International Journal of Biomedical and Health Sciences
Vol. 17, No.1, March 31, 2021
Printed in Nigeria

0794-4748/2021 $5.00 + 0.00

http://journals.niseb.org.ng

IJBHS 2020070/17102

Curcumin ameliorates alcohol-induced impaired locomotive activity of male mice

Lekan Sheriff Ojulari[1], Aisha Abubakar[2], Adam Moyosore Afodun[3] and Adaobi Geraldine Okudo[1]

[1]Department of Physiology, University of Ilorin, Ilorin, Nigeria [2]Physiology, University of Maiduguri, Maiduguri, Nigeria
[3]Anatomy and Cell Biology, Busitema University, Uganda

Corresponding author: Ojulari Lekan Sheriff email: ojularis@yahoo.com.+23403242067.

(Received November 16, 2020; Accepted January 4, 2021)

ABSTRACT**:** Acute alcohol intoxication results in diminished motor performance. Curcumin is the active ingredient in turmeric, a medicinal plant used as a food additive and preservative in Asian countries. This study examined the effects of curcumin on the locomotive activity of intoxicated male mice. 30 mice were divided into 3 groups and used in this experiment. Curcumin was administered for 14 days, followed by intoxication with 50% ethanol of 0.1 ml/kg and locomotive activity was assessed. Lipid peroxidation and antioxidant enzymes activity were also assessed from the brain tissue following assessment of locomotive activities. Curcumin increased ($p<0.05$) the time spent on the linear wire and caused a significant decrease ($p<0.05$) in number of times the mice attempted to reach their tail. Beam walk time following use also showed a significant decrease ($p < 0.05$) when compared to both positive and negative Control groups. Motor activities were also improved with decreased rearing time following. Antioxidant enzymes catalase and glutathione peroxidase showed significant ($p<0.05$) increase in activity following curcumin administration when compared to the negative control. This study showed considerable evidence that short-term curcumin supplementation can exert anti-intoxication effects.

Keywords: Curcumin, Alcohol Intoxication, locomotive activity, antioxidant

Introduction

Alcohol is the most common drug used among adults in the United States. Alcohol intoxication is a physiological state induced by the consumption of alcohol. The use of alcohol is associated with an increased risk of injuries and accidents (1). Alcohol is one of the most important personal risk factors for serious and fatal injuries, contributing to approximately one third of all deaths from accidents and it is also described that alcohol intoxication leads to a higher mortality in the clinical course (2).

Alcohol rapidly crosses cell membranes, resulting in ready equilibrium between intra and extracellular concentrations. Some of the signs that develop during or shortly after alcohol use include slurred speech, lack of coordination, unsteady gait, nystagmus, impairment of attention or memory and

stupor or coma (3). It also prevents muscle function by impairing the excitation/ contraction coupling because of the inhibition of calcium channels in the sarcolemma (4).

Curcuma longa or turmeric is a medicinal plant widely used and cultivated in tropical regions and Curcumin has been identified as its active principle (5 6). Curcumin may cross the blood–brain barrier and enter the central nervous system (7), a property that will support the use of curcumin as a therapeutic agent for neurodegenerative disorders. Curcumin appears to be nontoxic or it possess low toxicity, as doses as high as 12,000 mg have shown very little or no toxic effect (8) and its efficacy may be provided by its antioxidant or its free-radical scavenger action that offers neuroprotection. Since oxidative stress is one of the mechanisms through alcohol causes neurodegeneration, this study therefore examines the effect of curcumin on the locomotive activities of intoxicated male mice.

Materials and Methods

Chemicals and reagents:

Curcumin was purchased from Sigma-Aldrich. The ethanol and olive oil were purchased from Hamme-Olu Pharmacy in Ilorin, Kwara State, Nigeria.

Animal preparation:

Thirty (30) male mice with average weight of 200-220g were obtained from the animal house of The University of Ilorin, Ilorin, Nigeria, and were used for this experiment. The animals were kept in well ventilated plastic cages at room temperature. They were acclimatized for two weeks and provided with feed and water *ad libitum* throughout the experimental period**.** This work was ethically approved by the ethical approval committee, Department of Physiology, University of Ilorin, Ilorin, Nigeria.

Experimental design:

Thirty mice, grouped randomly into 3 groups of 10 animals each, were used for this study.

