Bioscene

Bioscene
Volume- 21 Number- 03
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

The Effects of Aqueous Extract of Alligator Pepper (Aframomummelegueta) on Alcohol Induced Motor Coordination Impairment in CD1 Mice

[*]Johnbull Martins Uket[1], Nkwa Oge[2] and Christiana Godwin Gekpe[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Nigeria

[2]Department of physiology, faculty of medical sciences, Abia state university, Uturu, Nigeria

* Correspondence Author: **Johnbull Martins Uket**

Abstract: There is a traditional claim that, Alligator pepper(Aframomummelegueta) a member of the ginger family can ameliorate alcohol intoxication. Aframomummeleguetacontain quercetin a form of antioxidant, gingerol and essential amino acids (tryptophan) a precursor of serotoninwhich had been known to affect neurobehavioral functions. However, whether administration of Aframomummeleguetaextract which contains quercetin, gingerol and tryptophancan affect neurobehavior notably, motor coordination and balance in CD1 mice has not been previously ascertained. Therefore, the effects of aqueous extract of alligator pepper (Aframomummelegueta) on alcohol induced motor coordination impairment in CD1 micewas studied. Twenty (20) experimental mice were randomly assigned into 4 groups namely; control, Aframomummelegueta only, Aframomummelegueta + ethanoland ethanol only groups. Beam walking apparatus was used to assess motor coordination and balance. Results showed that, group of mice treated with Aframomummelegueta extracts had improved motor coordination and balance compared ethanol only treated group ($p<0.05$). In conclusion, administration of Aframomummelegueta extract improved motor coordination and balance in CD1 mice. The improved motor coordination and balance effects observed may be attributed to quercetin, gingerol and tryptophan present in Aframomummelegueta.

Keywords: Aframomummelegueta, ethanol, tryptophan, gingerol, quercetin and motor coordination.

Introduction

Movement is an essential key componentfor the survival of every organism. The nervous system (NS) coordinates all movement of the organism directionally and regulated [1]. This coordinated movement is term motor coordination and balance. Motor coordination is the ability to execute smooth, accurate, controlled motor responses [2]. The neural centers that control and coordinate motor functions include: the motor cortex, basal ganglia, cerebellum and the anterior motor neurons in the spinal cord [3]. According to Adam Fitchett (2019),the loss of motorcoordination in humans is associated with a range of important diseases, including ataxia, Parkinson's Disease and motor neuron disease

[1].Model equation studies revealed that, motor coordination deficit affects emotional functioning through self-perceptions [4].In a survey, the financial burden of those living with coordination disorders is estimated at £700 in 6 months [5].

Alligator Pepper (Aframomummelegueta),is a member of the ginger family (Zingiberaceae) native to West Africa, particularly Nigeria, Ghana, and Cameroon [6]. Historically,Aframomummelegueta(AM) has been used in culinary practices, religious rituals, and traditional medicine for its various medicinal properties. In African folk medicine, AM species are used for alleviating stomach ache and diarrhea as well as hypertension, as an aphrodisiac, and against measles and leprosy [7]. There is a traditional claim that, consumption of AM before and during alcohol intake prevent intoxication. Studies showed that AM contain the following phytochemicals saponins, tannins, Alkaloids, Flavonoids and Phenols. It also contained carbohydrates, proteins and fat [8]. Further research revealed the presence of gingerol, paradol, shogaol[9]. The seed of AM contains various vitamins like: vitamin C, B(1,2,3,5,6&9) and others like tyrosine, phenylalanine and tryptophan[10].

Alcohol, sometimes referred to by the chemical name ‘‘ethanol’’, is one of the most widely used and abused psychoactive drugs in the world and falls under the central nervous system (CNS) depressantscategory [11]. According to World Health Organization (WHO) Alcohol is classified as a toxic, psychoactive, dependence-producing, and carcinogenic substance [12].Scientificallyalcohol is classified as a drug, whose effects has been linked to greater social harm than most illegal drugs [13]. Alcohol is found in fermentedbeverages such as beer, fruit wine, palm wine and distilled ethanol referred to asrectified spirit, and serves various purposes; it is used as a recreational refreshment or entertainment during events such as burial, wedding, naming ceremonies etc. It is also frequently involved in alcohol-related crimes such as drunk driving, public intoxication, and underage drinking. Most traditional religions in Africa incorporate the use of alcohol for their divinations [14].Alcohol consumption has short term and long term adverse effects onmost system especially the CNS. According toWHO 2024 report, the debilitating consequences of alcohol consumption had resulted in about 2.6 million deaths annually, accounting for 4.7% of all global deaths [15].The Short-term effectsfrom moderate consumption include relaxation, decreased social inhibition, and happiness while binge drinking may result in generalized impairment of neurocognitive function, blackout, and hangover. Excessive alcohol intake causes alcohol intoxication characterized by unconsciousness or, in severe cases, death.Long-term effects are considered to be a major global public health issue and includes alcoholism, abuse,withdrawal, fetal alcohol spectrum disorder (FASD), liver disease, hepatitis, cardiovascular disease (such as cardiomyopathy), polyneuropathy, hallucinosis, long-term impact on the brain (such as brain damage and dementia), and cancers such as breast cancer and head and neck cancer (especially laryngeal cancer). Alcohol works in the brain primarily by increasing the effects of γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain; by facilitating GABA's actions, alcohol suppresses the activity of the CNS [16].
Considering the debilitating consequences of alcohol consumptionglobally, there is need to search for natural remedies that are cheaper, safer and effective pharmacologically to

