting of infections. preventing cancer and reducing inflammation and antioxidative characteristics and by scavenging free radicals (Josling P, 2001; Aggarwal BB, 2004; Pedrazza-Chaverri J, 2006 and Anderson KJ, 2008).

Furthermore, report have supported the potentials of dietary garlic and ginger as alternative for management of Alzheimer's disease (Josling P, 2001) thou not in combination. Certain biomarkers has been identified in human bio fluids post-traumatic brain injury (TBI), such as neuron specific enolase (NSE), glial calcium-binding protein S100B, glial fibrillary acidic protein (gfap), myelin basic protein (MBP), p53, ubiquitin carboxyl hydrolase-like 1 (UCH-L1) and neurofilament proteins (Brophy GM, 2011; Mondello S, 2010 and Karen C, 2017). In a study by Kamphuis et al., (Kamphuis W, 2014) aimed at characterizing the expression pattern of different GFAP iso-forms in normal human hippocampal tissue and in conditions with AD-related gliosis, the number of GFAPю1-expressing astrocytes significantly increases during the progress of AD with lesser effects of gender and age. Also, the study equally showed that treatment of AD rats with Ab1e42 oligomers and fibrils increases the number of GFAPю1positive cells, suggesting that elevated antibodies level is a causative factor of neurodegeneration (Kamphuis W, 2014). The rationale of the study was based on the medicinal important of both plants within the region

and the increasing prevalence of neurological disorders in the world and yet no standard drug has been reported to completely cure this disorder. The study was aimed at evaluating the neuroprotective potentials of combined aqueous extracts of ginger and garlic in lead-induced neuronal loss.

#### MATERIALS AND METHODS

Thirty (30) Rattus norvegicus of average weight (149-179 g) were allowed to acclimatize for 14 days and then divided into 5 groups, n=6.  $LD_{50}$  was determined in the acute study for both treatment and control. Group A received 2 ml/kg water and normal feed, Group B (disease control), C, D and E all received 2 mls of 5000 mg/kg body weight of lead acetate once after 24 hours of starvation to induce neurodegeneration. Group C, D and E were treated (post 72 hrs) with 2 ml of 1000 mg/kg body weight of aqueous extract of garlic, ginger, and combined extracts of ginger and garlic for 4 weeks respectively. This study was conducted in accordance with the institute of health guideline for the care and uses of laboratory animals (NIH, 1985). The garlic and ginger extracts were prepared according to the method of Oboma et al., (Yibala IO, 2018). After 4 weeks of treatment with extracts the rats were anaesthetized using light dose of chloroform prior to sacrifice and the brain tissue collected and fixed immediately in 20% phosphate buffered formal saline for 48 hrs. The tissue were further processed by dehydrating in ascending grades of alcohol, cleared in two changes of xylene and embedded in molten paraffin wax and sectioned using rotary microtome, mounted on glass slide and stained with Heamatoxylin and eosin technique to demonstrate the tissue architecture. Glial fibrillary acidic protein (gfap), tumor suppressor gene (p53) immunohistochemistry antibodies were used to detect and localized the specific tissue antigens. Frozen brain tissues were further homogenized and used for lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) antioxidant activity study.

#### Semi-quantitative evaluation of histological sections

Ultra-sections from each group were stained with Heamatoxylin and eosin staining techniques examined by light microscopy for tissue damage using features such as shrinkage of the neuron, hyperchromasia, and nuclear pyknosis as previously described by (Stewart WF, 2002 and Oyinbo CA, 2016). Cerebellar Purkinje cells estimation was based on semi-quantitative scale described by (Oyinbo CA, 2016) was used to assess the extent of neurodegeneration in the cerebellar cortex with 400X magnification.

# Immunohistochemistry assessment of cerebellar damage

Glial fibrillary acidic protein (gfap) and tumor suppressor gene p53 proteins were used for immunohistochemical assessment of neuronal damage. Avidin Biotin Complex (ABC) method also referred to the Avidin biotin imuunoperoxidase method as described by (Oboma YI, 2016) was adopted. Colorectal cancer cells known to be positive for p53 were used as positive control while negative control was omission of the primary antibody. Appropriate negative controls were prepared by eliminating the primary antibody step for gfap and p53. Cells with specific brown colors in the cytoplasm, cell membrane or nuclei depending on the antigenic sites were considered to be positive for both gfap and p53. The stained cells without any form of brown colours were scored as negative.

