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Histological and Anti-oxidative Effects of Eugenol on Aluminium Chloride-Induced Neurotoxicity in Wistar Rats

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ABSTRACT

Accumulation of aluminium chloride results in damage to different brain regions, and has been used to model damage to the hippocampus which can be associated with various neurodegenerative diseases such as Alzheimer's and Parkinson's. The aim of this study was to assess the protective effects of eugenol on aluminium induced neurotoxicity in the hippocampus of adult Wistar rats. 30 adult Wistar rats were procured and divided into six groups with five animals in each group, namely: EGH (300 mg/kg eugenol), EGL (150 mg/kg eugenol), EGH+AL (300 mg/kg eugenol and 100 mg/kg $AlCl_3$), EGL+AL (150 mg/kg eugenol and 100 mg/kg $AlCl_3$), AL (100 mg/kg $AlCl_3$) and CTRL (2 mL/kg distilled water). All Groups were treated orally for 21 days after which they were humanely sacrificed under 0.8 mL/kg ketamine as an anaesthetizing agent. Thereafter, brain tissues were removed and processed for histological demonstration, while the frontal lobe was homogenized and the resultant homogenate obtained was used to assay the levels of superoxide dismutase (SOD) activity. Rat body weights were measured before and after treatment. Aluminium resulted in a significant ($p<0.05$) reduction in SOD activities. There was alteration in the histology of hippocampal neurons (CA1) and a significant ($p<0.01$) reduction in body weight of animals. However, the administration of Eugenol was able to restore the activity of SOD. The use of Eugenol offers a promising prospect in the management of neurodegenerative diseases associated with aluminium toxicity.

Key words: *Aluminium chloride, Eugenol, Neurotoxicity, Cytoarchitecture, Superoxide dismutase, Hippocampus*

INTRODUCTION

Aluminium is extremely reactive with carbon and oxygen, two of the leading elements of life on earth. For this reason, the widespread use of bioavailable aluminium may have immense and far reaching implications for the health of humans and animals. In fact, much evidence shows that aluminium seems to be toxic to all forms of life on earth, and where it appears in terrestrial biochemistry, it is invariably deleterious (Inan-Eroglu and Ayaz 2018).

Aluminium currently finds its way into virtually every aspect of our daily lives, where it is used in cans, cookware, aluminium foil, housing materials, and components of electrical devices, airplanes, boats, cars and numerous hardware items of all descriptions (Carson 2000; Hirata-Koizumi et al. 2011).

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In terms of bioavailability, aluminium is now found in drinking water and due to its action as a flocculant, as a common additive in various processed foods, added to cosmetics of many types, and increasingly, shows up in pharmaceutical products (Hirata-Koizumi et al. 2011). Exposure of humans and animals to aluminium from various sources can have deleterious consequences on the developing and adult nervous systems (Tomljenovic 2011; Mold et al. 2018). Al metal cannot pass through the blood-brain barrier; a natural filter of the brain, but its compounds such as the fluoride, sulphides and chlorides can pass through (Bingham and Cohrssen 2012).

Eugenol is widely applied in dentistry, anaesthetics, analgesics, and as anti-inflammatory and flavouring agents (Chaieb et al. 2007). Eugenol also has antioxidant properties with the ability to mop up reactive oxygen species associated with metal toxication (Masae et al. 2005). This study aimed at investigating protective effects of eugenol against cytoarchitectural and neurochemical changes associated with aluminium induced hippocampal neurotoxicity in adult Wistar rats.

MATERIALS AND METHODS

Experimental Animals

All protocols and treatment procedures were carried out according to the Ahmadu Bello University Research Committee (ABURC) guidelines, and approved by the Department of Anatomy Post Graduate Research and Ethics Committee.

A total of 30 adult Wistar rats of average weight 140-160 g were randomly grouped into six groups with five animals in each group. These rats were obtained from K-Vom National Research Institute, Jos, Nigeria, and allowed to acclimatize for two weeks before the commencement of this study, and were fed standard pelletized feed (Grand Cereals and Oil Mills Limited, Jos, Nigeria) and clean water *ad libitum*.