ameliorate the adverse effects of alcoholism. According to WHO statistical report, published in The LancetPublic Health in April 2023 that "there is no safe amount of alcohol consume that does not affect health" [17]. 80% of the world's population presently uses traditional medicine for some aspects of primary health care including mental health [18]. Therefore, natural products may provide a new source of beneficial neuropsychotropic drugs provided they had been scientifically validated and their mechanisms properly established.

Since there is a traditional claim that, consumption of AM before and during alcohol intake prevent intoxication. Following the traditional claims and the presence of phytochemicals saponins, tannins, Alkaloids, Flavonoids and Phenols as well as carbohydrates, proteins and fat [8] in AM that could help in building andstrengthening of body cells. it is, therefore, evident that, the consumption of AMcould improve motor coordination. Therefore, this study is to investigate the effects of alligator pepper (aframomummelegueta) extract on alcohol induced motor coordination impairment in CD1 mice

Materials and Methods

Preparation of alligator extract: The Alligator pepper pods were bought from Watt market (a local market in Calabar, Cross River State). The seeds were extracted and blended with an electric blender. 531g of the blended sample of Alligator pepper were dissolved in 1200ml of distilled water. The suspension was agitated with electric blender for about 10 minutes then allowed to stand for about 24 hours. Later the suspension was filtered with a chess- cloth and finally with what-man filters paper (No. 4). The filtrate was evaporated at 40°c temperature in an oven for complete dryness. The dried sample yielded8.4g of the crude extract that were encapsulated into a sample bottle using a spatula and stored in a refrigerator.

Experimental animals and design: Twenty (20) experimental adults CD1 mice both male and female sexes weighing 16 – 25g body weight were obtained from the animal house of Physiology Department, faculty of basic medical sciences, University of Calabar, Nigeria., were used for this study. The animals were assigned randomly into four (4) experimental groups of 5 mice in each group namely; control, AM only, AM plus ethanol and ethanol only groups. The control group was constituted by 3 males and 2 female mice, the AM only group was constituted by 3 males and 2 females, the AM plus ethanol group was constituted by 3 males and 2 females and the ethanol only group was constituted by 3 males and 2 females. The animals were housed individually in plastic cages with wired screen top illuminated on a 12-hour light-dark cycle. The animals were allowed access to clean water and food ad libitum. The animals were allowed to acclimatize for 72 hours. Approval for the use of laboratory animals was obtained from the faculty of basic medical sciences, college of medicine Ethical Committee of University of Calabar, Nigeria on the use of experimental animals and it was in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guide lines (EEC Directive of 1986; 86/609/EEC).

The mice in the control group were fed with normal rodent chow and administration of normal saline (1.7g/kg bodyweight intraperitoneally) according to the method of Berry and Matthews (2024) [19]., The AMonly group were administered AM extract (4.0g/kg bodyweight orally) and normal saline (1.7g/kg bodyweight intraperitoneally),the AM plus ethanol group were administered AM extract (4.0g/kg bodyweight orally) and 95% ethanol (2.25g/kg bodyweight intraperitoneally), The ethanol only group were fed normal rodent chow and administered 95% ethanol (2.25g/kg bodyweight intraperitoneally). All the experimental animals in the four groups were allowed access to free water and foodad libitum. The treatment was done for 7 days before laboratory test was done to assess motor coordination and balance.

Behavioural Protocols

Beam walking for determination of motor coordination: motor coordination was assessed using the beam walking test. The beam walking apparatus was used to test motor coordination and balance. The beam walking test is more sensitive than the mouse rotarod in determining motor coordination deficits [20].The beam has a length of 100 cm, a width of 2 cm and is elevated to a height of 40 cm. The beam is marked at 5 cm and 1 cm intervals. It is composed of wood and is coated with black paint. The animals were carried to the test room in their home cage. The mouse was removed from its home cage and placed at one end of the balance beam. After the mouse had secured its grip on the beam, the trial began. The maximum length of the trial is two minutes. The mouse was tested under white light, during the dark phase. The beam was cleaned with 70% ethanol and permitted to dry between each trial.

Behaviour scored were**:** Distance travelled, Foot Slips, Number of turns andLatency to fall.

Statistical Analysis

Data obtained were presented as mean ± SEM. Experimental data were analyzed using analysis of variance (ANOVA) followed by a post hoc test (Least Square Difference (LSD) test) to determine significant difference between means. Th e analysis was done with an SPSS 18 statistical package. The mean values were considered significant at$p<0.05$.