GROUP 1: Positive control, received normal saline daily through oral gavage for 14days.
GROUP 2: Negative control, received olive oil daily through oral gavage for 14days.
GROUP 3: Curcumin treated, receive 70 mg/kg of Curcumin daily through oral gavage for 14days.

Alcohol intoxication:

The mice were intoxicated on the 14th day, 30mins after the Curcumin and olive oil treatment. Groups 2 and 3 were intoxicated with 0.1 ml/10kg of 50% of ethanol. Group 1 was not intoxicated.

Assessment of locomotive activities:

Grip test:

The wire hang test was used to assess muscle performance in mice. The apparatus consists of a simple standard linear wire of 2.5 mm diameter wire was suspended on the top of a bench top held on both ends by a retort stand. The camera was positioned above the wire, and the recordings were initiated. Each mouse was placed in the center of the wire with only their forepaws, and a timer was set. The time taken for the mouse fall or crawl along the wire to reach the end was recorded.

Tail suspension test:

The tail suspension test was used to screen novel antidepressant or depression inducing treatment and procedures. A hook setup was suspended from a bar approximately 30 cm above the bench top. Adhesive tape was wrapped around the mouse's tail in a constant position three- quarters of the distance from the base of the tail. The recording was initiated and the mouse was then suspended on the

suspension hook through the adhesive tape close to the tail to ensure the mouse hangs in a straight line. The recording lasted for 10 minutes. The number of times the attempt to reach its tail was recorded.

Beam walking test:

The beam walking (BW) test was used to evaluate motor coordination, integration, balance performance and motor skills. The beam walking apparatus consisted of rectangular shaped base 100 cm long narrow wooden Beam (1-2 cm wide) suspended from its end at an elevation of 30 cm. A carton was placed underneath the beam walk apparatus to serve as cushion when the animal falls. The recording was initiated; the mouse was placed gently in the center of the beam, facing one of the ends. The mouse was allowed to walk to the end of the beam, and the time and speed was recorded.

Determination of lipid peroxidation and antioxidant parameters:

Lipid Peroxidation: After the 2-week treatment period, and completion of other physical evaluation tests, mice were anaesthetized using isoflurane and sacrificed by decapitation. Brains were quickly removed, cut along the midline, and a 10% homogenate was prepared in 100 mM Tris HCl (pH 7.4) using a Potter-Elvehjem homogenizer and used for the estimation of biochemical parameters.

Lipid peroxidation was estimated in brain tissue homogenate by the method of Hogberg *et al*. (9) using thiobarbituric acid. The release of malondialdehyde as an end product of peroxidation of lipids served as the index of the intensity of oxidative stress.

Enzymatic Antioxidants*:* Antioxidant enzymes were estimated in brain tissue homogenate of experimental groups. Superoxide dismutase (SOD) was assayed using the kit (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturers' standard procedures. Catalase activity was assayed by the method of Sinha (10). Glutathione peroxidase (GPX) was assayed by the method of Rotruck *et al*. (11). The utilization of glutathione was used to express the activity. Glutathione reductase (GR), that utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form was measured by the method of Staal *et al*. (12). The activity of glucose-6-phosphate dehydrogenase (G6PDH) was assayed by the method of Ells and Kirkman (13).

Statistical analysis:

Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a *post hoc* test according to the statistical package program (SPSS version 17.0). All values $p < 0.05$ was considered as significant for all statistical analysis in this study.

Results

Grip test: The grip test assayed for motor strength. The results showed a significant decrease ($p < 0.05$) in grip time for group 2 (17.00 ± 5.51 sec) when compared to group 1 (321.33 ± 9.96 sec) and. Group pretreated with curcumin (125.33 ± 8.2 sec) showed a significant increase ($p < 0.05$) in time spent on the linear wire as compared to groups 2