Reagent Preparation and Treatment Regimen

Aluminium chloride ($AlCl_3$, #7446-70-0; Guandong Guanghua Sci-Tech Co. Ltd., Shantou, Guandong, China) was prepared at a dose of 100 mg/kg bwt (Anil et al. 2009). Eugenol (#58-23-4; Yueyang Jiazhiyuan Biological Co Ltd, Hunan China) of 99.9% purity level and prepared at a dose of 300 mg/kg and 150 mg/kg bwt respectively (10 and 5 % LD_{50} of 3000 mg/kg). Rats were divided into six groups and were designated as groups 1-6. Group 1 was administered 300 mg/kg of Eugenol (EGH); Group 2 was administered 150 mg/kg of Eugenol (EGL); Group 3 was administered 300 mg/kg Eugenol and 100 mg/kg $AlCl_3$ (EGH + AL); Group 4 was administered 150 mg/kg Eugenol and 100 mg/kg $AlCl_3$ (EGL + AL); Group 5 was administered 100 mg/kg $AlCl_3$ (AL) and group 6 (CTRL) was administered 2 mL/kg of distilled water as placebo as shown in Table 1. Route of administration was via oral route except otherwise stated.

Table 1: Rat Grouping and Dosage

Groups	Dose
EGH	300 mg/kg Eugenol
EGL	150 mg/kg Eugenol
EGH+AL	300 mg/kg Eugenol; 100 mg/kg $AlCl_3$
EGL+AL	150 mg/kg Eugenol; 100 mg/kg $AlCl_3$
AL	100 mg/kg $AlCl_3$
CTRL	2 mL/kg

n=5, Eugenol high dose (EGH), Eugenol low dose (EGL), Aluminium chloride (AL), Control (CTRL)

Animal Sacrifice and Tissue Processing

Twenty-four hours following the termination of the treatment, the animals were weighed and then sacrificed using ketamine (40 mg/kg) as the anaesthetic agent, intraperitoneally. They were then decapitated and the skull carefully opened with a surgical blade and a pair of scissors. Part of the brain tissues were taken for biochemical analysis, and the remaining brain tissues were fixed in 10% formal saline. Histological staining was carried out in paraffin-embedded sections at a thickness of 5 µm (following the method of Canene-Adams 2013). Thin sections from paraffin-embedded tissues were processed histologically (haematoxylin and eosin stains), according to the methods of Fischer et al. (2008) as summarized:

Removal of wax with xylene was carried out in two turns with three minutes each, followed by hydration using descending grades of alcohol; absolute alcohol (1 minute), 95% alcohol (1 minute), 70% alcohol, followed by 50% alcohol (2 minutes and 1 minute respectively). Staining was done with haematoxylin (20 minutes), washed in distilled water, 35% alcohol (1 minute), and differentiated in acid alcohol (30 seconds). This was followed by distilled water washing for a minute and staining in 1% eosin for 2 minutes. Dehydration in ascending grades of alcohol 90% (15 seconds) was followed by absolute alcohol (1 minute). Clearing was done in xylene-alcohol (2 minutes), pure xylene I (3 minutes) and pure xylene III (3 minutes). Cleared tissue was mounted in dibutylphthalate polystyrene xylene (DPX).

Superoxide Dismutase Assay

The brain tissues for superoxide dismutase assay were cleared of any adhering structures, weighed and homogenized. Tissues were pulverized in 0.25 M sucrose in an automated homogenizer at 4 ºC. Lysates from the brain were centrifuged for 10 minutes at 12,000 rpm to obtain the supernatant. Superoxide dismutase activity was assayed using the superoxide dismutase lysate immunosorbent assay kit.

Light Microscopy and Data Analysis

The hippocampal sections were captured using an Olympus binocular light micro-scope connected to a 5MP Amscope camera. The change in body weight was calculated as the difference between initial and final body weights and analysed with statistical package for social sciences and Microsoft office excel 2007 for charts. Results were expressed as mean ± standard error of mean (SEM) and significant differences among means of the groups was deter-mined using one-way analysis of variance with the least significa-nt difference post hoc test for significance. A paired sample t-test was employed for comparison of means as appropriate. Values were considered signif-icant when $p \leq 0.05$.