Results

Behaviours scored in beam walking

Line crosses: Figure 1 shows the comparison of mean line crosses forcontrol, AM only,AM + ethanoland ethanol only in beam walking apparatus as 417.00 ± 16.26, 435.00 ± 20.55, 377.80 ± 15.64 and 140.80 ± 32.85 respectively.The line crosses of the ethanol only treated groupwas significantly decreased compared to control and other experimental groups ($p<0.05$). The result further showed a significant decreasedin the group administered AM + ethanol compared to control and AM only($p<0.05$). There was no significant difference between AM only and control.

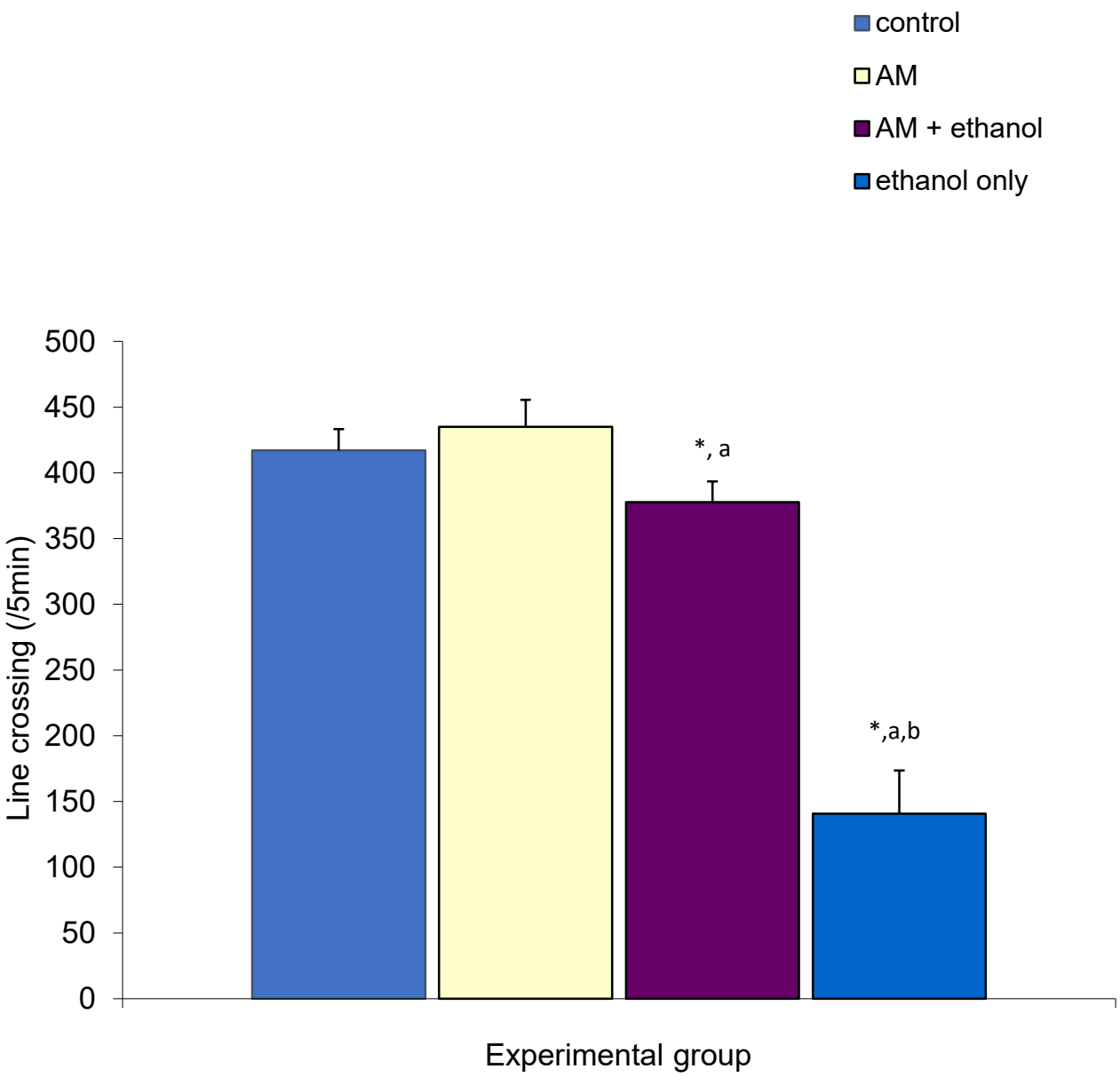

FIG. 1: Frequency of line crossing during the beam walking test in the different experimental groups .

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs AM only
b=p<0.05 vs AM + ethanol

Reversals: Figure 2 shows the comparison of mean reversals of control, AM only,AM + ethanoland ethanol only in beam walking apparatus as 7.20 ± 0.12, 9.40 ± 0.18, 7.60 ± 0.15and 3.20 ± 0.11 respectively.The results showed that, there was a significant decreased in mean number of reversals in the ethanolonly treated group compared to control and other experimental groups ($p<0.05$). There was also a significant decreasein the group administered AM + ethanol compared to AM only ($p<0.05$). the AM only group showed a significant increase in reversal compared to control ($p<0.05$).