#### **Biochemical assessment of neuronal damage**

The extent of brain damage associated with or without the treatment with extracts were assessed using the mean concentration of reduced glutathione (GSH) and malondialdehyde (MDA). The methods of (Lucky LN, 2018) was adopted for reduced glutathione assay while the method of Oyinbo et al. was used for malondialdehyde (MDA) determination. The cerebellar cortex (100 mg) were iced and homogenized in 100 mL of 5 mM Tris/HCl buffer (pH 7.4) in 1 mM EDTA, and completed in Mini, EDTA-free Protease inhibitor Cocktail. Homogenates were then centrifuged at 10,000 rpm for 10 min at 40°C and the clear supernatant was collected for the estimation of reduced glutathione (GSH). Also 0.2 mL of the homogenate was further mixed with 25% trichloroacetic acid and centrifuged at 3000 rpm for 10 min, then the supernatant (0.2 mL) was mixed with 10 mM DTNB in the presence of phosphate buffer (0.1 M,

pH 7.4), and the absorbance was read at 420 nm. The assay for lipid peroxidation (LP) was in accordance with the method of (Oyinbo CA, 2016), where 0.4 ml aliquot of the homogenate was mixed with 1.6 ml 0.15 M Tris-KCl buffer pH 7.4, 0.5 ml of TCA (10%), and 0.5 ml TBA (0.75%) was then added. All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were transferred to ice bath and then centrifuged at 3000 rpm for 10 min. The amount of MDA formed in each of the brain samples were assessed by measuring the optical density absorbance of the supernatant at 532 nm.

#### **Statistical analysis**

Results were presented as mean  $\pm$  standard deviation (SEM). Inferential statistical analysis was done using one-way ANOVA followed by Dun-net's multiple comparison. Graph Pad Prism 6 Soft-ware, San Diego California USA). Differences between compared data were considered significant at p<0.05. Ethical permit was obtained from the department of medical laboratory sciences. MLS/BMS/18/018.

### RESULTS

Table 1 presents the final weight gained and initial weight of rats studied after 4 weeks of chronic toxicological evaluation. All the groups were administered with lead except control, 72 hrs post lead administration. The extracts were administered to study their effects on some biochemical parameters and tissue morphology. All the groups showed increased in final body weight and were statistically significant at  $p \leq 0.05$ . Table 2 presented oxidative stress markers in lead induced neurodegeneration in rat model. The mean MDA level in the control was 4.03  $\pm$  0.96 and there was an increase in the level of MDA in the GP B administered with lead acetate only compared with the control (Figure 1-3).

 Table 1. Mean body weight of rats (g) following Lead acetate and extracts administration.

Groups	Final	Initial	t-test	p<0.05	Mean diff		
Gp A	210.4 ± 18.0	168.4 ± 7.0	2	0.05	42.5		
Gp B	203.4 ± 17.6	161.0 ± 6.7	1.8	0.01	42.4		
Gp C	210. 0 ± 11.4	174.0 ± 5.5	2.8	0.02	36		
GP D	194.6 ± 6.1	149.0 ± 7.9	4.4	0.02	45.6		
Gp E	190.4 ± 17.6	169.7 ± 4.8	2.4	0.03	20.7		
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Values expressed as mean ± SD, p.value 0.05, GP A=Normal control received water only, GP B=Lead administered group only, GP C=Lead acetate and garlic administered group, GP D=Lead acetate and ginger administered group, GP E=Lead acetate and garlic+ginger combine administered group.

The group that received lead acetate with aqueous extract of ginger and garlic combined showed a statistically significant decrease in MDA level at P<0.05. Also there was a drop in reduced glutathione

antioxidant activity in the group administered with lead acetate (Group B) which significantly increased level

post treatment with garlic and ginger combined compared with lead acetate treated group (Group B).

