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Research Article

Protective Effects of *Camellia sinensis* on Alcohol-Induced Oxidative Stress in Postnatal Developing Cerebellum of Rats

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Abstract
This study investigated the protective effect of *Camellia sinensis* (green tea) on alcohol-induced oxidative stress in developing rat cerebellum. Twenty-five female Wistar rats weighing between 160 and 210 g were made pregnant and divided into five groups (n=5). Group A, received water (control), Group B, received 1mL of 10% alcohol (Alc) (at a dose of 0.5 mL/100 g body weight), Group C, received 0.5 mL (0.5mL/100 g) of 15% *Camellia sinensis* extract only, Group D, received 0.5 mL of 10% Alc + 0.5 mL *Camellia sinensis* extract and Group E, received 0.5 mL of 10% Alc + 0.5 mL of 200 mg/kg of vitamin C. All administration was done orally from the 1st day of gestation to postnatal day 21. Neurobehavioural evaluation for muscular strength, balance and coordination was carried out on day 21 pups. Pups of days 1, 7, 14, 21, and 28 were sacrificed and the cerebellum dissected out for oxidative stress and morphological studies. In the alcohol-treated group, there was decreased forelimb grip strength, increased negative geotaxis, increased lipid peroxidation (LPO) and decreased GSH levels, SOD, CAT and GPx activities in the cerebellum compared with the control and other treated groups. Histologically, in the cerebellar cortex of day 21 pups, there was persistent external granular layer (EGL), reduced molecular layer (ML) thickness, Purkinje cell depletion and astrogliosis in the alcohol-treated group. Administration of *Camellia sinensis* extract and vitamin C to alcohol restored the altered neurobehavioural, biochemical and morphological deficits observed in the alcohol group. *Camellia sinensis* and vitamin C ameliorated maternal alcohol-induced oxidative stress and in the postnatal developing rat cerebellum.

Key Words: *Camellia sinensis* , alcohol, neurobehaviour, oxidative stress, developing cerebellum

INTRODUCTION

Alcohol (ethanol) is one of the most toxic and widely abused substances in our society today (Carvajal and Lerma-Cabrera, 2015). Early exposure to alcohol during pregnancy, leads to Foetal Alcohol Syndrome (FAS), which is a major factor for poor development of the nervous system and is one of the main causes of intellectual disability in most parts of the world (Popova *et al*., 2016). Foetal Alcohol Syndrome could be identified by numerous abnormalities such as stunted growth, microcephaly, reduced coordinating ability, mid-facial and joint anomalies (Archilbald *et al*., 2001). Ethanol has been reported to readily cross the placenta of the mother to the fetal blood circulation and almost assuming equilibrium in both the maternal and fetal blood circulation (Goodlett *et al*., 2005). Persistent maternal abuse of ethanol during gestation has serious teratogenic effects on embryonic development resulting in congenital malformations and mental retardation. The gravity of malformations however depends on consumption dose and period during gestation (Mattson *et al*., 2001). Alcohol use disorders (AUDs), causes neurodegeneration, which is responsible for the cognitive and behavioral impairment that leads to alcohol addiction (Liput, 2013). The foetus is limited in its ability to metabolize alcohol as a result of low activity of hepatic dehydrogenase. Furthermore, alcohol slowly diffuses out of amniotic fluid which may serve as reservoir for alcohol and exposing the foetus to alcohol syndrome in the long run (Goodlett *et al*., 2005). Alcohol is highly diffusible through cell membranes and metabolized by most tissues. Thus, its toxicity affects most organs especially the liver, a major site of alcohol metabolism (Gao and Bataller, 2011), kidney (Schaeffner and Rtitz, 2012), testes and ovaries (WHO, 2011), and brain (Harper, 2009).

The cerebellum helps in motor coordination as well as motor learning (Lamont and Weber, 2012). The cerebellum among other brain regions has high vulnerability to alcohol consumption because its weight reduced severely with alcohol consumption (Harper, 2009). In addition, literatures have it that ethanol poses more harmful effect on the brain in adolescence, this may be hinged on the fact that many central nervous system (CNS) organs are developing during this period (Toga *et al*., 2006). Ethanol intoxication also reportedly caused cerebellar atrophy as well as neurodegeneration in a

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peri-adolescence (postnatal day 25 to 70) or adulthood (postnatal day 180 to 225) of both males and females mouse (Huang *et al*., 2012). Pascual *et al*. (2007) reported that pre-adolescence and adolescence exposure to ethanol resulted in cerebellar damage, causing long-lasting behavioural and motor consequences during adulthood. In another report, alcohol induced apoptosis in the developing brain and increased neuronal loss within different regions of the brain (Light *et al*., 2002). These neuronal deficits may be permanent, extending into adulthood, and causing increase in oxidative stress and caspase-3 effector proteins activation (Marino *et al*., 2004). In rats, alcohol has been found to trigger widespread apoptotic neurodegeneration in the developing rat forebrain, causing damage to the olfactory bulbs (Crews *et al*., 2000). Reports have also shown that chronic ethanol exposure caused increased brain oxygen radical formation and lipid peroxidation in adult/juvenile/neonatal rats (Montoliu *et al*., 1994).

Nutraceutical supplementation has been found to significantly lower the levels of histologic and biochemical indices of neurodegeneration in rat brain exposed to alcohol (Charles *et al*., 2016). *Camellia sinensis* leaf extract, a nutraceutical and polyphenol, has been shown to reduce inhibition induced by pesticide on cellular proliferation (Cho *et al*., 2003; Tai and Truong, 2010) and offers protective effect in many neural cells (Pan *et al*., 2003). Studies have shown that *Camellia sinensis* has a protective effect against smoke-induced oxidative stress as the affected guinea pig lung microsomes was inhibited by 93% upon exposure to the *Camellia sinensis* infusion (Misra *et al*., 2003).

