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Biochemical and Histological Evaluation of Chronic Alcohol Administration in Wistar rats

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ABSTRACTS
Alcohol is consumed worldwide as a drink by humans. Regular and unlimited use of alcohol leads to toxicity and alcohol-induced pathological problems and can constitute a menace in the society. This study was aimed at evaluating the effects of chronic alcohol administration on antioxidant and histology of cerebral cortices in Wistar rats. Thirty-two (32) adult Wistar rats of both sexes were divided into 4 experimental groups with 8 animals (4 males and 4 females housed separately) in each group: Group 1 serve as control administered with 1 ml of distilled water, Group 2 was administered with 0.12g/kg, Group 3 was administered with 0.16g/kg and group 4 was administered with 0.24g/kg of alcohol respectively for 11 weeks orally. No significant difference was obtained between the control and the groups administered with alcohol in all the antioxidant markers in males and females Wistar rats and histological study revealed no histo-architecture distortion in the cerebral cortices of the treated group compared to control in male Wistar rats while distortion in the histo-architecture in the group 4(0.24g/kg bw of ethanol) compared to the control group in female Wistar rats. in conclusion, chronic daily oral administration of lose dose of alcohol does not significantly increase the production of reactive oxygen species which can cause oxidative damage in male and female Wistar rats. It also causes loss of neuronal processes of the cerebral cortices of female Wistar.

Keywords: Alcohol, Reduced glutathione, Catalase, Superoxide dismutase, Malonaldehyde

INTRODUCTION
Alcohol is consumed worldwide as a drink by humans. Regular and unlimited use of alcohol leads to toxicity and alcohol-induced pathological problems and can constitute a menace in the society[1]. Several reports on the association between chronic alcohol consumption and variety of pathological conditions varying from simple intoxication to severe life-threateningpathological states have been reported[2-7].Alcohol is absorbed into the blood stream in the gastrointestinal tract and is primarily metabolized in the liver[8].

Alcohol affects many organs of the body including the nervous system[9]. The molecule is metabolized via two pathways: The alcohol dehydrogenase (ADH) pathway and the microsomal ethanol oxidizing system (MEOS) pathway. Both pathways generate potentially dangerous by-products such as acetaldehyde and highly reactive molecules called free radicals or reactive oxygen species (ROS) which cause oxidative stress and are capable of attacking cell membranes and biomolecules[10]. Oxidative stress occurs when the production of ROS exceeds the level the body's natural antioxidant defense mechanisms can cope with; causing damage to macromolecules such as DNA, proteins and lipids[11]. This is often characterized by high level of malondialdehyde (MDA).

High MDA level is a marker of lipid peroxidation which is a fallout of oxidative damage. Reduced glutathione (GSH) is an endogenous antioxidant which plays a vital role in the detoxification of xenobiotics and scavenging of free radicals or reactive oxygen species (ROS) in cells[12]. Superoxide Dismutase is an enzyme that catalyzes the dismutation of the superoxide anion to hydrogen peroxide, which is then decomposed by catalases primarily located in the peroxisomes[13].Catalaseis also responsible for the majority of acetaldehyde production in the brain[14,15]. Moreover, modulation of catalase levels can alter behavioral responses to ethanol, presumably by controlling levels of acetaldehyde[16-18] and influence the rate of ethanol elimination within thebrain[19].

This study was aimed at evaluating the effects of chronic alcohol administration on antioxidant and histology of cerebral cortices in Wistar rats.

Experimental Animals
Thirty-two apparently healthy Wistar rats of both sexes (Weighing 80 - 120g) were obtained from the Department of Human Anatomy, Bello University, Zaria. The animals were housed in clean environment in the animals House of the Department and fed with standard animal diet fed and water ad libitum.

Alcohol Procurement
Laboratory graded absolute ethanol manufactured by Guangdong GannghuaSci- Tech Co., Ltd, Shantoua,

Guangdong, China was purchased from a reputable chemical store, Zaria, Nigeria.