**Table 2.** Oxidative stress pattern in oral lead inducedneurodegeneration.

Parameters	GP A	GPB	GP E	p value				
MDA	4.03 ± 0.96	9.23 ± 1.29	5.03 ± 1.3	0.01				
GSH	10.10 ±1.74	5.28 ± 1.39	8.05 ± 0.12	0.01				
MDA=Malondialdehyde, GSH=Reduced Glutathione, GPA=control received water only GP B=lead administered group, GP E=Lead acetate and garlic+ginger combine administered group.								

The histological of the cerebellar cortex shows a molecular layer (ML) with poor cellularity, Purkinje neurons layer (PL) with prominent nuclei and the third layer, the granular layer (GL) containing numerous cells of various sizes and shapes. There was little or no neuronal damage in the control group as shown in plate 1. Plate 2 represents lead induced neuronal degeneration group without treatment. Photomicrograph shows reduction in purkinje neuron population and granular cell layer with increased eosinophilia cytoplasm revealing neurodegenerative activity.



**Figure 1.** Photomicrographic plates of Heamatoxylin and Eosin stained sections of the brain tissues.

Also, Plate 3 was treated with combined extracts of ginger and ginger showing increased neuronal population compared with the lead group with some of the neurons showing vacuolation, pyknosis and decreased cellularity of the granular layer compared with the lead group. Histological results revealed that combined aqueous garlic and ginger extract offers purkinje neurons protection. Plate 4 and 5 represented group treated with garlic and ginger and independently, the extracts shows decreased neuronal population compared with the control. The extract showed no purkinje neurons protection compared with protection observed in group treated with garlic and ginger combined.

Representative photomicrographs of the effect of combined aqueous extracts of garlic and ginger on lead exposed rat. GFAP positive neurons show cell body blue and astrocytes brownish. It was over expressed in the disease control group labeled 1, but moderately expressed in the garlic and ginger treated groups (plate 3 and plate 4). Results revealed that combined aqueous extract of garlic and ginger aqueous decreases gfap protein activity in the lead induced brain tissue sections as labeled in plate 2.



**Figure 2.** Photomicrograph plates of GFAP stained sections of the cerebral tissues.

The p53 labeled (positive) neurons shows brownish dots. P53 gene was over expressed in the positive control lead group (labeled 1), but moderately expressed in the garlic and ginger groups group (plate 2 and 3). The protein was suppressed in the group treated with combined extracts of ginger and garlic as shown in plate 4 indicating a protective activity.



**Figure 3.** Photomicrograph plates of p53 stained sections of the cerebral tissue.

#### DISCUSSION

Neurodegeneration is a slow progressive dysfunction, loss of neurons and axons in the central nervous system. It is the principal pathological feature of acute and chronic neurodegenerative conditions such as Alzheimer's, and Parkinson's disease, Stroke, traumatic brain injury, multiple sclerosis and paraneoplastic disorders (Sandra A, 2009). It was observed that lead acetate poisoning reduced Purkinje neurons population in rats examined and is in agreement with previous works (Wilson MA, 2000 and Sandra A, 2009). Previous research have shown that the Purkinje neurons are the most susceptible part of the cerebellar cortex to toxic substances (Fonnum F, 2000 and Luo J, 2015) and that adult brain are more venerable to the insult and this is in agreement with this current study on the effect of lead on the purkinje neuronal loss.

The present study examined the neuroprotective potential and antioxidant activities of combined aqueous extracts of ginger and garlic in rats exposed to lead acetate poisoning. Combined extracts of ginger and garlic showed a significant neuroprotective effect as demonstrated by heamatoxylin and eosin technique as shown in the Figure 1. The study showed reduced neuronal cells destruction in rats treated with combined aqueous extracts of ginger and garlic evidenced by reduced apoptotic cells and increased neuronal cell population within the purkinje layer of the cerebral tissue. Previous studies has reported significant neuroprotective potentials on the cerebellar cortex by some medicinal plants (Stewart WF, 2002; Oyinbo CA, 2016; Noorafshan A, 2013 and Salavati P, 2013). Although previous studies have highlighted the neuroprotective potentials of ginger and garlic aqueous extracts independently in mammalian brain, the present study is perhaps a novel study on the combined effect of both extracts.