Despite the available literatures, there is paucity of data on the protective effects of *Camellia sinensis* extract on alcohol-induced oxidative stress and morphological alteration in postnatal developing cerebellum in rats, hence this study. Results from this study will contribute to knowledge, on the health benefits of *Camellia sinensis* which may be an approach to the prevention and management of alcohol-induced neurodegeneration.

MATERIALS AND METHODS

Experimental animals: Twenty-five (25) adult female Wistar rats (160-210 g) were obtained from the Animal house of the Department of Physiology, University of Ibadan. They were housed in plastic cages at room temperature with natural 12 hours' light/dark cycle. The rats were fed with pelleted chow obtained from Ladokun Feeds®, Ibadan and drinking water provided *ad libitum*. Wood shaven beddings were changed daily to maintain a hygienic environment. 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).

Grouping and Mating of Experimental Animals: The female rats were mated, and pregnancy confirmed by the presence of vaginal plug and smear, taken as the first day of gestation.

Experimental Design: The pregnant rats were divided into five groups as follows:
Group A: Rats were administered distilled water (control group).
Group B: Rats were administered 0.5mL/100 g body weight of 10% ethanol (Lodhi *et al*., 2014).
Group C: Rats were administered 0.5 mL/100 g body weight of 15% extract of *Camellia sinensis* extract (Lodhi *et al*., 2014).
Group D: Rats were administered ethanol (0.5 mL/100 g body weight) + *Camellia sinensis* extract (0.5 mL of 15% extract).
Group E: Rats were administered Vitamin C, 0.5 mL (200 mg/kg body weight) + ethanol.
The doses administered were calculated using the average weight of the animals in each group. The interventions were administered by oral gavage from day 1 of gestation through day 21 after delivery (i.e. pre- and post-natal life). *Camellia sinensis* leaf extract and vitamin C were administered one hour before administration of ethanol.

Dosage Determination
Preparation and administration of alcohol: Absolute ethanol was obtained from the Department of Pharmacognosy, University of Ibadan, Ibadan Nigeria and diluted to 10%. Each preparation was freshly made and administered orally using an oral gavage at a dose of 0.5 mL/100 g body weight, daily.

Preparation and administration of *Camellia sinensis* : The dried leaf of *Camellia sinensis* manufactured by Qualitea Ceylon (PVT) Ltd, Sri Lanka, was purchased, blended into powder and sieved. One hundred grams of the powdered plant material was steeped in 600 mL of distilled water and heated in water bath for 3 h at 90°C. The mixture was allowed to cool to room temperature, filtered, and dried. A total of 3 g yield of dried *Camellia sinensis* extract was obtained and used to treat rats at 0.5 mL /100 g body weight, daily (Lodhi, *et al*., 2014).

Dosage of Vitamin C: Standard vitamin C manufactured by Nuel Pharm Ltd, Owode-Egba, Ogun State was purchased from a reputable Pharmacy Store, at Ojoo, Ibadan, Oyo State, Nigeria. Ten (10) tablets of 100 mg each (i.e. 1000 mg) was dissolved in 2.5 mL of tap water to a concentration of 2.5 mg/mL and then 0.5mL (200mg/kg body weight) was administered to the animals daily (Imosemi *et al*., 2010; Uchendu, 2018).

Sacrifice of the Experimental Animals and Brain Tissue Harvest: After birth, the pups of days 1, 7, 14, 21, and 28 were weighed, neurobehavioural studies done (on pups of day 21) and sacrificed by cervical dislocation. The brains and cerebella of the pups dissected out, weighed and some cerebella were preserved in phosphate buffered saline at 4^0C and pH 7.2 for oxidative stress evaluation, while others were fixed in 10% formol-saline for histological, histomorphometric and immunohistochemical studies.

Neurobehavioural Studies: Pups of day 21 for each group were made to perform forelimb grip strength and negative geotaxis. Time spent in achieving these tests were recorded using a stop watch.

Forelimb grip Test: This test (for muscular strength and balance) was carried out in accordance with Owoeye and Farombi (2015) with slight modifications. Briefly, a steel wire of about 1m long and 7 mm thick was placed horizontally across two wooden beams above a landing area filled with soft bedding. The pup was allowed to grip the wire with its forepaws and afterwards released. The grasp duration (time) for each pup (Latency to fall) was taken and recorded using a stopwatch. The pups were randomly tested, each given two trials of with a maximum of two minutes and five minutes

respectively, and a minimum of twenty minutes interval between trials.

Negative Geotaxis: This test (for balance/equilibrium) was also done following the method described by Owoeye and Farombi (2015). Pups were placed head down on a 45° (forty-five degrees) inclined plane and then observed for the time taken to orient in a head-up direction (Latency to turn). Time taken (in seconds) was recorded using a stopwatch.

Oxidative stress and antioxidant assays: The cerebellar tissue of day 21 pups was homogenized in ice cold phosphate buffer at pH 7.4. The resulting homogenate was centrifuged at 4°C at 1500 rpm for 10 minutes. The supernatant collected from the centrifugation was used for all oxidative stress and antioxidant biomarkers using colorimetric assay. The following oxidative stress and antioxidant biomarkers were assayed for; 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 method (1972) and glutathione peroxidase (GPx) activity by the method of Rotruck *et al.* (1973).