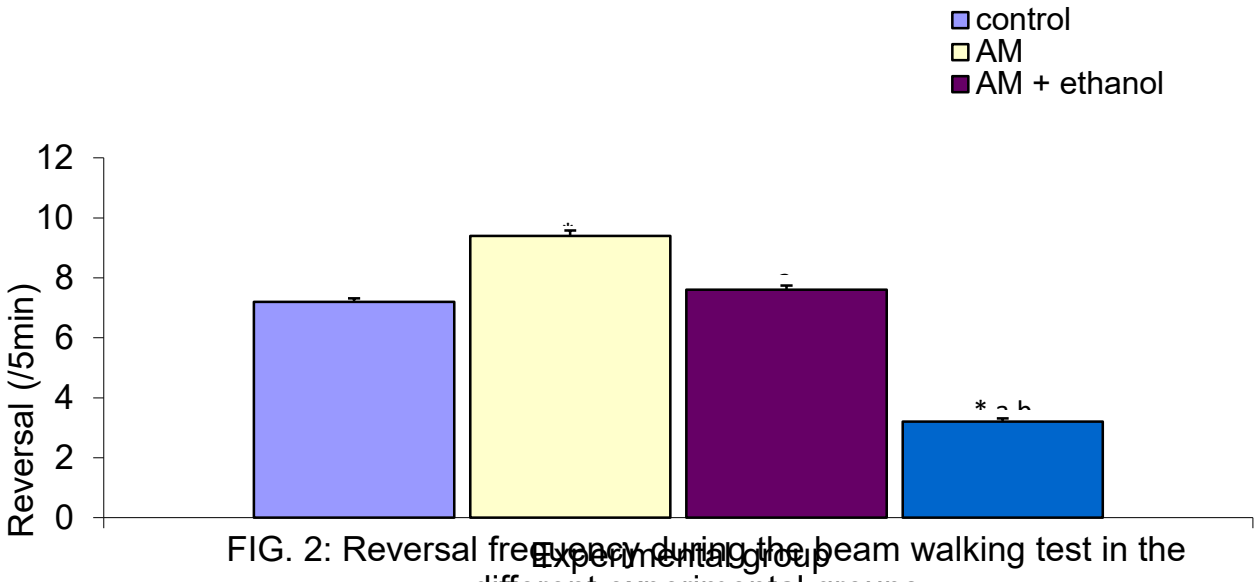

FIG. 2: Reversal frequency during the beam walking test in the different experimental groups .

Values are expressed as mean +SEM, n = 5.
* = $p<0.05$ vs control
a = $p<0.05$ vs AM only
b=$p<0.05$ vs AM + ethanol

Foot Slips: The mean frequency of foot slips of the different experimental groups was recorded as follows: 4.63 ± 0.93, 3.68 ± 0.87, 8.42 ± 0.40 and 18.67 ± 0.51 for control, AM only,AM+ ethanoland ethanol only in beam walking apparatus respectively. The result represented in figure 3 showed that, the number of foot slips in the group administered ethanolonly was significantly higher compared to control and experimental groups ($p<0.05$). The result further showed that, the group administered AM + ethanol showed a significant increase when compared to control and AM only groups ($p<0.05$). there was no significant difference between the control and the AM only group.

