



IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com



Original Article

Histochemical Evaluation of the Activities of Vitamin C on Ethanol Administration in Rat; Implication on the Cytoarchitectural and Some Neurochemical Indices of the Prefrontal Cortex

Adekeye A.O^{1*}, Akintayo C.O², Enye L.A¹, Ogendengbe O.O¹ and Adeniyi A.I³

¹Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Nigeria

²Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Nigeria

³Department of Physiology, College of Medicine and Health Sciences, Ahmadu Bello University, Zaria, Nigeria

ARTICLE INFO

Article history:

Received: 12 Apr, 2015

Revised: 27 May, 2015

Accepted: 25 Jun, 2015

ePublished: 30 Jul, 2015

Key words:

Ethanol

Apoptosis

Oxidative markers

Prefrontal cortex

Neurodegeneration

ABSTRACT

Objective: This study was to evaluate the effect of Vitamin C on the histology and histochemistry of the prefrontal cortex of ethanol-induced rats. **Methods:** Male Sprague-Dawley rats were used for the study. Ethical approval was obtained from the University's ethical committee. The rats were randomly divided into 6 groups of 10 rats each. Rats in group A= free access to normal saline. Rats in group B= treated with 4.25 ml ethanol. Rats in group C= treated with 100 mg/kg Vit. C. Rats in group D= pre-treated with 100 mg/kg Vit. C followed by 4.25 ml ethanol. Rats in group E=co-treated with 100 mg/kg of Vit. C and 4.25ml ethanol. Rats in group F=post-treated with 4.25ml ethanol followed by 100 mg/kg Vit.C. 24hrs after the last administration, the rats were sacrificed by cervical dislocation: the fraction of the brain for tissue histochemistry was fixed in formol calcium and later processed for Heamatoxylin and Eosin with Cresyl fast violent staining techniques and the other fraction meant for enzyme and/or marker histochemistry was processed accordingly for some neurochemical indices for oxidative stress. **Results:** The markers of oxidative stress were statistically increased in the rats in group D, E and F compared with the rats in group B. There is a significant reduction of TBARS when compared with ethanol induced group (group B). The histological profile of the prefrontal cortex of rats in group A and C were preserved while that of the rats in group B displayed distorted cytoarchitecture profile with a marked increase in apoptotic bodies, lateral deviation of neurons and a marked increase in the activities of oxidative markers.

1.INTRODUCTION

Ethanol is the most psychoactive substance used after caffeine. Chronic alcoholism is a major public health problem and causes multiorgan diseases and toxicities (Subir *et al.*, 2007) and also leads to oxidative stress

(Sullivan *et al.*, 2005; Sullivan and Pferfferbaum, 2005). The etiology of some oxidative stress-based pathological conditions in the brain and liver has implicated excessive alcohol consumption. More than ever before, there is an upsurge in alcohol abuse as a result; alcohol-related disorders are becoming increasingly important causes of morbidity and mortality globally (Rukkumani *et al.*,

*Corresponding Author: Adekeye A.O, Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Nigeria (cyberdex21@gmail.com)