Tissue processing for histological and immunohistological studies:

Histological preparations: Cerebellar tissues from the pups of all groups were fixed in 10% formol saline, processed employing routine paraffin embedding and stained with Haematoxylin and Eosin for histomorphological evaluation. The slides were examined and evaluated under a 500-pixel Leica digital binocular microscope and the following were evaluated in the cerebellar cortex; thickness of the external granular layer (EGL) and molecular layer (ML), and Purkinje cell density and astrocyte population using the computer software, image-j.

Immunohistochemistry: Cut formalin-fixed paraffin cerebellar tissues were immunostained with Glial fibrillary acidic protein (GFAP) for astrocyte population (neuroglia) using the Avidin biotin immunoperoxidase method (Wasowicz *et al*, 1994) with horseradish peroxidase (HRP), diaminobenzidine (DAB) and counterstained with Haematoxylin solution. Images were captured from the cerebellar cortex with a 500-pixel Leica binocular microscope. Astrocyte population was counted using the software, image-j.

Statistical analysis: Data collected was further analysed as mean ± SD employing one-way analysis of variance (ANOVA) followed by Tukey Posthoc for multiple comparison using the GraphPad prism® 6.0 at $p<0.05$.

RESULTS

Neurobehavioural studies

Forelimb Grip Strength Test: A decreased forelimb grip strength was observed in the Alc- and AlcVc- treated day 21 pups compared with the control at $p<0.05$. Administration of *Camellia sinensis* to the alcohol-treated rats, increased the forelimb grip strength when compared with the Alc-treated rats at $p<0.05$ (Figure 1).

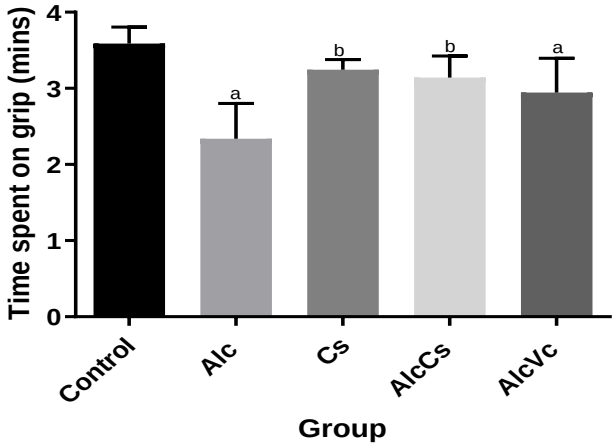

Figure 1:
Forelimb grip strength test (minutes) of day 21 pups.
Values are expressed as mean±SD (n=5), Alc–Alcohol, Cs-Camellia sinensis , AlcCs-Alcohol+Camellia sinensis , AlcVc-Alcohol+Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

Negative geotaxis

Increased time of negative geotaxis was observed in the Alc- and Alc*Cs*-treated day 21 pups compared with the control at $p<0.05$. However, there was significantly decrease time of negative geotaxis in the Cs-, Alc*Cs*- and and AlcVc-treated groups compared with Alc-treated day 21 pups (Figure 2).

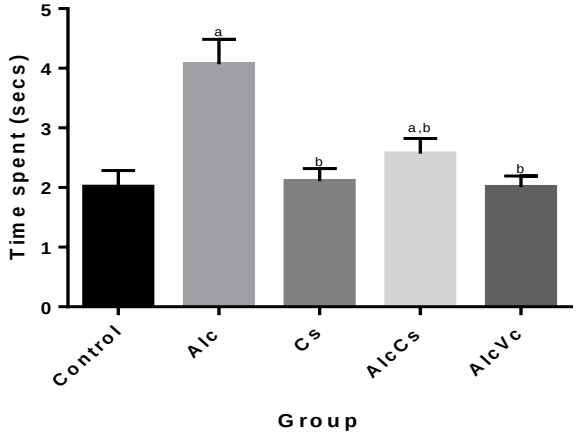

Figure 2:
Negative geotaxis of day 21 pups.
Values are expressed as mean ± SD (n=5), Alc–Alcohol, Cs-Camellia sinensis , AlcCs-Alcohol+Camellia sinensis , AlcVc-Alcohol+Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

Oxidative stress marker and antioxidants: The data analysed on day 21 pups, compared with the control at $p<0.05$ indicated an increased lipid peroxidation (LPO) in the Alc-treated group (Figure 3) and decreased GSH in the Alc- and Alc*Sc* (Figure 4), SOD in the Alc- and Alc*Sc* (Figure 5), CAT (Figure 6) and GPx (Figure 7) groups. *Camellia sinesis* and vitamin C administered to the Alc-treated rats decreased the LPO levels, and prevented the depletion of SOD, CAT and GPx activities compared with the Alc-treated day 21 pups rats at $p<0.05$

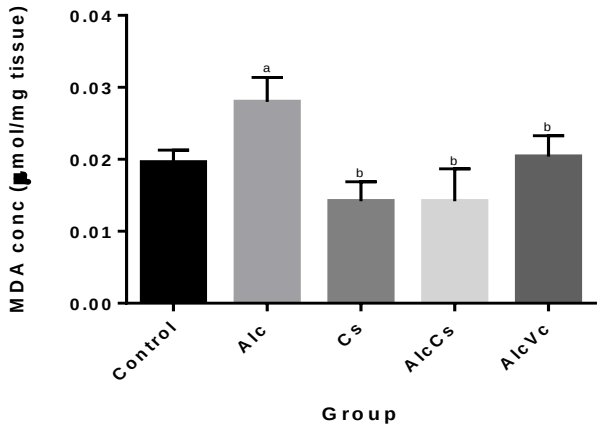

Figure 3:
Lipid Peroxidation (LPO).
Values (n=5) are presented as Mean±SD, MDA- Malondialdehyde, Alc–Alcohol, Cs-Camellia sinensis , AlcCs-Alcohol+Camellia sinensis , AlcVc-Alcohol+Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