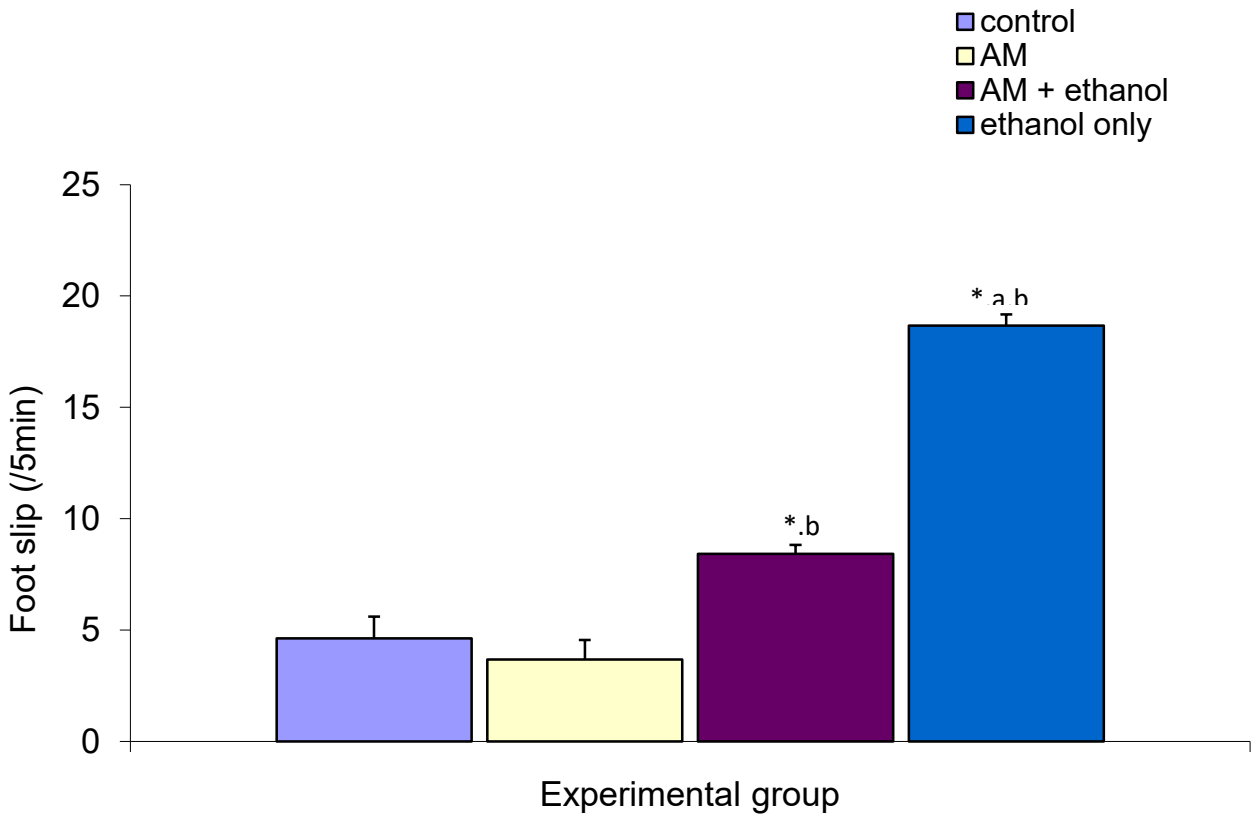

FIG. 3: Frequency of foot slip during the beam walking test in the different experimental groups .

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs AM only
b=p<0.05 vs AM + ethanol

Latency of fall: Figure 4 represent the mean frequency of fallfor control, AM only,AM+ ethanoland ethanol only in beam walking apparatus. The frequencies were 0.00 ± 0.06, 0.00 ± 0.11, 1.50 ± 0.21 and 17.50 ± 0.29for control, AM only,AM+ ethanoland ethanol only respectively.The frequency of fallin the group administered ethanolonly was significantly higher compared to control and other experimental groups ($p<0.05$). The result further showed that, the group administered AM + ethanol showed a higher significant difference when compared to control and AM only groups ($p<0.05$). there was no significant difference between the control and the AM only group.