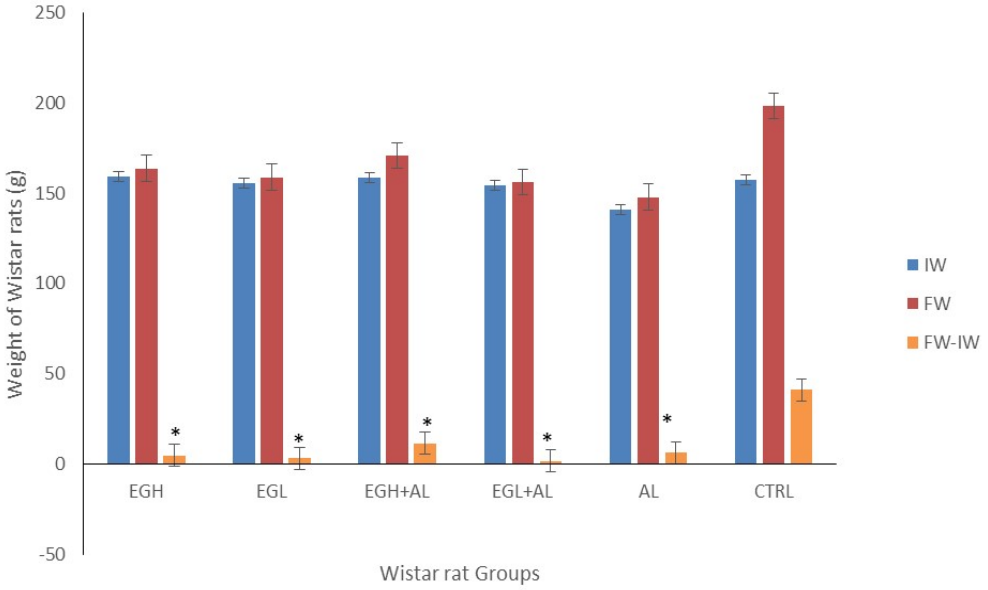
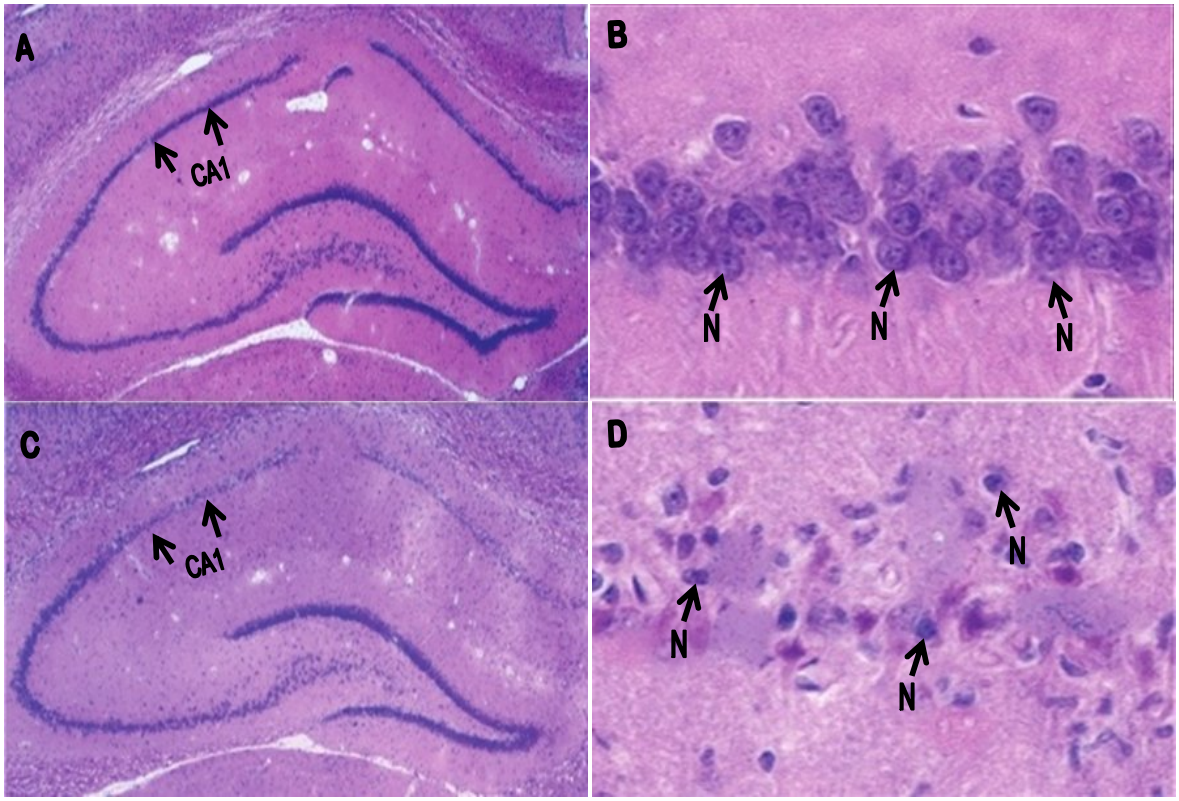