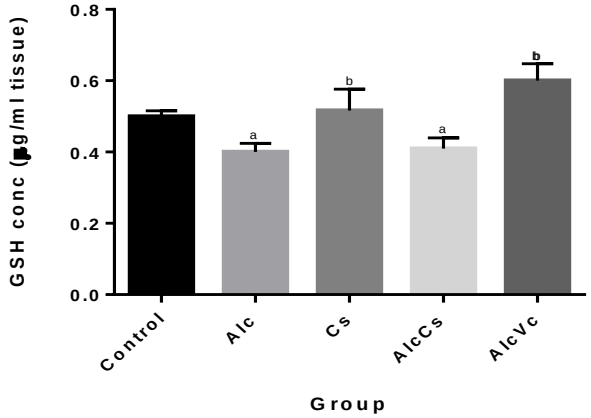

Figure 4:
Reduced glutathione levels (GSH).
Values (n=5) are presented as Mean±SD, Alc-Alcohol, Cs-Camellia sinensis , AlcCs- Alcohol+Camellia sinensis , AlcVc-Alcohol+ Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

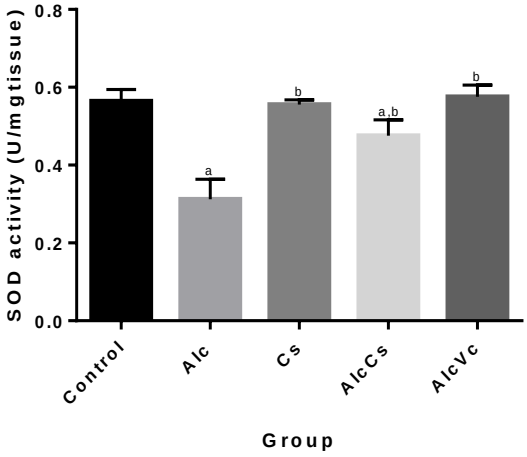

Figure 5:
Superoxide dismutase (SOD) activity.
Values (n=5) are presented as Mean±SD, Alc-Alcohol, Cs-Camellia sinensis , AlcCs- Alcohol+Camellia sinensis , AlcVc-Alcohol+ Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

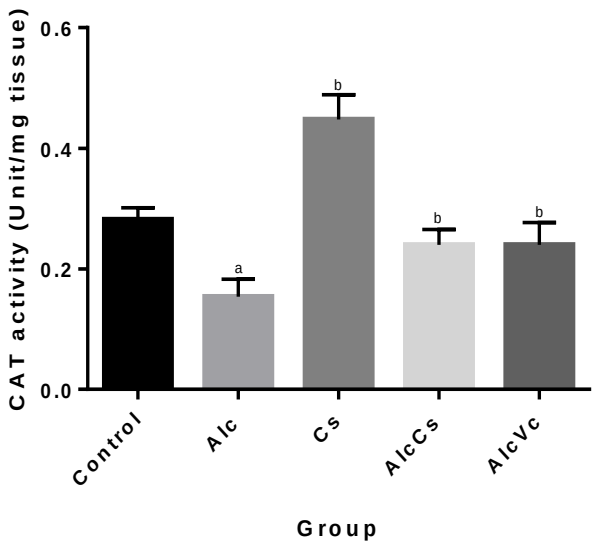

Figure 6:
Catalase (CAT) activity.
Values (n=5) are presented as Mean±SD, MDA- Malondialdehyde, Alc–Alcohol, Cs-Camellia sinensis , AlcCs-Alcohol+Camellia sinensis , AlcVc-Alcohol+Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

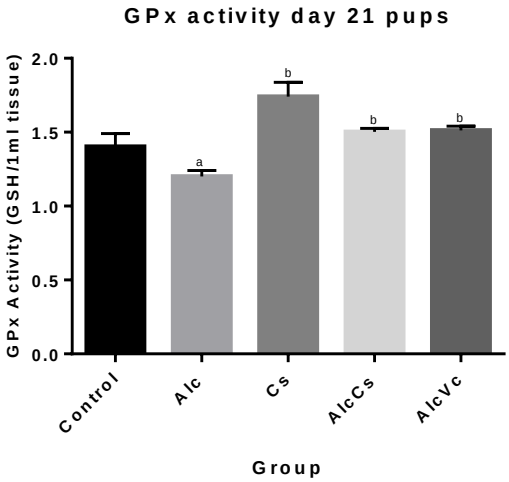

Figure 7:
Glutathione peroxidase (GPx) activity.
Values (n=5) are presented as Mean±SD, Alc–Alcohol, Cs-Camellia sinensis , AlcCs-Alcohol+Camellia sinensis , AlcVc-Alcohol+Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

Histological and histomorphometric studies: The cerebellar cortex of the pups stained with H & E showed normal folia comprising, external granular layer (EGL), molecular layer (ML), Purkinje layer (PL) with normal Purkinje cells and granular layer (GL). No pathological lesion was seen in all the groups, however, histomorphometric evaluation showed some significant differences.