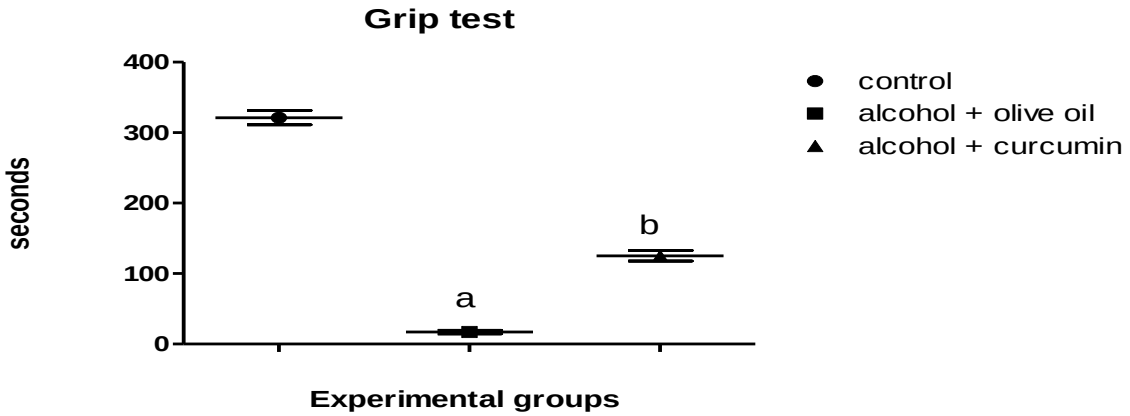

Fig 1: Effects of curcumin on the grip test of intoxicated male mice.
[a]Significant difference when compared to control at $p<0.05$. [b]Significant difference when compared to olive oil treated group at $p<0.05$.

Tail suspension test: Under the tail suspension test, there was a significant decrease ($p < 0.05$) in number of times the mice attempted to reach their tail in group 3 (1.76 ± 0.23 sec) as compared to groups 1 (6.00 ± 0.8 sec) and 2 (20.67 ± 1.06 sec) respectively

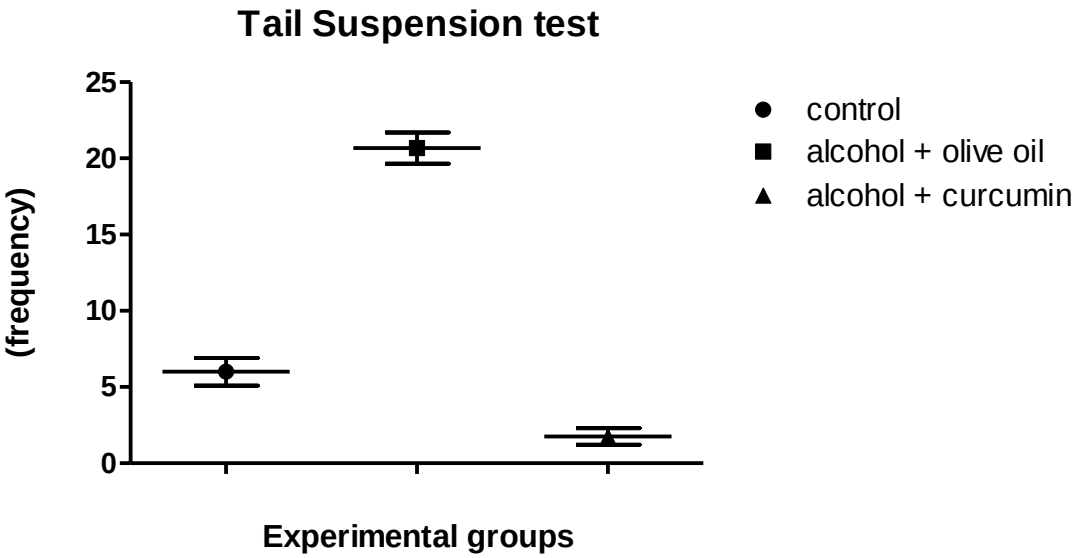

Fig 2: Effect of curcumin on the number of times the mice attempts to reach their tail following alcohol intoxication.

Beam walk: Motor coordination was evaluated using a stationary beam test. Analysis of beam walk time in group 3 (12.67 ± 1.76 sec) showed a significant decreased ($p < 0.05$) as compared to control groups 1 and 2 (125.67 ± 4.70 sec; 57.00 ± 5 sec) respectively. The speed time in group 5 (34.87 ± 2.20 cm/s) showed a significant increase ($p < 0.05$) in comparison to group 4 (5.00 ± 0.25 cm/s). Group 1 recorded the least speed time (0.47±0.01sec)