2004). Many research reports have linked chronic alcohol consumption and variety of pathological conditions varying from simple intoxication to severe life-threatening pathological states (Tsukamoto and Lu, 2001; Mcdonough, 2003; Tuma, 2002; Lieber, 2003; Molina *et al.*, 2002). It has been observed that progressive increases in ethanol consumption lead to alterations in brain structures that reduce behavioral control to promote further alcohol abuse and neurodegeneration. The frontal lobes are the most insulted region in the alcoholic brain with the superior frontal cortex showing significant neuronal loss (Kubota *et al.*, 2001; Sullivan and Pfefferbaum, 2005). Alcohol-induced neurotoxicity has been observed to develop mainly through generation of free radicals and reactive oxygen species, as well as impaired anti-oxidant defense mechanism; conditions which result in oxidative stress with attendant health problems (Wu and Cederbrum, 2003). Excessive alcohol consumption also has a negative effect on the nutrition status of the alcoholics, interfering with the digestion, absorption and utilization of essential nutrients such as vitamins, minerals and proteins (Gruchow *et al.*, 1985; Lieber, 2003). Characteristics of alcohol consumption include the following: impaired judgement, blunted affection, social withdrawal, reduced motivation, distractibility, impaired anatomy of walking, blurred vision, slurred speech, slowed reaction times, impaired memory and impulse – control deficits (Parsons, 1987; Oscar-Berman and Hutner. 1993, Sullivan *et al.*, 2005; Sullivan and Pfefferbaum, 2005). Conversely, vitamin C is an important water – soluble antioxidant and enzyme co-factor in animals (Mandi *et al.*, 2009; Soujanya *et al.*, 2012), thus possesses the ability to scavenge free radicals and reactive oxygen species (ROS). It also protects low-density lipoprotein from oxidation and reduces harmful oxidants in the CNS. Therefore, this study seeks to evaluate the effects of Vitamin C on the histology and histochemistry of the prefrontal cortex of ethanol-exposed rats.

2. MATERIALS AND METHODS

2.1. Preparation and administration of ethanol

30% v/v ethanol solution was used as chronic dose in this experiment. 20 g absolute ethanol was dissolved in distilled water and made up to 100 ml. 4.25 ml of the solution was administered daily for 14 days to each rat treated with ethanol.

2.2. Preparation and administration of Vitamin C

Vitamin C tablets (100 mg) from Evans Medical Laboratories, Lagos, Nigeria were dissolved in distilled water to obtain 100 mg/ml suspension prior to daily administration.

2.3. Animal Care and Treatment

A total of 60 Wistar rats weighing between 140 – 160 g were used for the study. The rats were randomly divided into 6 groups (10 rats in each) designated as A, B, C, D, E, and F.

2.4. Histological Procedure

After the last administration, the animals were anesthetized with ether and then sacrificed by cervical dislocation, the frontal cortex were excised and coronally divided into anterior and posterior regions for histological examination. The anterior region were fixed in 10% formalin and embedded in paraffin, and were cut in 15 μ m thick in serial coronal sections using a rotating microtome; stained with hematoxylin and eosin, after which they were dehydrated and cover slipped. Then the sections were photomicrograph with a digital camera equipped to a microscope. However, the neurons were identified based on cell morphology.

2.5. Statistical analysis

All data were expressed as mean \pm standard error of mean (SEM) and statistical analysis was carried out using statistical software (SPSS version 15). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons among groups were done using Duncan post hoc analysis. P values <0.05 were considered as significant.

3. RESULTS

In the present study, effects of vitamin C on oxidative stress and toxicity related biochemical parameters in brain tissues were investigated. Fig 1 showed effect of vitamin C and ethanol on body weight of rats. Rats that were administered ethanol only showed lower body weight when compared with the control group and other treated groups with vitamin C which may be due to essentially fat mass reduction. The body weight of rats in group co-treated, pre-treated and post-treated showed a significant increase (P<0.05). Effect of Vitamin C on rats brain showed a decrease in oxidative stress by significantly increasing (P<0.05) the glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels, and decrease in lipid peroxidation, thiobarbituric acid reactive substance (TBARS) in brain (Fig 2) when comparing group A, D, E and F with group B that had an increased oxidative stress values as shown by decrease (P<0.05) GSH and increase lipid peroxidation (TBARS). Elevated level of TBARS in group B might be due to relatively high concentration of easily peroxidizable fatty acids in the brain as suggested by Carney *et al* (1999).