Thickness of the EGL of days 7 and 14 pups: There was no significant difference in the cerebellar EGL thickness between the control and treated pups on day 7 (Figure 8A and Plate 1) at $p>0.05$. However, an increased EGL thickness was seen in the Alc-treated pups on day 14 (Figure 8B and Plate 2) compared with the control and AlcVc groups at $p<0.05$.

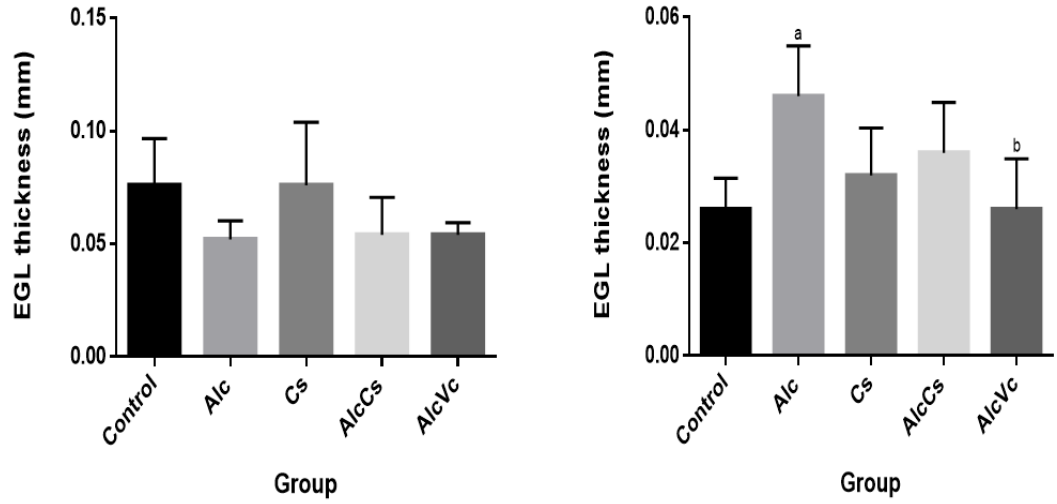

Figure 8:
Cerebellar External Granular Layer (EGL) thickness (mm) of (A) day 7 and (B) day 14 pups. Values are expressed as mean±SD (n=5) Alc–Alcohol, *Cs-Camellia sinensis* , Alc*Cs*-Alcohol+*Camellia sinensis* and AlcVc-Alcohol + Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

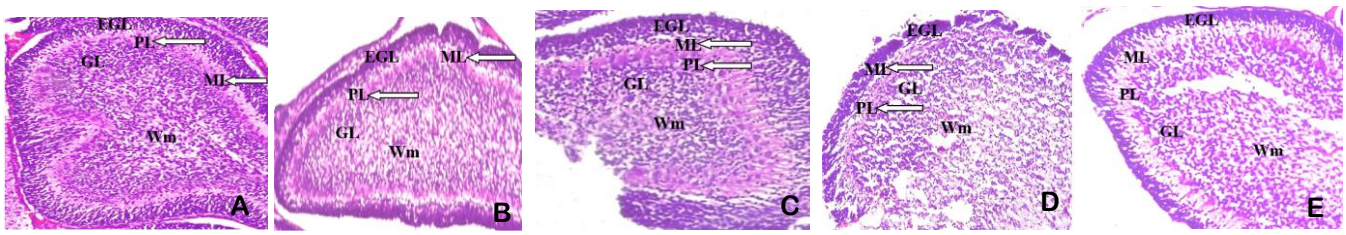

Plate 1:
Photomicrographs of the cerebellar cortex of day 7 pups. H&E X100. A. Control, B. Alc–Alcohol only, C. *Sc-Camellia sinensis* , D. Alc*Sc*-Alcohol+*Camellia sinensis* , E. AlcVc- Alcohol+Vitamin C. EGL-External Granular Layer, ML-Molecular layer, PL-Purkinje Layer, GL-Granular Layer, Wm-White matter.

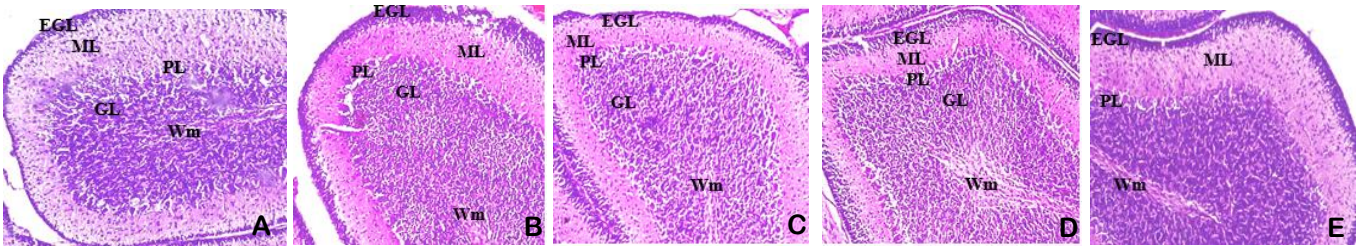

Plate 2:
Photomicrographs of the cerebellar cortex of day 14 pups. H&E X100. A. Control, B. Alc–Alcohol only with thicker EGL, C. *Sc-Camellia sinensis* , D. Alc*Sc*-Alcohol+*Camellia sinensis* , E. AlcVc- Alcohol+Vitamin C. EGL-External Granular Layer, ML-Molecular layer, PL-Purkinje Layer, GL-Granular Layer, Wm-White matter.