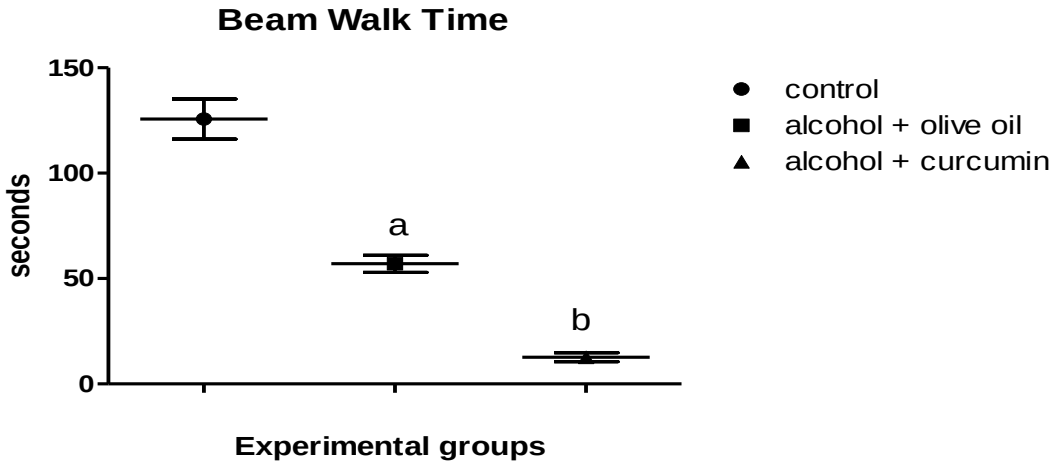

Fig 3: Effect of curcumin on the beam walk time of intoxicated male mice.
[a]Significant difference when compared to control at $p<0.05$. [b]Significant difference when compared to olive oil treated group at $p<0.05$

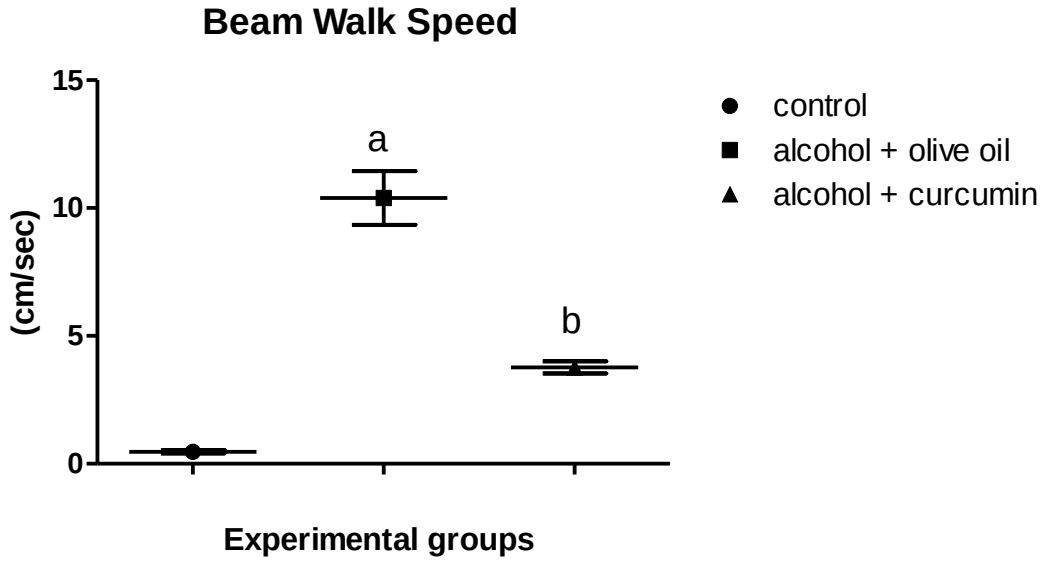

Fig 4: the effect of curcumin on the beam walk speed of intoxicated male mice.
[a]Significant difference when compared to control at $p<0.05$. [b]Significant difference when compared to olive oil treated group at $p<0.05$

Lipid peroxidation and antioxidant enzyme activity following alcohol intoxication

MDA: The effect of curcumin on the level of MDA in the brain of control and experimental rats is expressed in Fig 5. As shown in Fig 40, the MDA level was 6.83±0.6 in animals in the control group. There was an insignificant increase ($p>0.05$) in MDA level following intoxication and treatment with olive oil (7.8±0.9) and curcumin (7.2±0.49).