Experimental Design

Thirty-two Wistar rats used for this study were selectively categorized into four groups (Group 1, Group 2, Group 3 and Group 4) with eight animals of both sexes in each group (Table 1). The drug was administered orally through oral cannula once daily for the period of 75days.

Table 1: Experimental Design

Group	Dosage	Duration
Group 1	1 ml of Distilled Water	75 days
Group 2	0.12g/kg body weight	75 days
Group 3	0.16g/kg body weight	75 days
Group 4	0.24g/kg body weight	75 days

Biochemical assay: Wistar rats were euthanize using ketamine injection (75 mg/kgbw Intraperitoneal), Brain was removed and homogenized in pH 7.4 phosphate buffer solution (PBS) and centrifuge at 4000 rpm for 5 min and supernatant was separated for the estimation of Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondaldehyde (MDA).

Histological evaluation: Brain tissue was quickly fixed in Bouin's fluid for haematoxylin and eosin (H and E) and Golgi staining techniques.

Data analysis: Data obtained was expressed as mean ± SEM and analyzed using Statistical Package for Social Sciences (SPSS) version 20. One-way analysis of variance was used to obtained across the group. *p* value of 0.05 was considered significant.

RESULTS

Biochemical assay: No significant difference was obtained between the control and the groups administered with alcohol in all the antioxidant markers in males (table 2) and females (table 3) Wistar rats.

Table 2: Antioxidant enzymes activities in male Wistar rats as mean ± SEM

	Group A (distilled water)	Group B (0.12 g/kg ethanol)	Group C 0.16 g/kg ethanol	Group D 0.24g/kg ethanol	*F*	*p*
SOD(U/ml)	9.47 ± 2.12	9.48 ± 1.58	10.41 ±0.85	10.99 ±0.62	0.84	0.84
CAT(U/mg)	65.04 ±10.37	49.14±9.69	59.52±9.21	66.67±12.14	0.57	0.65
GSH(ug/ml)	7.75 ± 0.52	8.13 ±0.90	8.62 ± 0.34	8.35 ± 0.63	0.34	0.80
MDA(nMols/mg)	6898.53±675.19	6522.07±1669.05	4375.33±1235.88	3148.23±896.62	2.28	0.16

SOD = Superoxide dismutase, CAT = Catalase, GSH = Reduced Glutathione, MDA = Malonaldehyde

Table 3: Antioxidant enzymes activities female Wistar rats as mean ± SEM

	Group A (distilled water)	Group B (0.12g//kg ethanol)	Group C (0.16g/kg ethanol)	Group D (0.24g/kg ethanol)	*F*	*p*
SOD(U/ml)	10.53 ± 0.41	10.53 ± 0.00	11.23 ± 0.20	10.76 ±0.62	0.74	0.56
CAT(U/mg)	53.84 ± 14.20	63.51 ± 19.47	70.11 ± 13.57	69.35 ± 0.31	0.30	0.83
GSH(ug/ml)	8.94 ± 0.19	8.57 ± 0.60	9.16 ±0.33	9.65 ± 0.24	1.46	0.30
MDA(nMols/mg)	17698.57±6742.86	14624.53±3845.03	13799.53±3794.28	13430.60±4849.10	0.15	0.93

SOD = Superoxide dismutase, CAT = Catalase, GSH = Reduced Glutathione, MDA = Malonaldehyde

Histological studies: The result from the histological study reveal no histo-architecture distortion in the frontal lobe (internal Pyramidal cell layer) of the administered group compared to control in male Wistar rats while distortion in the histo-architecture in the group 4(0.24 g/kg bw of ethanol) compared to the control group in female Wistar rats.