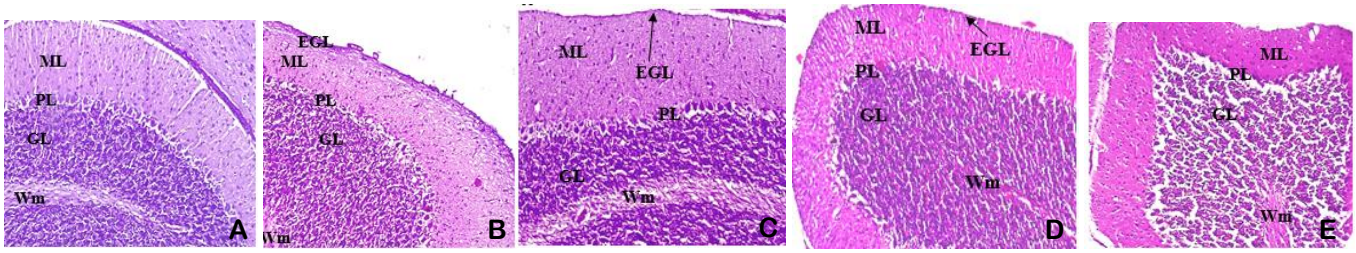

Plate 3:
Photomicrographs of the cerebellum at day 21. H&E X100. A. Control, B. Alc–Alcohol only, C. *Cs- Camellia sinensis* , D. Alc*Cs*- Alcohol+*Camellia sinensis* , E. AlcVc-Alcohol+Vitamin C. EGL-External Granular Layer, ML-Molecular layer, PL-Purkinje Layer, GL-Granular Layer, Wm-White mater

Thickness of the Molecular layer and Purkinje cell density of day 28 pups: Reduced thickness of the molecular layer (ML) of the cerebellar cortex and a decreased Purkinje cell density was seen in the Alc-treated group (Figures 12, 13 and 14B) compared with the control (Figures 9, 10 and Plate 4A) at $p<0.05$. Administration of *Cs* and Vit C to Alc-treated rats, significantly increased the ML thickness (Figures 9, Plate 3D, E ($p<0.05$) and non-significantly increased Purkinje cell density ($p>0.05$) compared with the Alc-treated rats (Figure 10).

Cerebellar astrocyte population on day 21 pups: Significantly increased astrocyte population was seen in Alc-treated pups on day 21 (Figures 15 and 16B) compared with the control at $p<0.05$. *Camellia sinesis* and vitamin C administered to Alc-treated rats, decreased the astrocyte population (Figures 11, Plate 5C and D) compared with the Alc-treated group at $p<0.05$.

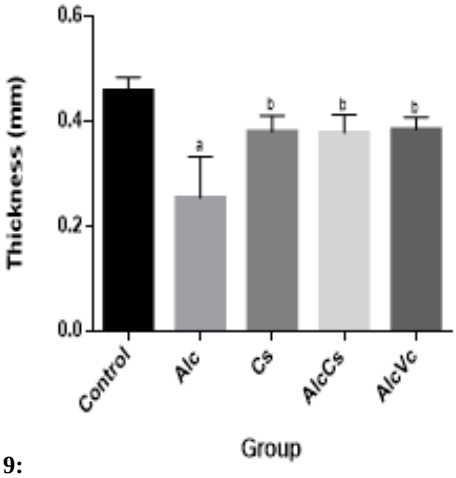

Figure 9:
Cerebellar molecular layer (ML) thickness of day 28 pups. *Values (n=5) are presented as Mean±SD, Alc-Alcohol, Cs-Camellia sinensis, AlcCs- Alcohol+Camellia sinensis, AlcVc-Alcohol+ Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.*

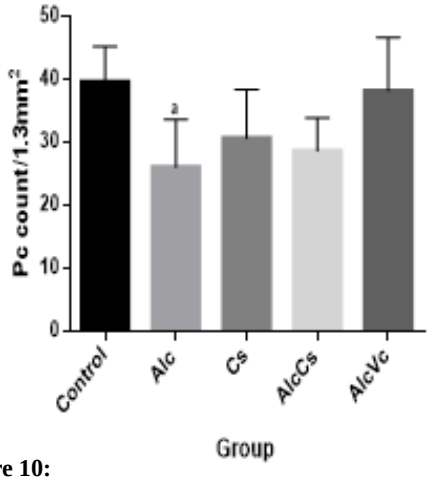

Figure 10:
Purkinje cell (Pc) count of day 28 pups.
Values (n=5) are presented as Mean±SD, Alc-Alcohol, Cs-Camellia sinensis, AlcCs- Alcohol+Camellia sinensis, AlcVc-Alcohol+ Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group

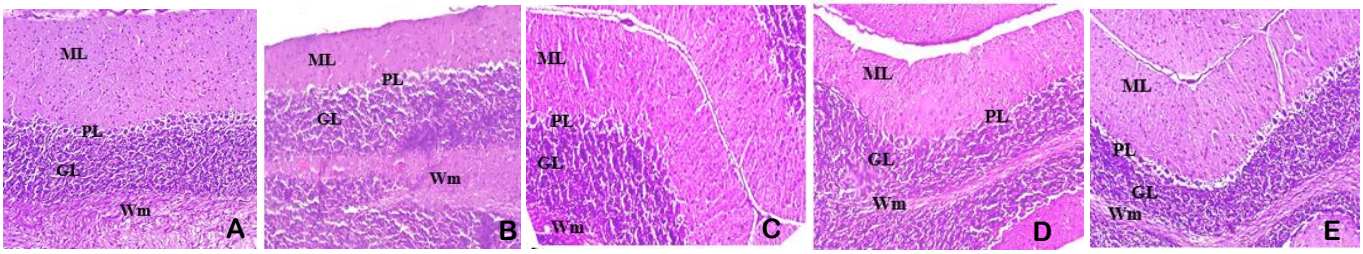

Plate 4:
Photomicrographs of the cerebellar cortex of day 28 pups. H&E X100. A. Control, B. Alc–Alcohol with decreased ML and depleted Purkinje cells, C. *Cs-Camellia sinensis* , D. Alc*Sc*-Alcohol+*Camellia sinensis* , E. AlcVc-Alcohol+Vitamin C. EGL-External Granular Layer, ML-Molecular layer, PL-Purkinje Layer, GL-Granular Layer, Wm-White matter.