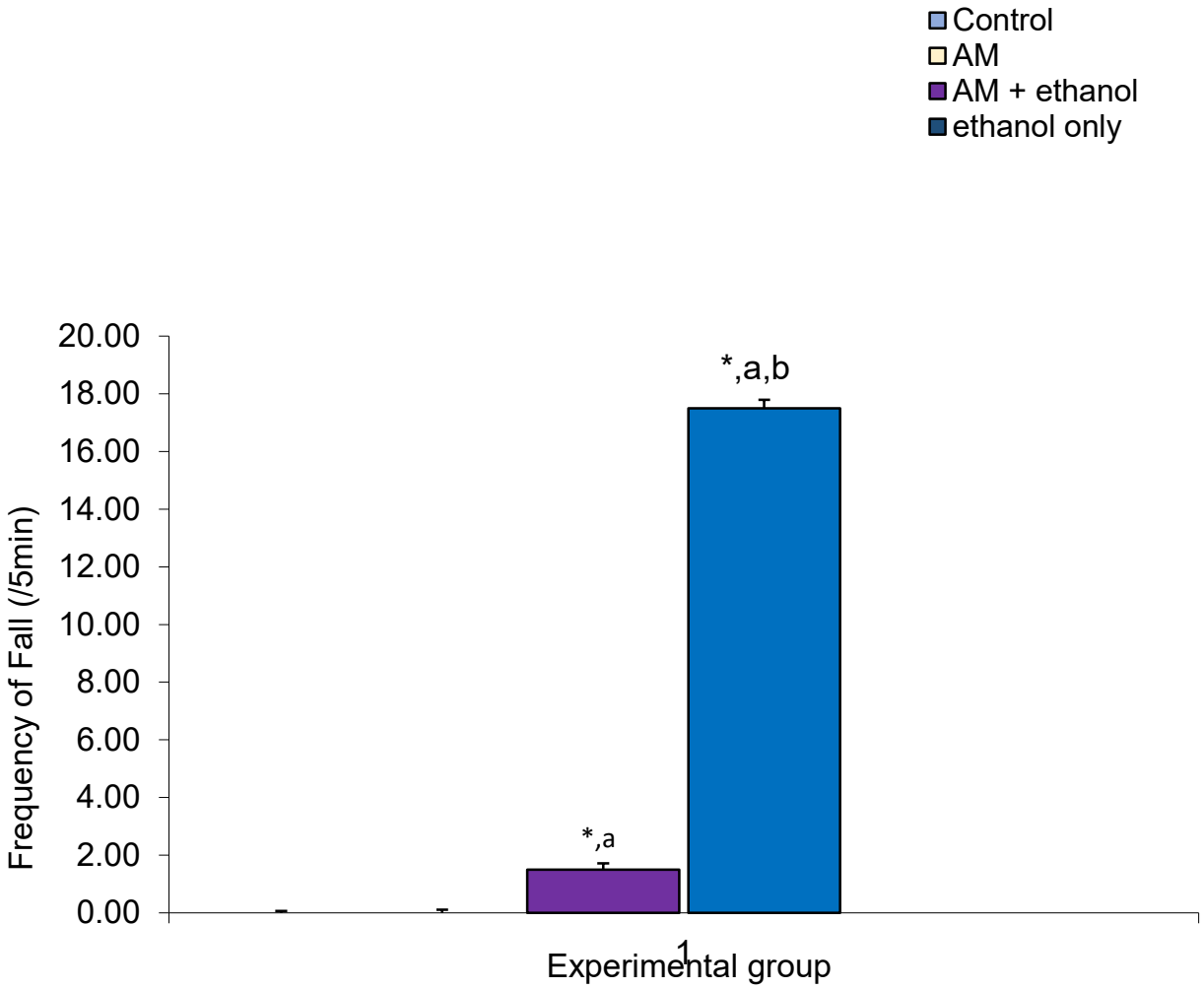

FIG. 4: Frequency of fall during the beam walking test in the different experimental groups .

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs control
a = p<0.05 vs AM only
b=p<0.05 vs AM + ethanol

Discussion

This study was to investigatethe effects of aqueous extract of alligator pepper (Aframomummelegueta) on alcohol induced motor coordination impairment inCD1 mice.The parameters considered in the study were line crossing, reversals,foot slipsand latency to fall for assessment of motor coordination and balance.The beam walking apparatus was used to study motor coordination and balance. Before motor coordination was studied, feeding and administration of various regiments were done for 7 days. The treatment period of 7 days was to enable sufficient time for bioavailabilityofcompounds like saponins, tannins, Alkaloids, Flavonoids,Phenols as well as carbohydrates, proteins, fat, gingerol, paradol, shogaol, vitamins, tyrosine, phenylalanine and tryptophanpresent in the seed of alligator pepper to assess the brain tissues.Recent studies showed that, the seed of alligator pepper contains flavonoid 4.29%, saponin 3.98%, allkaloids 3.85%, phenol 3.54%,tanin 2.35%, terpenoid 1.85%, and steroid 1.25 [21].Hence transient time is needed for biogenesis of various nutrients present in alligator pepperto be available in the brain tissues.

Motor coordination: The results obtained fromthe beam walking in this test showed that, the group(s) of mice administered AM extract only and AM extract + ethanol showed better motor coordination compared to ethanol only treated group ($p<0.05$). In figure(s)1and 2 representingthe number of line crosses and number of reversals respectively.,the group of mice treated with AM only and the group treated with AM + ethanol showed significantly higher number of line crosses and reversals compared to the group administered ethanol only ($p<0.05$). In figure(s) 3 and 4, representing number of foot slips and latency to fall respectively., the groups treated with Aframomummelegueta showed significant decreased number of foot slips and absence of fall whereas the group administered ethanol only showed significant increased number of foot slips and fall during the beam walking test($p<0.05$). It is possible that ethanol suppressed neurons in motor cortex, basal ganglia, cerebellum and the anterior motor neurons in the spinal cord [3] responsible for motor coordination and balance in the brain as indicated in the results of this study. These effects of ethanolcould be explained by itsactions on different neurotransmitters, including the stimulation of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the CNS, and the inhibition of glutamate, the main central excitatory neurotransmitter [22].Research showed that ethanol potentiates the effects of GABA by acting directly on its receptors, enhancing their inhibitory effectsof sedation, loss of self-control and relaxation as a result of allopregnanolone(neuroactive steroids)production in the CNS that is a positive allosteric modulator of neurotransmitter receptors [23].From the results obtained above, it could be suggestive that, alligator pepper seedarenutraceuticals that may have exerted effects on the cerebellum, motor cortex, basal ganglia and even the spinal cord neuronsin the mice administered alligator pepper extract., sinceduring alcohol intoxication neuro-excitatory transmitter receptors (glutamate) the main central excitatory neurotransmittersare inhibited throughout the central nervous system and GABA the main inhibitory neurotransmitter of the CNS is stimulated,it could be suggestive that, alligator pepper seeds exerted excitatory effects on glutamate receptors and inhibitory effects on