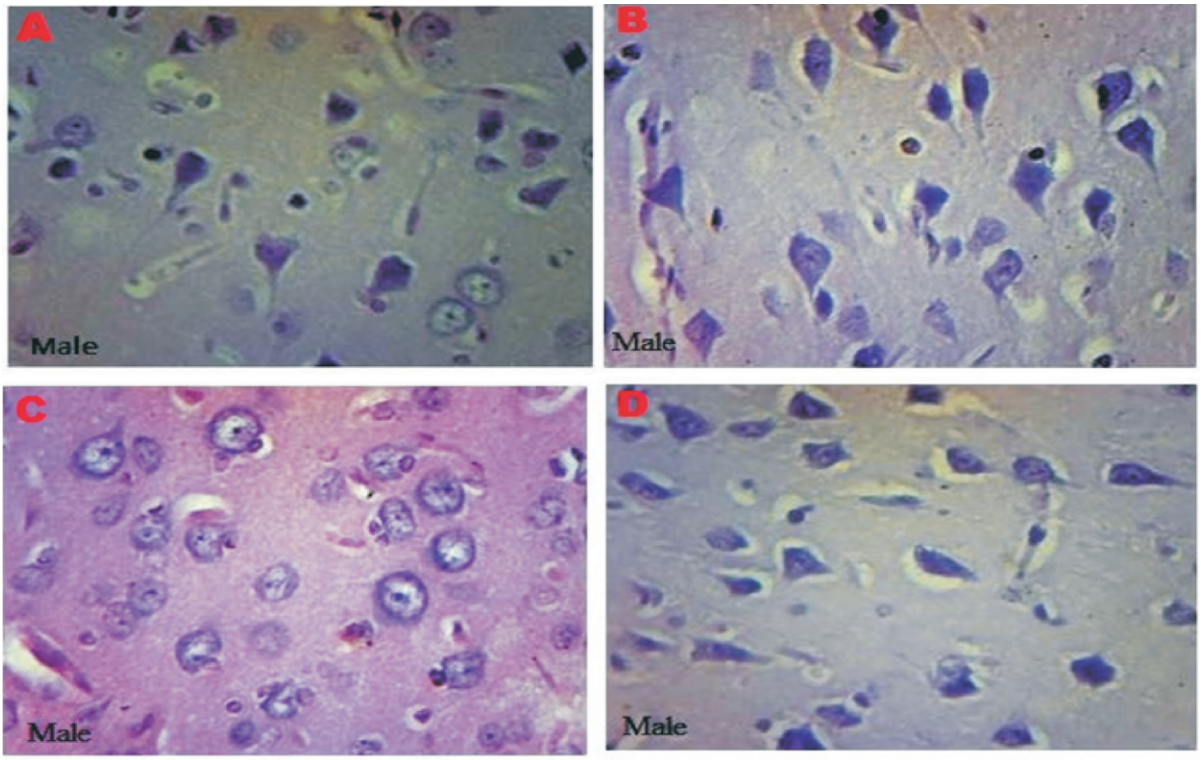

Figure1: Section of cerebrum Male Wistar rat (Group **A, B, C, D**) with normal histology of the layer V of cerebral cortices. Pyramidal Neuronal cells (P). H and E (Mag. X 250).