a
a c

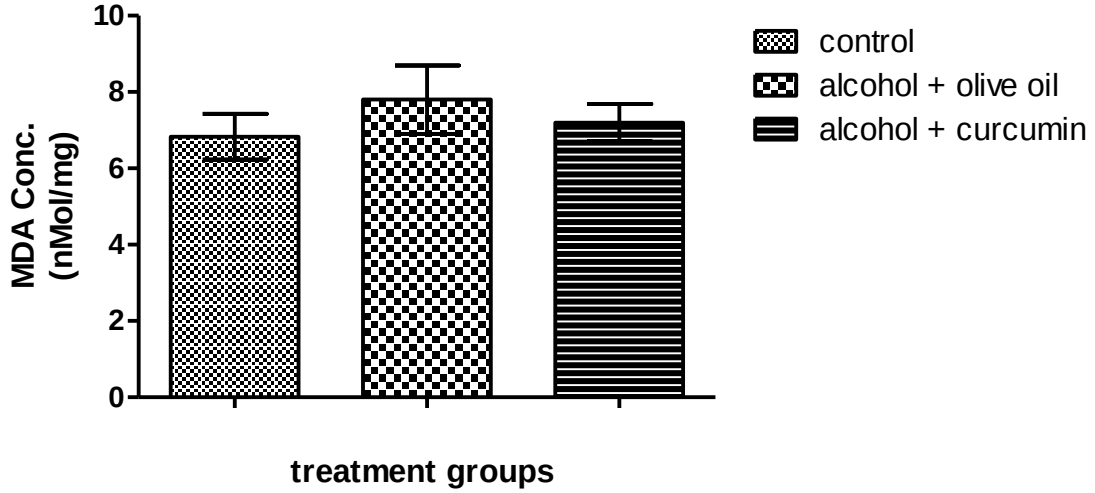

Fig 5: Concentration of malondialdehyde in the brain of curcumin treated rats.
There was no significant difference ($p>0.05$) when experimental groups were compared to control.
MDA- malondialdehyde

SOD activity: Fig 6 shows the effect of curcumin on the activity of SOD in the brain following alcohol-induced intoxication. No significant ($p>0.05$) difference was observed in the SOD activity in experimental groups (II-5.98±0.21 and III- 6.29±0.24) when compared to animals in the control group (I-5.35±0.18).

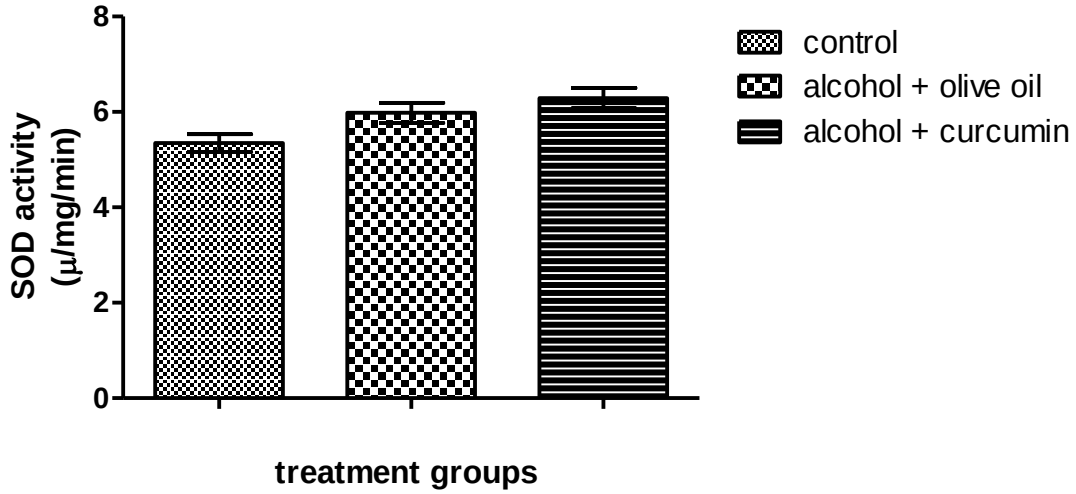

Fig 6: Activity of superoxide dismutase in the brain following treatment with curcumin.
There was no significant difference ($p>0.05$) when the experimental groups were compared to control.
SOD- superoxide dismutase

CAT activity: Fig. 7 shows the effect of curcumin on the level of CAT activity in the brain alcohol-induced intoxicated rats. All experimental groups (II- 1.97±0.49 and III- 3.46±0.20) showed a significant ($p<0.05$) decrease in CAT activity when compared to control (group I-

4.80±0.18). However, the curcumin treated animals showed a significant ($p<0.05$) increase in CAT activity when compared to the olive oil treated intoxicated animals.