GABA receptors in the CNS especially on the brain centers responsible for motor coordination and balance. Alligator pepper contains flavonoids a neuroprotective antioxidant that had been reported to improved motor coordination and balance in various studies. Grossoet al., (2013), reported that flavonoidsprevent oxidative stress, one of the causes of disorders affecting the central nervous system through enzymes and receptors activities, being regarded as multi-target botanical therapeutics or drugs [24].Flavonoids enhances neurotransmitters secretion (glutamate)thereby providing neuro-protection against glutamate-mediated excitotoxicityone of the major causes of neurodegeneration disordersthat can affect motor coordination [25].Phytochemical studies showed that, alligator pepper seed contains 12-15mg/g of quercetin a form of flavonoid. It is possible that, the ameliorating effects of alligator pepper seed extract on our alcohol intoxicated mice models observed in this study could be attributed to quercetin present in the extract. According to Jung and Lee(2013), high dose of quercetin may reduce GABA's effects by blocking GABA receptors [26]. Recentstudies revealed that,quercetinhas potential therapeutic propertiesthat could be used for the managementof nervous system illnesses arising from its protective role against oxidative damage and neuroinflammations [27].According to Jakariaet al., (2019), quercetin acts through several molecular signalsand regulates neurotrophic and anti-oxidative signalling molecules [27].

Another bioactive ingredient suspected to influence neurobehavior is gingerol present in the seeds of alligator pepper.Gingerol influences neurotransmitters like serotonin, dopamine and GABA, which regulate mood, motivation and other functions.Serotonin plays essential role in neurobehavior, and theunderlying basis of neurobehavior is sensory-motor integration [28].,and that, the serotonergic system is anatomically organized to play that role.Recent Studies showed that Gingerol is involved in antidepressant-like effects through serotonergic system in mice model [29]. High serotonin levels lead to altered mental status, deliria, rigidity and myoclonus, together recognized as serotonin syndrome which are symptoms displayed by an alcohol intoxicated individual [30]. It could be suspected that, the gingerol present in alligator pepper may have acted in the brain centers involved in motor coordination through the serotonergic pathways to prevent Symptoms of alcohol intoxicationwhich include sedation, altered speech, temporal loss of memory, poor motor coordination etc.During alcoholintake there is usually an increase in dopamine production that keeps the individual feeling good and generallyrelaxed. The feel good and relaxation prompt the brain to seeking more drink and continue drinking [31].Research has shown that the brains of alcoholics have dopamine levels that are significantly below average [32]. This explains why alcoholics would continue to seek more and more alcohol in order to achieve the same pleasure.Qianet al., (2009) reported that, gingerol has good activity against cisplatin-induced emesis in minks possibly by inhibiting central or peripheral increase of serotonin, dopamine and substance P [33].It is possible that gingerol present in our extract exhibited inhibitory interaction with dopaminergic neurons in the central nervous system.

Research investigation by Adefeghaet al., (2016), shown that, aqueous extract of alligator pepperthat was analyzed using Ultra-performance liquid chromatography (UPLC)-

photodiode array detection (PDA) indicated the presence of amino acid at 254 nm.One of the amino acids identified was tryptophan [34]. Tryptophan as one of the essential amino acids had been reported to improve motor coordination through involvement in neurotransmitter synthesis and neuronal functions.Example include: tryptophan is converted into serotonin, a neurotransmitter that helps regulate motor control, balance and coordination [35]., tryptophan influences the activity of other neurotransmitters like dopamine and GABA which are involved in motor control. Costardiet al., (2015), reported that, the effects of alcohol on the central nervous system is through the stimulation of GABA, the main inhibitory neurotransmitter in the CNS [22]andfurther research had shown that the brains of alcoholics expressed low dopaminelevel [32]. It can be suspected that, tryptophan present in seed of alligator pepper (1.3-1.5%) have positive effects on GABA and dopamine in the brains of the mice treated with ethanol and alligator pepper extracts as expressed inour results presented in figures 1, 2, 3 and 4. This study was to investigatethe effects of aqueous extract of alligator pepper (Aframomummelegueta) on alcohol induced motor coordination impairment inCD1 mice.
The effects of administration of alligator pepper (Aframomummelegueta) onalcohol induced motor co-ordination impairment in CD1 mice was studied.Motor coordination was impaired in miceadministeredethanol only compared to control andother experimental groups ($p<0.05$). Administration ofaqueous extract of alligator pepper improved motor coordination and balance. Fromthe results obtained from this research, it is suspected that alligator pepper hadexcitatory effects on motor cortex, basal ganglia, cerebellum andanterior motor neurons of the spinal cord.

Conclusion: Administrationof aqueous seed extract of alligator pepper improvedmotor coordination and balance. Motor coordination enhancementobserved may be attributed to quercetin, gingerol, tryptophan, etc.. present in present in alligator pepper seed.

References

1. September AF. Need Motor Coordination Mazes for your research?.
2. Davis K, Williams JL, Sanford BA, Zucker-Levin A. Assessing lower extremity coordination and coordination variability in individuals with anterior cruciate ligament reconstruction during walking. Gait & Posture. 2019 Jan 1;67:154-9.
3. Uysal SA, Düger T. Motor control and sensory-motor integration of human movement. InComparative Kinesiology of the Human Body 2020 Jan 1 (pp. 443-452). Academic Press.
4. Rigoli D, Piek JP, Kane R. Motor coordination and psychosocial correlates in a normative adolescent sample. Pediatrics. 2012 Apr 1;129(4):e892-900.
5. Cleaton MA, Lorgelly PK, Kirby A. Developmental coordination disorder in UK children aged 6–18 years: Estimating the cost. British Journal of Occupational Therapy. 2020 Jan;83(1):29-40.
6. Inegbenebor U, Ebomoyi MI, Onyia KA, Amadi K, Aigbiremolen AE. Effect of alligator pepper (Zingiberaceaeaframomummelegueta) on first trimester pregnancy in Sprague Dawley rats. Nigerian journal of physiological sciences. 2009;24(2).