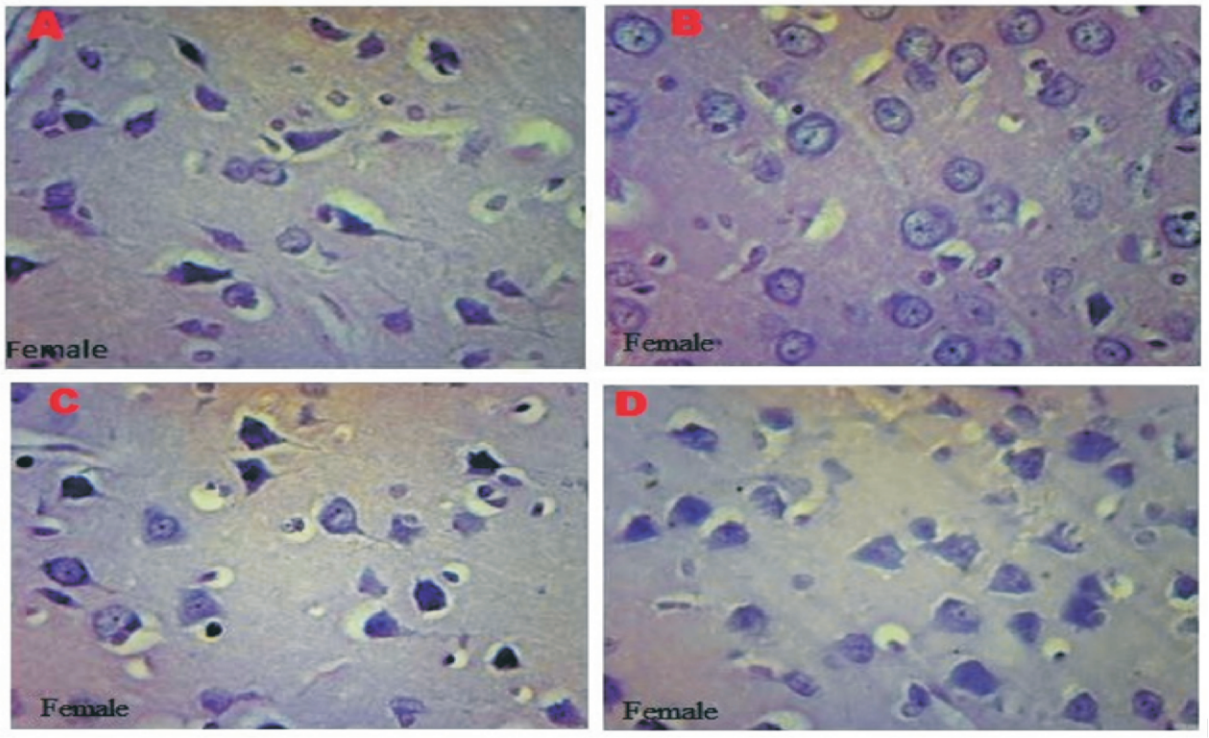

Figure2: Section of cerebrum female Wistar rat (Group **A, B, C**) with normal and distorted (Group **D**) histology of the layer V of cerebral cortices. Pyramidal Neuronal cells (P). H and E (Mag. X 250).