Figure 1: Effects of Eugenol on Body Weight before and after Administration of Aluminium Chloride. n = 5; mean ± SEM, One way ANOVA, LSD post hoc test: *p<0.01 when weight change difference (FW-IW) across all treated groups are compared to the control. CTRL (control), AL (aluminium chloride) EG (Eugenol), Initial weight (IW), Final weight (FW), Weight change difference (FW-IW)



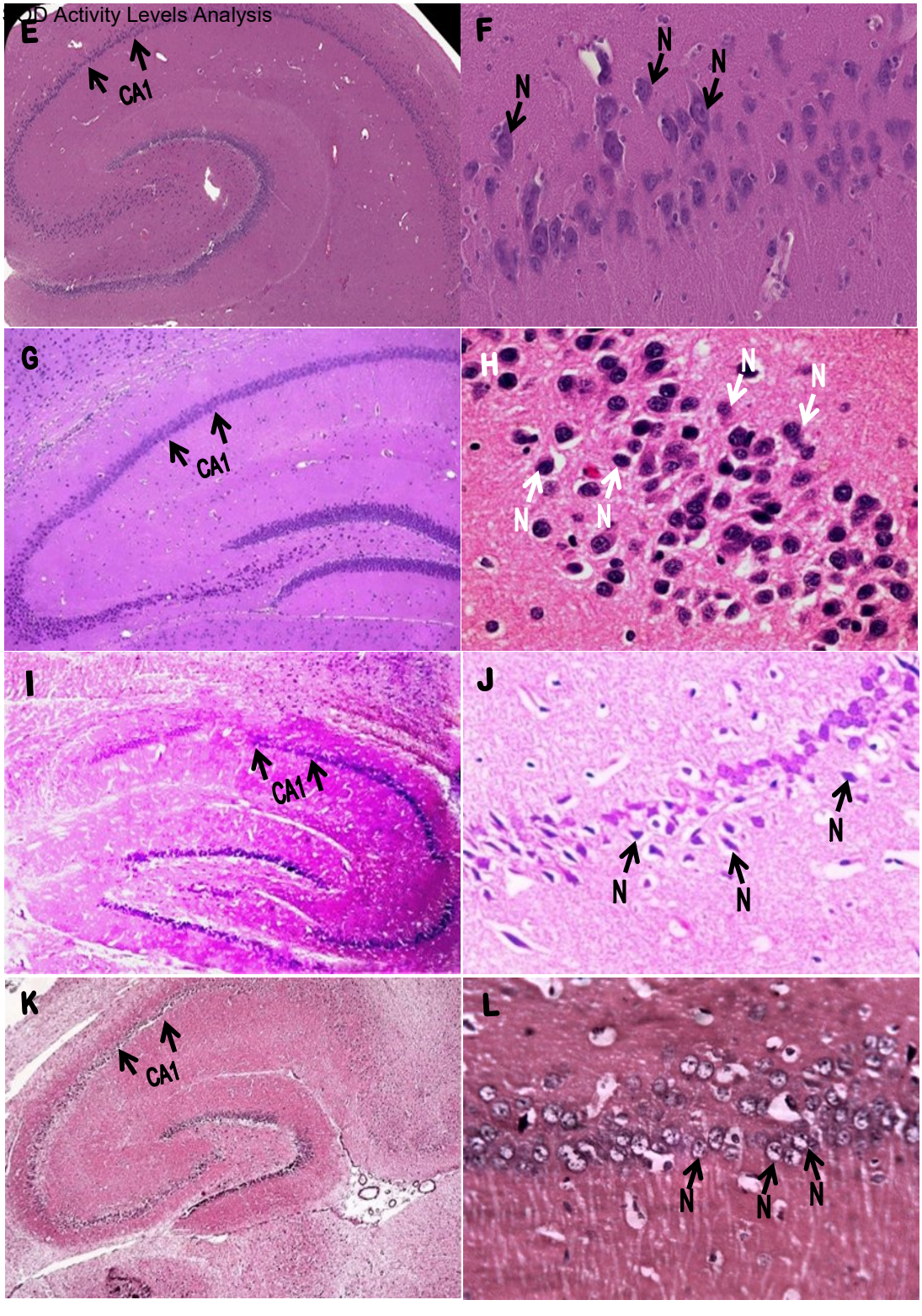


Figure 2: Histological section of the hippocampus (CA1) of rats:
A) 2.0 mL/kg of distilled water revealing a normal cytoarchitecture of the hippocampus
B) 2.0 mL/kg of distilled water revealing a normal cytoarchitecture of the hippocampus (CA1).
C) 100 mg/kg of AlC_{l3} revealing a distorted cytoarchitecture.
D) 100 mg/kg of $AlCl_3$ revealing a distorted cytoarchitecture of the hippocampus (CA1) with necrosis, chromatolysis and perineuronal vacuolations.
E) 300 mg/kg of eugenol revealing an almost normal cytoarchitecture of the hippocampus.
F) 300 mg/kg of eugenol revealing an almost normal cytoarchitecture of the hippocampus (CA1).
G) 150 mg/kg of eugenol revealing an almost normal cytoarchitecture of the hippocampus.
H) 150 mg/kg eugenol revealing an almost normal cytoarchitecture of the hippocampus (CA1).
I) 300 mg/kg eugenol and 100 mg/kg AlCl3 revealing a mild distortion in the cytoarchitecture of the hippocampus.
J) 300 mg/kg eugenol and 100 mg/kg AlCl3 revealing a mild distortion in the cytoarchitecture of the hippocampus (CA1).
K) 150 mg/kg eugenol and 100 mg/kg AlCl3 revealing a mild distortion in the cytoarchitecture of the hippocampus.
L) 150 mg/kg eugenol and 100 mg/kg AlCl3 revealing a mild distortion in the cytoarchitecture of the hippocampus (CA1).

SOD Activity Levels Analysis

Data obtained from the SOD activity levels across all groups were analysed using the statistical package for social sciences and Microsoft office excel 2007 for charts. Results were expres-sed as mean ± Standard error of mean (SEM) and the presence of significant differences among means of the groups was determined using one-way analysis of variance with the least significant difference (LSD) post hoc test for significance. significant difference (LSD) post hoc test for significance. Values were considered significant with $p \leq 0.05$.

RESULTS

Morphological Observations

The body weights obtained at the onset of this study were designated as initial weight, and 24 hours after the termination of experiment, before their sacrifice was designated as final weight. There was significant ($p < 0.01$) weight change in the group administered $AlCl_3$ compared to the control group. Other groups' weight changes were significantly ($p < 0.01$) different compared to the control as shown in Figure 1.