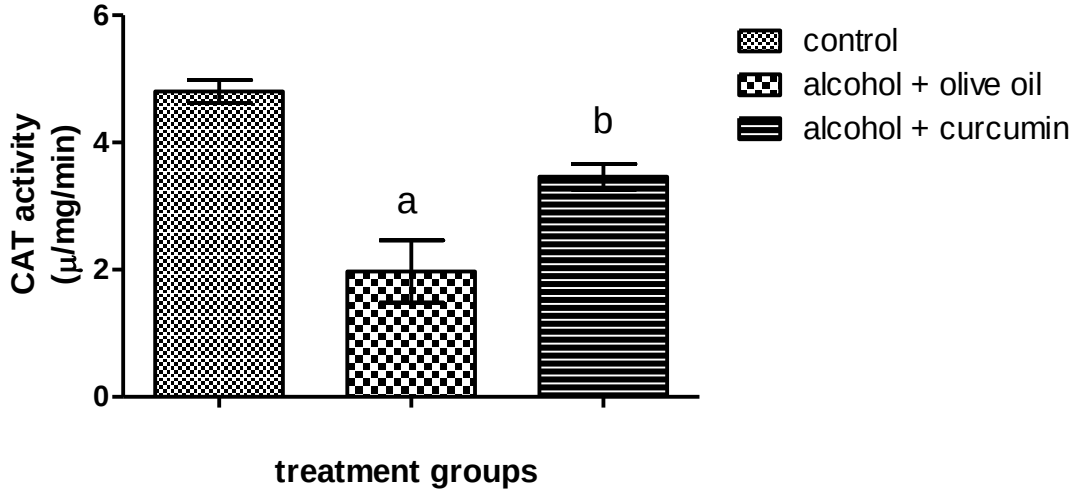

Fig 7: Activity of catalase in the brain following treatment with curcumin.
[a]Significant difference when compared to control at $p<0.05$. [b] Significant difference when compared to olive oil treated group at $p<0.05$. **CAT**-catalase.

GPx activity: Fig 8 shows the effect of curcumin on the activity of GPx in the brain following alcohol intoxication. Significantly ($p<0.05$) reduced levels of GPx activity were recorded in the animals following intoxication (II- 4.96±0.78 and III- 6.96±0.30) groups when compared to control (I- 8.81±0.59). Difference in the level of GPx activity in was significant ($p<0.05$) between animals in the intoxicated groups.

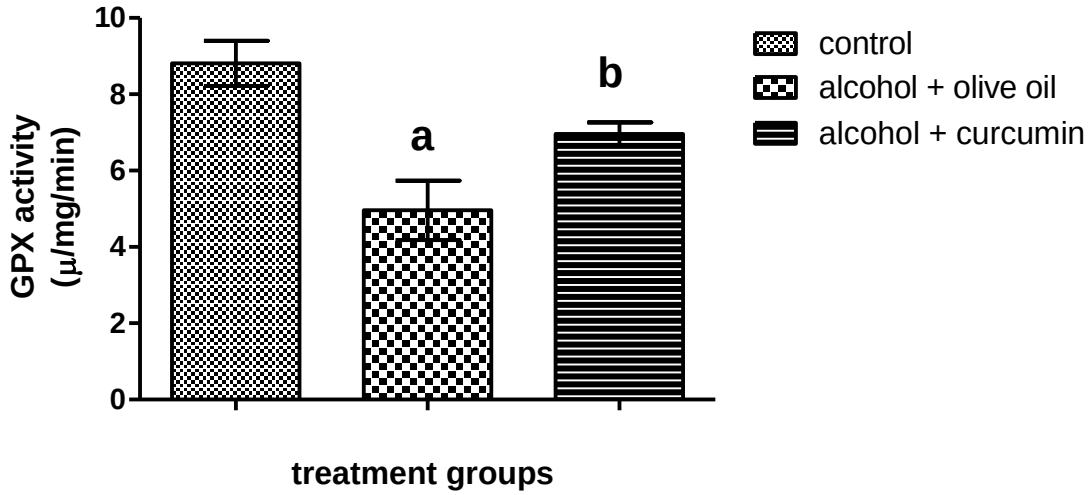

Fig 8: Activity of glutathione peroxidase in the brain of alcohol-induced intoxicated rats.
[a]Significant difference when compared to control at $p<0.05$. [b]Significant difference when compared to olive oil treated group at $p<0.05$. **GPx**- glutathione peroxidase.