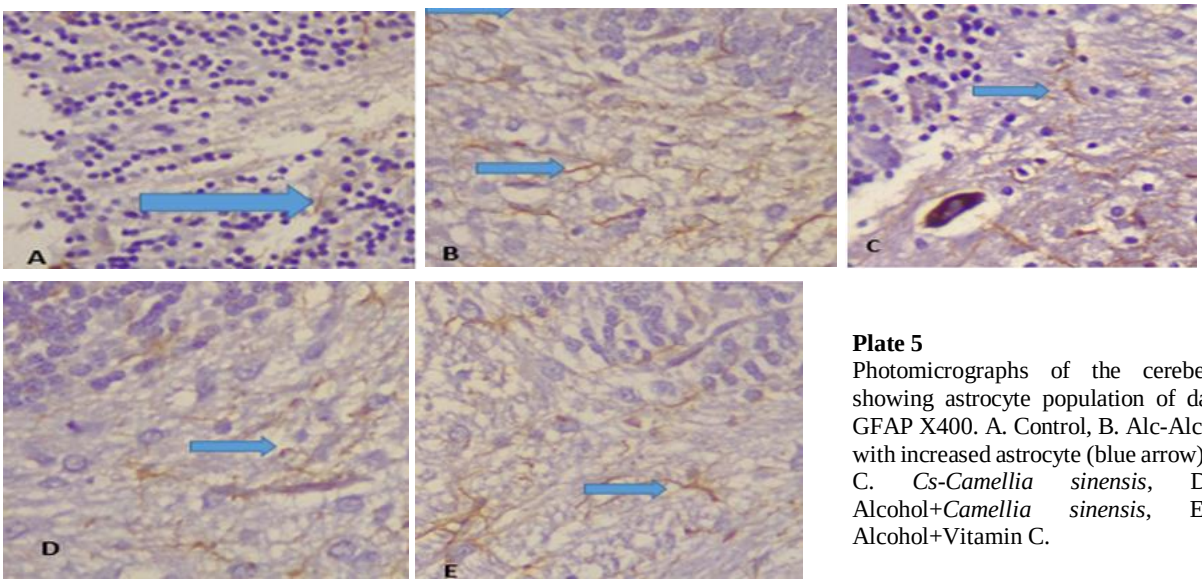

Plate 5
Photomicrographs of the cerebellar cortex showing astrocyte population of day 21 pups. GFAP X400. A. Control, B. Alc-Alcohol-treated with increased astrocyte (blue arrow) population, C. *Cs-Camellia sinensis*, D. Alc*Cs*-Alcohol+*Camellia sinensis*, E. AlcVc-Alcohol+Vitamin C.

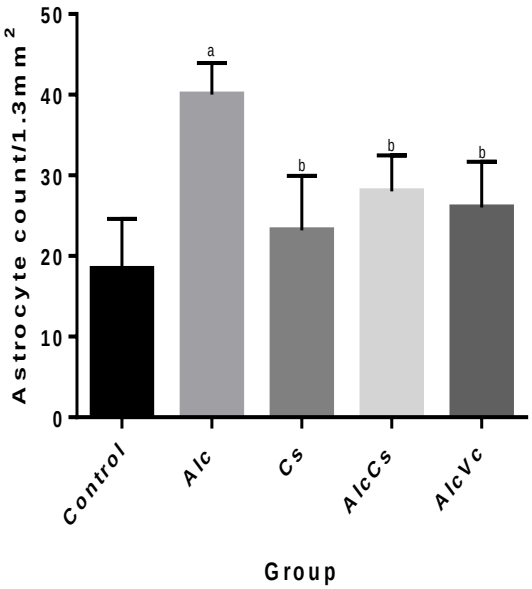

Figure 11:
Cerebellar astrocyte count of day 21 pups. GFAP X400. Values are expressed as mean±SD (n=5). Alc–Alcohol, *Cs-Camellia sinensis* , Alc*Sc*-Alcohol+*Camellia sinensis* , AlcVc-Alcohol+Vitamin C. [a]p<0.05 vs control, [b]p<0.05 vs Alc group.

DISCUSSION

Excessive alcohol use can cause structural and functional abnormalities of the developing brain and this has significant health, social and economic implications for most countries in the world (Larue-Achagiotis *et al*., 1990). The cerebellum functions in controlling various motor activities and exposure of the developing cerebellum to active oxidants may result in developmental irregularities induced by embryonic hypoxia with vascular necrosis and tissue damage as a result of ischaemia. In the present study, there was decreased forelimb grip strength and increased latency to turn in the negative geotaxis test in the alcohol-treated pups. The function of the cerebellum in modulating the sequence and strength of muscular contractions during posture and voluntary movements as well as control of proprioceptive impulses from joint receptors and Golgi tendons is highly dependent on the development of granule cell (Noback *et al*., 2005). The result of the reduction in forelimb grip strength is in agreement with Steiner *et al.* (2011) who reported decreased grip strength in rat in a dose-dependent manner after alcohol injection. The increased negative geotaxis observed in the pups of the alcohol-treated group may be due to the effect of alcohol on the proprioceptive function of the brain, occasioned by poor development of the cerebellar neurons. Motz and Alberts (2005) reported that negative geotaxis is considered diagnostic of vestibular and/or proprioceptive function. Hannigan (1995) reported that pre-natal alcohol exposure had a significant negative impact on several developmental indices, including grip strength and negative geotaxis. *Camellia sinensis* and vitamin C increased the latency to fall in forelimb grip test, and decreased latency to turn in the negative geotaxis test.