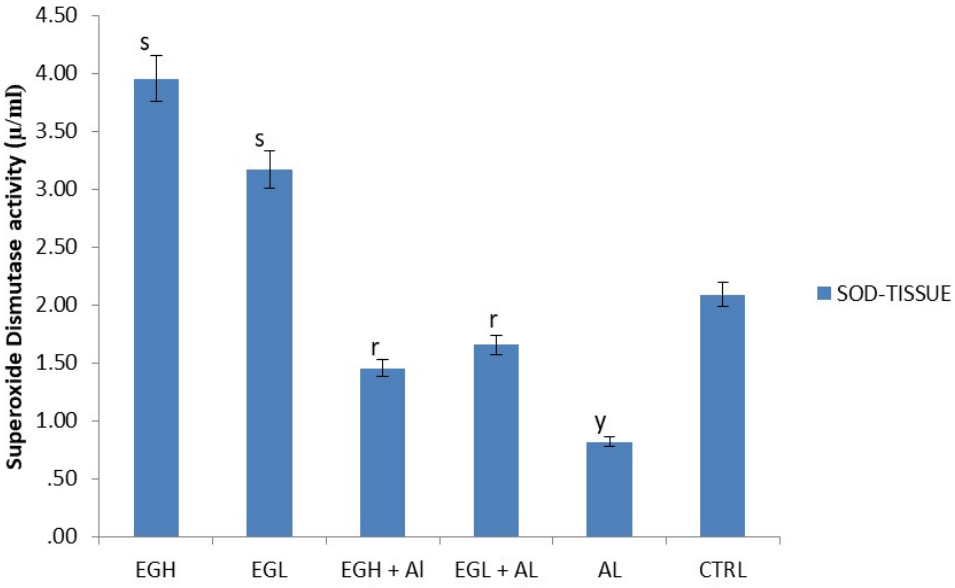


Figure 3: Effect of Eugenol on tissue (brain) SOD concentration following administration of aluminium chloride in Wistar rats. n = 5; mean ± SEM One way ANOVA LSD post hoc test: r = p<0.01; s = p<0.001; when EGH, EGL, EGH+AL and EGL+AL groups respectively are compared to the AL treated group and y = p<0.01; when AL treated group is compared to the control group (CTRL). SOD = Superoxide dismutase; EGH = Eugenol (300 mg/kg); EGL (150 mg/kg), AL = Aluminium chloride (100 mg/kg) CTRL = Control (distilled water 2.0 mL/kg.

Histological Findings

Sections of the hippocampus (CA1) were studied qualitatively using haematoxylin and eosin stains to characterize histopathological changes associated with aluminium intoxication. High power magnification of the CA1 region of the hippocampus of the rats treated with Eugenol and the control group revealed similar histomorphological findings. The pyramidal neurons exhibited normal morphology within their neuropils surrounded by supporting cells as observed in Figure 2.

Animals treated with $AlCl_3$ revealed degenerative changes within the CA1 region of the hippocampus such as perineuronal vacuolations and chromatolysis. However, Eugenol intervention at 300 mg/kg and 150 mg/kg was able to protect the hippocampus CA1 region from the deleterious effect of $AlCl_3$ as observed in Figure 2.

Eugenol Suppresses Aluminium Induced Oxidative Stress (SOD)

In the present study, we assayed the activity of superoxide dismutase to quantify the generation of reactive oxygen species (ROS) within the brain of Wistar rats. In the $AlCl_3$ group, $AlCl_3$ induced a significant ($p<0.01$) reduction in the activity of SOD relative to the control group. However, the administration of Eugenol resulted in a significant ($p<0.01$) increase in tissue activity of SOD when EGL + AL and EGH + AL groups compared to the AL treated groups. EGH and EGL group also showed a significant ($p<0.05$) increase in tissue activity of SOD compared to the control as shown in Figure 3.

DISCUSSION

In the present study, we evaluated the role of Eugenol on aluminium induced- weight change, oxidative stress, cytoarchitectural distortion and chromatolysis in the CA1 region of the hippocampus. $AlCl_3$ caused a significant weight change difference. This suggests that the administration of $AlCl_3$ may have raised the basal metabolic rate of the animals thereby causing such animals to use or burn more adipose tissues compared to animals in the other experimental groups. Observed histoarchitectural distortion of the hippocampal region (CA1), such as an irregular arrangement of CA1 hippocampal neurons, pyknosis and basophilic necrosis is indicative of treatment associated with administration of aluminium chloride

induced neurodegenerative changes. Findings in accordance with previous reports on aluminium induced neurotoxicity (Buraimoh et al. 2011). Neurodegenera-tive changes have been reported as a direct toxic effect of aluminium chloride as anoxic poison (Rui and Yongjian 2010; Buraimoh et al. 2011; Akinrinade et al. 2013).

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical radical scavenging and antioxidant system by utilizing enzyme such as SOD (Semiz and Sen 2007; Uma et al. 2012). SOD belongs to the members of enzymatic antioxidative defence mechanisms against reactive oxygen species (ROS) and protects macromolecules, cells and cell membranes from peroxidative damage. SOD catalyses the dismutation of superoxide anion radicals into oxygen and hydrogen (Kankofer 2002). The activity of SOD is a sensitive index in oxidative damage as it scavenges the superoxide anion to form hydrogen peroxide leading to diminishing toxic effects (Khan et al. 2012). Administration of Eugenol in this study maintained and elevated the levels of SOD activity. This is consistent with the reports of (Debapriya et al. 2015). Eugenol is believed to have an aromatic ring. This phenolic group stabilized a radical formed on a-carbon with conjugation in the eugenol molecule. It has been reported that the main mechanism of action of phenolic antioxidants was the scavenging of free radicals, although other mechanisms may be involved (Nenandis et al. 2003).

Conclusion

Owing to $AlCl_3$-induced oxidative stress, weight loss, microarchitectural distortion and chromatolysis of the hippocampus (CA1), Eugenol inhibited free radical generation enhancing the activity of SOD in the biological system. Eugenol therefore has potential in the management of neurodegenerative diseases associated with aluminium toxicity.

Conflict of Interest

None declared.

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