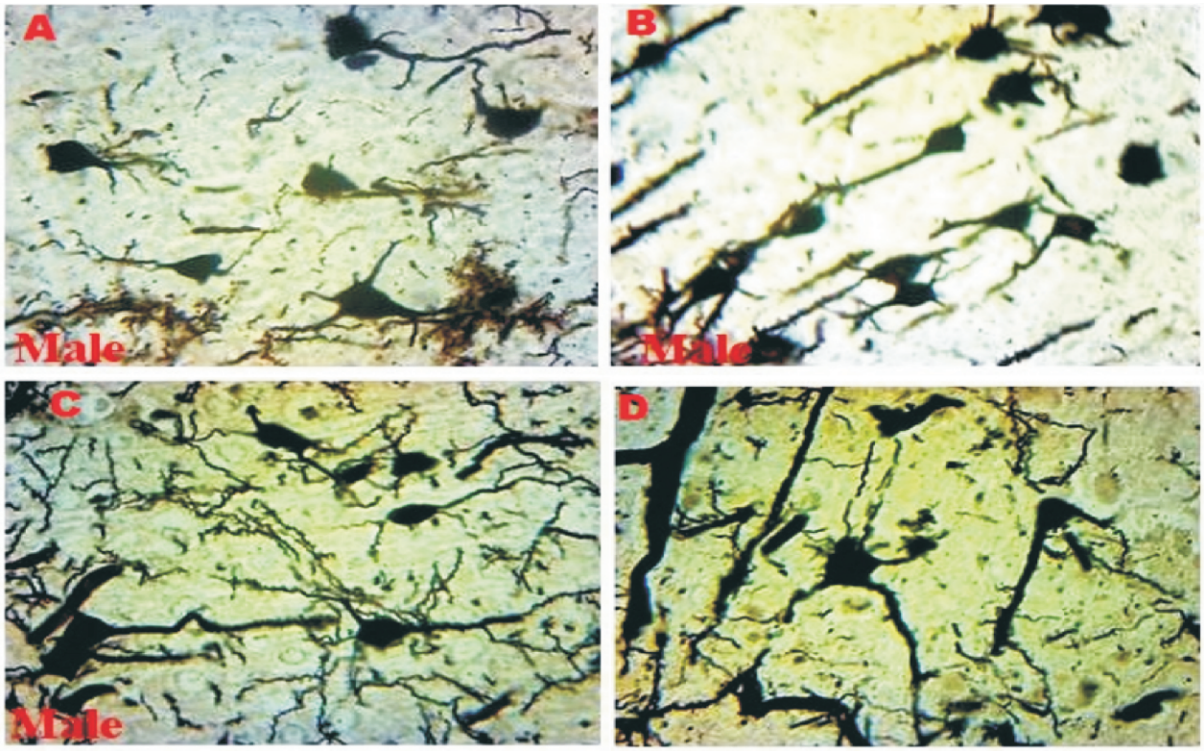

Figure3: Section of cerebrum Male Wistar rat (Group **A, B, C, D**) with normal neuronal processes of the layer V of cerebral cortices. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250).

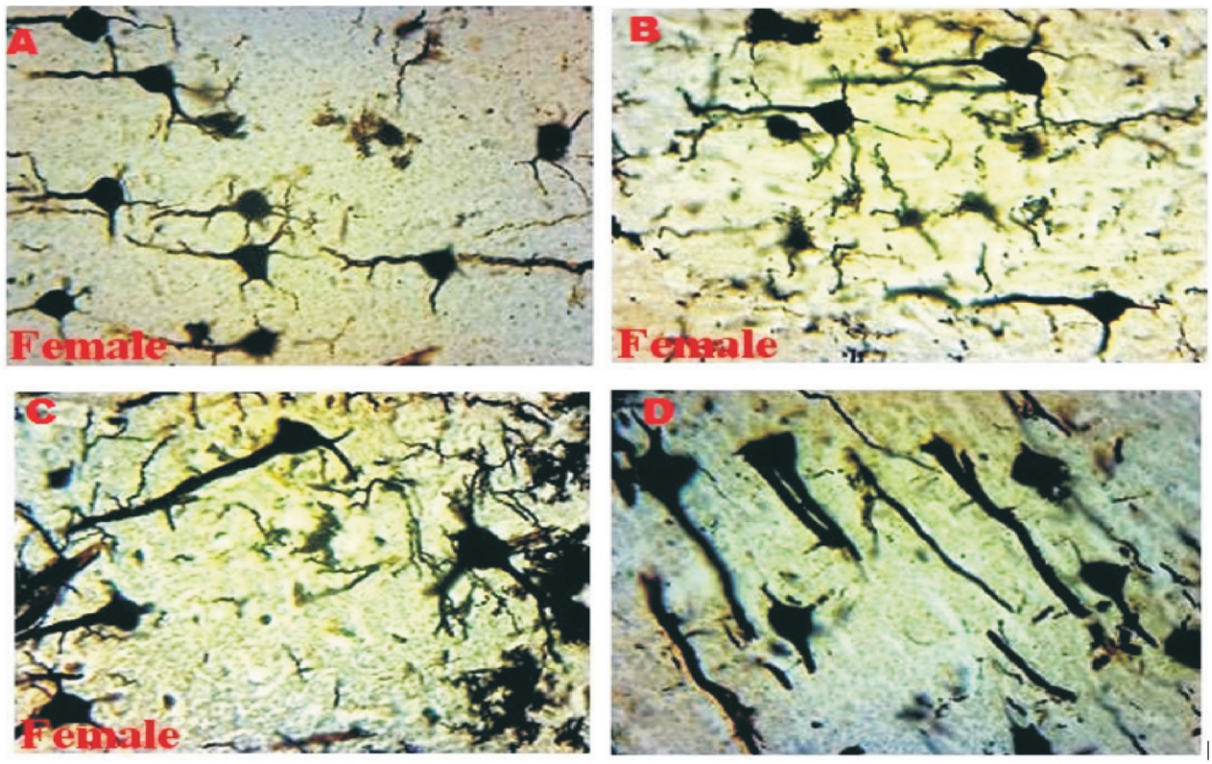

Figure4: Section of cerebrum Male Wistar rat (Group **A, B, C**) with normal and loss (Group **D**) of neuronal processes of the layer V of cerebral cortices. Pyramidal Neuronal cells (P). Golgi stain (Mag. X 250)