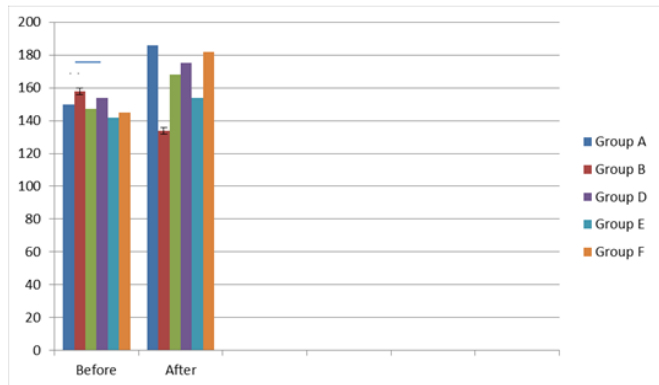


Fig 1: Showing effect of vitamin C on body weight of ethanol-induced rats

Rats in group B shows significant decrease in the body weight when compare with body weight before and after under normal condition which may be due to ethanol administration only. Other groups treated with vitamin C before and after administration of ethanol had a progressive increase in weight under normal condition ($P < 0.05$).

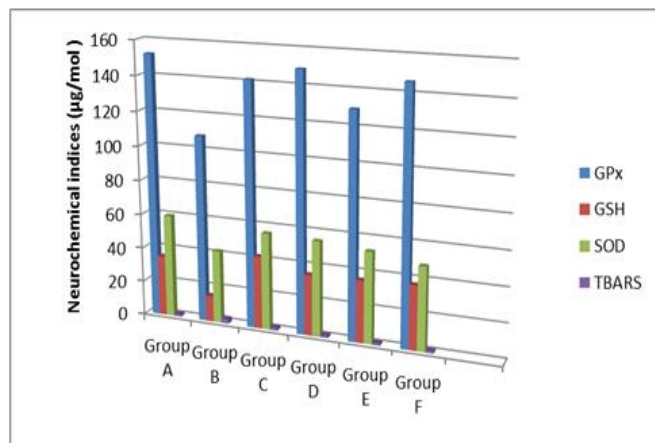


Fig 2 : Showing statistical inferences of the effects of Vitamin C on neurochemical indices of ethanol induced rats in $\mu\text{g/mol}$ ($p < 0.05$, $n = 10$)

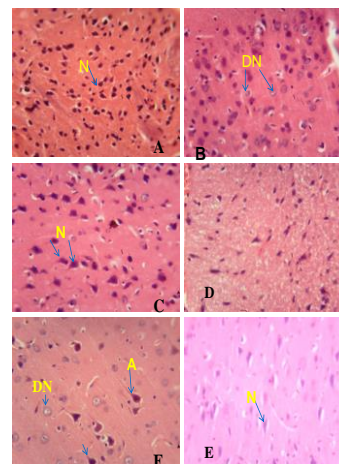


Fig 3: Showing effects of vitamin C on glial cells and neurons of prefrontal cortex of experimental rats. (A and E-neurons are preserved; B-neuronal cytoarchitectural profile of degenerating neurons; C-neurons with a preserved integrity and with conspicuous axons; D-minimal decrease in the number of distorted neurons apoptotic bodies with vacuolation, neuronal cell death; F-degenerating neurons with axon). H&E stain, Mag. x400

4. DISCUSSION

Alcohol-induced neurotoxicity has been observed to develop mainly through generation of free radicals and reactive oxygen species, as well as impaired anti-oxidant defense mechanism; conditions which result in oxidative stress with attendant health problems. Vitamin C is an important water-soluble antioxidant and enzyme co-factor in animals, thus possesses the ability to scavenge free radicals and reactive oxygen species (ROS). It also protects low-density lipoprotein from oxidation and reduces harmful oxidants in the CNS. The effects of Vitamin C on rats brain indicate a decrease oxidative stress by significantly increasing ($P < 0.05$) the glutathione (GSH) level, GPx and SOD, and decreased lipid peroxidation, thiobarbituric acid reactive substance (TBARS) in brain (Fig 2) when comparing group A, D, E and F with group B that had an increased oxidative stress values as shown by decrease ($P < 0.05$) GSH and increase lipid peroxidation (TBARS). Elevated level of TBARS in group B might be due to relatively high concentration of easily peroxidizable fatty acids in the brain as suggested by Carney *et al* (1999). Histologically, degenerating neuron observed in group B was actually preserved in the other groups treated with vit C. This indicates that vitamin C has an ameliorative effect on ethanol induced rats, perhaps due to its antioxidant and free radical scavenging activities which is also supported by Soujanya *et al*, 2012 and Maret, 2011.