Discussion

Alcohol intoxication has been well documented and it remains one of the world's most widespread recreational drugs. In general, alcohol intoxication affects physiological processes such as memory, cognitive abilities and motor functions.

Following successful induction of alcohol intoxication in rat models, this study showed significant increase in forepaw grip following treatment with curcumin, reflecting an increase in forepaw strength. This reflects the overall performance and health of the musculoskeletal system and can also be used to evaluate the motor skill associated coordination. This corresponds to studies by Wen-Chun-Hung *et al* (14), who reported that the grip strength could be dose-dependently increased with curcumin supplementation. Findings by Lam *et al.* (15) strongly contradict ours; they reported that curcumin inhibits skeletal muscle force production most likely through inhibitory effects of excitation-contraction coupling upstream from the sarcoplasmic reticulum.

Beam walk test assayed for muscular coordination and balance. It is an integrated form of behaviour requiring pertinent level of consciousness, memory, sensorimotor and cortical functions mediated by the cortical areas (16). The loss of equilibrium followed by erratic movements was observed together with imbalanced body activity and muscular incoordination was seen following 0.1 ml/10kg of 50% of ethanol within 30 min after administration. The progressive increase in which the mice slipped off the beam indicates impairment of motor co-ordination. This could be as a result of the widespread effects of alcohol as it travels through the body, reaching every organ including the brain which controls motor functions (17).

Tail suspension test was used to subject mice to an inescapable aversion situation whereby failure to exhibit actions aimed at escape represented an effective coping strategy in a despaired situation (18) induced by intoxication. Following treatment with curcumin, animals assumed an immobile posture sooner than their counterparts. This suggested an improved tail suspension test and significant antidepressant effects of treatment with curcumin.

The excessive intake of alcohol causes damage which is related to ethanol oxidation in the brain. The metabolism of ethanol to acetaldehyde and then to acetate is associated with the reduction of reactive oxygen species that accelerate the oxidative state of cells. This metabolism of ethanol can induce the oxidation of fatty acids in phospholipids and the bioactive aldehyde produced is known to be associated with neurotoxicity and neurodegeneration (19). The cellular antioxidant defence mechanism against reactive oxygen species includes enzymatic defence systems such as SOD, catalase, and GPX. SOD converts the superoxide radical to H_2O_2, which, in turn, is further eliminated by catalase and GPX. A significant rise was also recorded in the level of CAT and GPx in the curcumin treated intoxicated rats. The combined effect of an elevation in the levels of both CAT and GPx would lead to an increase in intracellular antioxidant defence with a subsequent reduction in molecular damage caused by free radicals. These would suggest curcumin ameliorates oxidative stress and could improve damage caused by ethanol oxidation to the brain; this supports the popular notion of dietary supplements that possess antioxidant properties may be favourable in maintaining brain function (20-23).

Conclusion

Here we found that curcumin supplementation had significant benefits for physiological indicators after alcohol intoxication via improved locomotor activities. Curcumin supplementation may have a wide spectrum of bioactivities, and could help ameliorate alcohol-induced impaired motor and muscular coordination and promote health safety.