DISCUSSION

Superoxide dismutase (SOD) activity, catalase (CAT) activity, reduced glutathione (GSH) and Malondialdehyde (MDA); which is a make for lipid peroxidation were estimated and it was found that even though there was slight increase in the level of these antioxidant mark, the increase was not significant. The increase in the antioxidant mark indicate that metabolism of alcohol lead to more production of reactive oxygen species as reported in the literature. There is evidence that ethanol produces alterations in the mitochondrial structure and function of several organs, both in laboratory animals and humans[24]. The mitochondria play an important role in the alcohol metabolism via the enzyme ALDH; this enzyme catalyzes the conversion of acetaldehyde into acetate. When this enzyme reaches a saturation point, the acetaldehyde escapes into the blood stream and leads damage to biomolecules such lipids, proteins and nucleic acids which results of the toxic side effects that are associated with alcohol consumption[25]. These changes affect the mitochondrial function decreasing respiratory rates[12] and ATP levels, and might result in increased production of reactive oxygen species (ROS)[26]. Numerous studies show that mitochondrial levels of ROS may be increased by chronic alcohol consumption as a consequence of increased mitochondrial CYP2E1 levels[27,28] as well as a by-product of the matrix enzyme α-ketoglutarate dehydrogenase[29].The ROS generated from the metabolism is not enough to cause oxidative damage and therefore could be further use to benefit the cells, which is line with the concept mitohormetics as report, by Ristow and Schmeisser[30].

Sections of cerebral cortices of Wistar rats reveal that no histo-architecture distortion in male Wistar rats while in female Wistar rats, vacuolation and loss of neuronal dendrite in the group administered with 0.24g/kg of ethanol compared to his male counterpart and female control.

The result obtained indicated that there are gender differences in alcohol pharmacokinetics, in agreement with Mumenthaler et. al.[31] that reported that Women appear to become more impaired than men do after drinking equivalent amounts of alcohol, achieving higher blood alcohol concentrations even when doses are adjusted for body weight. This finding may be attributable in part to gender differences in total body water content. Significant gender differences in alcohol pharmacokinetics appear to include increased bioavailability and faster disappearance rates in women. Women have proportionally more body fat and less water than do men of the same body weights.

Because alcohol is dispersed in body water, women reach higher peak BACs than men do after consuming equivalent doses of alcohol, even when doses are adjusted for body weight[32,33]. Higher peak BACs in women may also reflect lower rates of FPM by gastric[32,34] or hepatic ADH[35]. The result of the male rats was also in agreement with the studies on Moderate ethanol consumption and increases hippocampal cell proliferation and neurogenesis in the adult mouse by Aberg et al.[36] and reported that Moderate ethanol consumption increased the proliferation of cells, which survived and developed a neural phenotype and ethanol consumption did not induce apoptosis, neither did it change differentiation nor the distribution patterns of the newly formed cells. The cell proliferation rate in the dentate gyrus returned to basal levels 3 d after ethanol withdrawal. Moreover, concluded that voluntary ethanol intake by mice can change the rate of cell proliferation in the dentate gyrus. Since there was no increase in apoptosis concomitant with the ethanol-induced increase in neurogenesis, it is possible that the new cells in the dentate gyrus will contribute to the long-lasting changes of brain function after ethanol consumption.

CONCLUSION

Chronic daily oral administration of lose dose of alcohol does not significantly increase the production of reactive oxygen species which can cause oxidative damage in male and female Wistar rats. It also causes loss of neuronal processes in the cerebral cortices of female Wistar.

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