CONCLUSION

In conclusion, data obtained from this study showed that the administration of Vitamin C (Ascorbic acid) mitigate against neurotoxicity evoked by consumption of ethanol in rats through its antioxidant properties and this can be indicated by the increase in the enzyme markers (GPx, GSH and SOD), reduction of lipid peroxidation (TBARS), increase of body weight and its ameliorative effect on the cytoarchitectural damaged of brain tissues exposed to ethanol administration.

ACKNOWLEDGMENT

I sincerely want to appreciate member of staff of Anatomy, Physiology and Biochemistry Department of Afe Babalola University, Ado-Ekiti for their assistance towards making sure the research is a huge success. I will also like to recognize Society of Neuroscientists of Africa (SONA) and IBRO for their support.

REFERENCES

Carney JM, Strake-Reed PE, Oliver CN, Landum RW, Chang MS, Wu JF, Floyd RA (1999). Reversal of age-related increase in brain protein oxidation. *PNAS*:88:3633-6

Gruchow HW, Sobocinski KA, Barboriak JJ (1985). Alcohol consumption, nutrients intake and relative body weight among US adults. *Am. J. Clin. Nutr.* 42(2):289-295.

Ighodaro OM, Omole JO (2012) Ethanol-induced hepatotoxicity in male wistar rats: effect of aqueous leaf extract of *Ocimum gratissimum*. *Journal of Medicine and Medicinal science* Vol 3(8): 499-505

Kubota M, Nakazaki S, Hirai S (2001). Alcohol consumption and frontal lobe shrinkage: Study of 1432 non-alcoholic subjects. *J Neurosurg Psychiatry* 71:104-6

Lieber CS (2003). Relationships between Nutrition, Alcohol Use and liver disease. *Alcohol Health & Research World*; 27(3):220-231

Mandi J, Szarka A. and Banhegyi G (2009). Vitamin C: Update on physiology and pharmacology. *British Journal of Pharmacology* 157:1097-1110

McDonough KH (2003). Antioxidant nutrients and alcohol. *Toxicology*, 189:89-97

Molina P, McClain C, Villa D (2002). Molecular pathology and Clinical aspects of alcohol-induced tissue injury. *Alcoholism: Clin. Exp. Res.* 26(1):120-128

Oscar-Berman M, Hutner N (1993) Frontal lobe changes after chronic alcohol ingestion. In Hunt W.A, Nixon SJ (eds)

Parsons OA (1987) Intellectual impairment in alcoholics: Persistent issues. *Acta Med Scand* 717 (suppl)33-46

Rukkumani R, Aruna K, Suresh VP, Menon VP (2004). Influence of folic acid on circulatory peroxidant-antioxidant status during Alcohol and PUFA-induced toxicity. *J. physiol. Pharmacol.* 55(3):551-561

Soujanya S, Lakshman MA, Gopala R (2012) Histopathological and ultrastructural changes induced by imidacloprid in brain and protective role of Vitamin C in rats. *Journal. Chemical and Pharmaceutical Research.* 4(9):4307-4318.

Subir KD, Hiran KR, Sukles M and Vasudevan DM (2007). Oxidative stress is the primary event: Effects of ethanol consumption in brain. *Ind. J. Clin. Biochem.* 22(1)99-104

Sullivan EA, Pfefferbaum A (2005) Neurocircuitry in alcoholism: a substrate of disruption and repair. *J. Psychopharmacology* 180:583-594

Tsukamoto H, Lu CS (2001). Current concepts in the pathogenesis of alcoholic liver injury. *The FASEB J.* 15: 1335-1349.

Tuma DJ, Casey CA (2003). Dangerous byproducts of alcohol breakdown-focus on adduct *Alcohol Health & Research World.* 27(4):285-290.

Wu D, Cederbaum AL (2003). Alcohol oxidative stress and Free Radical Damage. *Alcohol Res. Health.* 27(4):277-284.