The link between the neuroprotective effect of combined aqueous extracts of ginger and garlic and its antioxidative properties was established in this study. Antioxidant activity in the rat's brain tissues was estimated using reduced glutathione level and malondialdehyde mean concentrations. The mean value of Malondialdehyde (MDA) was elevated in rats with lead toxicity. There was significant reduction in the levels of antioxidant activities in rats treated with combined aqueous extracts compared with lead induced group only. Reduced glutathione level was decrease in the group administered with lead acetate in agreement with previous report that a drop in reduced glutathione levels kills brain cells and a corresponding increase in group treated with group treated with combined extract. The protective effect of the combined aqueous extracts in the cerebellar neuronal cell degeneration is possibly akin to its antioxidative properties. The etiology of most neurodegenerative conditions or diseases is linked with free radical (Floyd RA, 1992; Lewen A, 2000). It is observed that in neuropathology, free radicals are over produced and these overwhelm the endogenous antioxidant defenses, leading to oxidative stress. This subsequently induces cellular or subcellular membrane damage resulting in organelle or organ dysfunction in combating the menace of free radical assault on the CNS.

Biomarkers are important for accurate diagnosis of complex disorders of neurodegeneration. It is likely that a single biomarker will not reflect the full spectrum of the response of brain tissue to injury. Glial fibrillary acidic protein (gfap) and p53 proteins are among the most widely studied biomarkers in neurodegenerative disorders. Combined aqueous extracts of garlic and ginger was able to ameliorate cerebellar cortex neuronal degeneration by inhibiting p53 and gfap expressions in the cerebral tissues. The p53 protein was highly suppressed in rats treated with the combined aqueous extract of garlic and ginger compared with the groups treated with garlic and ginger extracts independently. This finding is in line with Carstein et al. (Carsten C, 2001) who reported a drug protection and correlated it with decreased p53 DNA-binding activity, decreased expression of the p53 target gene Bax and suppression of mitochondrial dysfunction and caspase activation. Accumulating evidence indicates that p53 is perturbed in the central nervous system in a number of neurodegenerative disorders (Miller FD, 2000 and Lanni C, 2008). Furthermore, previous postmortem studies suggested an involvement of p53 in degenerating neurons in traumatic brain injuries. These reports included de la Monte et al. (De la Monte SM, 1997) reported increased p53 and Fas expression in specific populations of cortical neurons; Kitamura et al. (Kitamura Y, 1997) increased amounts of p53 in the temporal cortex, mainly localized in glial cells; and Seidl et al. (Seidl R, 1999) higher levels of p53 in the frontal and temporal lobes in Down syndrome patients.

The study also showed that combined aqueous extracts of ginger and garlic reduces gfap expression collaborating with other reports that neuronal degeneration initiates glial cell proliferation by triggering various growth factors that result in astrocytes proliferation (Kamphuis W, 2014; Du C, 2002). These results suggest that gfap persist within degenerating astrocytes in the lead brain injury and of purkinje neurons to excitotoxic damage. Previous lines of evidence have documented that the density of gfappositive astrocytes appears to be inversely related to the magnitude of dopaminergic neuronal loss across the different main dopaminergic areas of the brain in Parkinson disease post-mortem samples (Du C, 2002). In study by Kamphuis (Kamphuis W, 2014) aimed to characterize the expression pattern of different GFAP isoforms in normal human hippocampal tissue and in conditions with AD-related gliosis, the number of GFAPю1-expressing astrocytes significantly increases during the progress of AD with lesser effects of gender and age. Also invitro studies showed that treatment of AD rats with Ab1e42 oligomers and fibrils increases the number of GFAPю1- positive cells, suggesting that elevated antibodies level is a causative factor (Kamphuis, 2014). This study was limited to

histopathology studies using haematoxylin and eosin and immunohistochemistry markers, behavioural studies was not included due lack of equipment.

#### CONCLUSION

The results of this study suggested that combined aqueous ginger and garlic extracts ameliorated leadinduced cerebellar neurodegeneration by increasing glutathione (GSH) and reduction of malondialdehyde (MDA) levels and suppressing gfap and p53 tissue proteins in the injured tissues. All histological and biochemical evaluations revealed improved protective effect in rats treated with combined aqueous extracts of ginger and garlic.

Therefore this study provided a template in the production of drugs for the management of lead-induced cerebellar neurodegeneration.

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