Several studies have demonstrated that alcohol increases lipid peroxidation as well as the modification of proteins (Montoliu *et al*., 1994; Marino *et al*., 2004) however, it is not always clear if these changes are the causes rather than consequences of alcohol-induced tissue injury. Previous studies have shown that lipid peroxidation has a role in pathogenesis of several pathologies as in neurodegenerative diseases (Dominguez *et al*., 2008). In this study, maternal alcohol consumption induced oxidative stress by decreasing glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in the developing cerebellum, and increasing lipid peroxidation (LPO), thereby generating free radicals. The decreased GSH levels and the antioxidant enzymes represent increased utilization due to oxidative stress (Matcovi *et al*., 1982). These observations agree with the report of Łuczaj *et al.* (2015) that catalase and superoxide dismutase activities significantly decreased in brain tissues following high concentration of alcohol intake. Consumption of *Camellia sinesis* and vitamin C decreased the rate of oxidative stress in the developing cerebellum of rat induced by pre- and post natal consumption of alcohol probably by increasing the GSH levels and stimulating the antioxidant enzymes, resulting in more activity and reducing free radicals to prevent oxidative stress.

The cerebellar cortex develops from the cerebellar plate, and cells from its neuroepithelial layer migrate to form the external granular layer (EGL), from which cells later migrate past the Purkinje layer to form the granule cells in the internal granular layer (Sadler, 2010). Cerebellar development begins early in the fetus, but its maturation, in particular, inward migration of the granule cells, continues late into the infant year in animals. Thus, making the cerebellum particularly susceptible to pre- and postnatal insults (Volpe, 2009). The external granular layer (EGL) consists of highly metabolic cells whose differentiations result in the synthesis of the outer stellate, basket, Golgi type II and granule cells of the cerebellar cortex. Hatten and Heintz (1995) reported that the EGL disappears on day 20 in rats. In this study, there was complete disappearance of EGL in the control and other treated groups on day 21 pups but 2-3 layers thick of EGL persisted in alcohol group. The mechanism for the persistent EGL in the alcohol group is not clearly understood but delayed maturation of cells of the EGL have been reported by some workers (Malomo *et al.*, 2004; Imosemi and Osinubi, 2011) which may be due to the oxidative stress induced by alcohol.

The Molecular layer becomes the most superficial layer of the cerebellar cortex after the complete disappearance of the EGL (Marzban *et al*., 2014). The Molecular layer thickness of the cerebellar cortex on day 28 pups in the alcohol-treated group was reduced. The mechanism involved for the reduction of the ML thickness is not clear but neuronal cell death induced by oxidative stress in the granule layer or delayed parallel fibre formation caused by delayed granule cell formation may have affected the density of unmyelinated parallel fibres in the ML. Rakic and Sidman (1970) reported that the thickness of the ML is determined by the amount of cells and fibres present, while Rakic (1971) showed that its thickness is mainly by accretion of new parallel fibres. Administration of *Camellia sinensis* extracts and vitamin C increased the thickness of the ML probably by decreasing the rate of oxidative stress by mopping up the free radicals produced by alcohol.

The cerebellar Purkinje neurons constitute the most visible neurons in the CNS of the vertebrates which solely send signals out of the cerebellar cortex (Hirano, 2018). In this study, there was decreased Purkinje cell density in the alcohol-treated day 28 pups. Studies have shown that decreased level

of antioxidants in the brain increases their susceptibility to ethanol's teratogenicity (Abel and Hannigan, 1995) and the migration, differentiation and maturation of cerebellar neurons are affected by exposure to alcohol (Chedotal, 2010). *Camellia sinensis* extracts and vitamin C restored the depleted Purkinje cells probably by boosting their free radicals scavenging property which are well documented.

Astrocytes are the most populated glial cells. They play a major role in the metabolism and supply of energy for synaptic transmission. They provide support for surrounding neurons, help in neuronal functioning and signal processing in the CNS. The astrocytic process terminal ends encapsulate blood vessels and participate in forming blood brain barrier (Ambrosi *et al*., 1995). In this study, employing immunohistochemical technique using the GFAP marker, there was increased astrocyte population in the alcohol group when compared with the control and other treated groups. Rossi (2015) reported that activation of astrocyte seems to be important in the initiation of synaptic mechanisms. Consumption of alcohol can lead to oxidative stress which causes activation of astrocyte. Extracts of *Camellia sinensis* and vitamin C decreased the astrocyte population probably due to the presence of flavonoid and antioxidant property.

In conclusion. from the study, maternal alcohol consumption during pregnancy and nurturing of the pups induced oxidative stress resulting in neurobehavioural changes and morphological alterations in the postnatal developing cerebellum of rat. However, treatment with *Camellia sinensis* extract and vitamin C reduced the oxidative stress caused by alcohol, and as such, may offer some degree of protection to the developing cerebellum of rats due to its antioxidant activity.

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