References

1. Moss HB. The impact of alcohol on society: a brief overview. *Social Work Public Health* 2013; 28 (3-4): 175-7.
2. Stübig T, Petri M, Zeckey C, Brand S, Müller C, Otte D, *et al*. Alcohol intoxication in road traffic accidents leads to higher impact speed difference, higher ISS and MAIS, and higher preclinical mortality. *Alcohol* 2012; 46 (7): 681-6.
3. Addolorato G, Leggio L, Ferrulli A, Cardone S, Vonghia L, Mirijello A, *et al*. Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol-dependent patients with liver cirrhosis: randomised, double-bind controlled study. *Lancet* 2007; 370 (9603): 1915- 1922
4. Pesta HD, Angadi SS, Burtscher M and Roberts KC. The effects of caffeine, nicotine, ethanol, and tetrahydrocannabinol on exercise performance. *Nutrition and metabolism* 2013; 10 (1): 71.
5. Aggarwal BB, Kumar A, and Bharti AC. Anticancerpotential of curcumin: preclinical and clinical studies. *Anticancer Res* 2003; 23 (1A): 363-98.
6. Aggarwal BB, Sundaram C, Malani N and Ichi-kwara, H. Curcumin: the Indian solid gold. *Advances in Experimental Medical Biology* 2007; 595: 1-75.
7. Tayyem RF, Heath DD, Al-Delaimy WK and Rock CL. Curcumin content of turmeric and curry powders. *Nutri Cancer* 2003; 55 (2): 126-31.
8. Muthian G, Mackey V, Prasad K and Charlton, C. Curcumin and an antioxidant formulation protect C57BL/6J mice from MPTP- induced Parkinson's disease like changes: potential neuroprotection for neurodegeneration. *Dove press 2018;* 8: 49-59.
9. Högberg J, Larson RE, Kristoferson A and Orrenius S. NADPH-dependent reductase solubilized from microsomes by peroxidation and its activity. *Biochem Biophys Res Commun*. 1974; 56 (3):836-42.
10. Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1972; 47(2):389-94.
11. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra DG, "Selenium: biochemical role as a component of glutathione peroxidase," *Science* 1973; 179, (4073): 588–590.
12. Staal GEJ, Visser J, and Veeger C, "Purification and properties of glutathione reductase of human erythrocytes," *Biochimica et. Biophysica Acta* 1969; 185 (1): 39–48.
13. Ells HA and Kirkman HN, "A colorimetric method for assay of erythrocytic glucose-6-phosphate dehydrogenase," *Proceedings of the Society for Experimental Biology and Medicine* 1961; 106 (3): 607–609
14. Wen CH, Chiu W, Chuang H, Tang D, Lee Z, Wei L. Effect of Curcumin Supplementation on Physiological Fatigue and Physical Performance in Mice. *Nutrients* 2015; 7(2): 905–921.
15. Lam J, Pinniger GJ and Bakker AJ. Curcumin inhibits skeletal muscle force production, most likely through inhibitory effects on aspects of E-C coupling upstream from the SR. *Proceedings of the Australian Physiological Society* 2016; http://aups.org.au/proceedings/47/5P
16. Delatour B, Faure A, and Le Cudennec C. One-Year longitudinal evaluation of sensorimotor functions in $APP_{751}SL$ transgenic mice Genes. *Brain and Behaviour 2008*; 7 (1): 83-91.
17. Acevedo MB, Molina JC, Nizhnikov ME, Pautassi RM, and Spear NE. Ethanol induced locomotor activity in adolescent rats and their relationship with ethanol induced conditioned place preference aand conditioned taste aversion. *Dev Psychobiol 2013;* 55(4): 429-442.
18. Engidawork E, Fekadu N, and Shibeshi W. Evaluation of the antidepressant like activity of the crude extract and solvent fractions of Rosa Abyssinica Lindley (Rosaceae) using rodent models of depression. *Journal of clinical and experimental pharmacology* 2016; 3: 6.
19. Hernandez JA, Sanchez RL and Ramirez AR. Lipid and oxidative stress associated with ethanol-induced neurological damage. *Oxidative Medicine and Cellular Longevity* 2016 15p
20. Liu R, Liu IY, Bi X, *et al*. Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100 (14): 8526-8531
21. Sun AY, Chen YM, James-Kracke M, Wixom P and Cheng Y. Ethanol-induced cell death by lipid peroxidation in PC12 cells. *Neurochemical Research* 1997; 22 (10): 1187–1192.
22. Bruce-Keller AJ, White CL, Gupta S, Knight AG, Pistell PJ, Ingram DK, Morrison CD and Keller JN. NOX activity in brain aging: Exacerbation by high fat diet. *Free Radical Biology and Medicine* 2010; 49 (1): 22-30
23. Gemma C, Vila J, Bachstetter A and Bickford PC. Oxidative stress and the aging brain: from theory to prevention," in *Brain Aging: Models, Methods, and Mechanisms* 2007, D. R. Riddle, Ed., Chapter 15, CRC Press, Boca Raton, Fla, USA.