7. Okonkwo EE. Traditional healing systems among Nsukka Igbo. Journal of Tourism and Heritage Studies. 2012;1(1):69-81.
8. Okunade AF, Agboola TI, Ogunsina BS, Salau AO, Salami A. Physicochemical properties of four selected food spices of Nigerian origin: alligator pepper, black pepper, ginger and cloves. Ife Journal of Technology. 2019 Nov 1;26(1):7-12.
9. Usman YO, Abechi SE, Benedict OO, Victor O, Udiba UU, Ukwuije NO, Anyahara SE. Effect of solvents on [6]-Gingerol content of ginger rhizome and alligator pepper seed. Annals of Biological Research. 2013;4(11):7-13.
10. Adefegha SA, Oboh G, Adefegha OM, Henle T. Alligator pepper/Grain of paradise (Aframomummelegueta) modulates Angiotensin-I converting enzyme activity, lipid profile and oxidative imbalances in a rat model of hypercholesterolemia. Pathophysiology. 2016 Sep 1;23(3):191-202.
11. Crocq MA. Alcohol, nicotine, caffeine, and mental disorders. Dialogues in clinical neuroscience. 2003 Jun 30;5(2):175-85.
12. World Health Organization. No level of alcohol consumption is safe for our health. Date last accessed. 2023 Oct;7.
13. Nutt DJ, King LA, Phillips LD. Drug harms in the UK: a multicriteria decision analysis. The Lancet. 2010 Nov 6;376(9752):1558-65.
14. Nutt DJ, King LA, Phillips LD. Drug harms in the UK: a multicriteria decision analysis. The Lancet. 2010 Nov 6;376(9752):1558-65.
15. World Health Organization. Department of Mental Health, Substance Abuse. Global status report on alcohol 2004. World Health Organization; 2004.
16. Lobo IA, Harris RA. GABAA receptors and alcohol. Pharmacology Biochemistry and Behavior. 2008 Jul 1;90(1):90-4.
17. World Health Organization. No level of alcohol consumption is safe for our health. Date last accessed. 2023 Oct;7.
18. Gureje O, Nortje G, Makanjuola V, Oladeji BD, Seedat S, Jenkins R. The role of global traditional and complementary systems of medicine in the treatment of mental health disorders. The Lancet Psychiatry. 2015 Feb 1;2(2):168-77.
19. Berry RB, Matthews DB. Acute ethanol administration selectively impairs spatial memory in C57BL/6J mice. Alcohol. 2004 Jan 1;32(1):9-18.
20. Luong TN, Carlisle HJ, Southwell A, Patterson PH. Assessment of motor balance and coordination in mice using the balance beam. JoVE (Journal of Visualized Experiments). 2011 Mar 10(49):e2376.
21. GC O. Phytochemical, Mineral, and Proximate Characterization of Seed and Pod of Aligator Pepper [Aframomummelegueta]. Asian Journal of Food Research and Nutrition. 2024 Feb 7;3(1):92-7.
22. Costardi JV, Nampo RA, Silva GL, Ribeiro MA, Stella HJ, Stella MB, Malheiros SV. A review on alcohol: from the central action mechanism to chemical dependency. Revista da associaçãomédicabrasileira. 2015;61(4):381-7.
23. Charlton ME, Sweetnam PM, Fitzgerald LW, Terwilliger RZ, Nestler EJ, Duman RS. Chronic ethanol administration regulates the expression of GABAA receptor α1 and α5 subunits in the ventral tegmental area and hippocampus. Journal of neurochemistry. 1997 Jan;68(1):121-7.

24. Grosso C, Valentão P, Ferreres F, B Andrade P. The use of flavonoids in central nervous system disorders. Current medicinal chemistry. 2013 Nov 1;20(37):4694-719.
25. dos Santos Souza C, Grangeiro MS, Pereira EP, Dos Santos CC, da Silva AB, Sampaio GP, Figueiredo DD, David JM, David JP, da Silva VD, Butt AM. Agathisflavone, a flavonoid derived from Poincianella pyramidalis (Tul.), enhances neuronal population and protects against glutamate excitotoxicity. Neurotoxicology. 2018 Mar 1;65:85-97.
26. Jung JW, Lee S. Anxiolytic effects of quercetin: Involvement of GABAergic system. Journal of Life Science. 2014;24(3):290-6.
27. Jakaria M, Azam S, Jo SH, Kim IS, Dash R, Choi DK. Potential therapeutic targets of quercetin and its derivatives: its role in the therapy of cognitive impairment. Journal of clinical medicine. 2019 Oct 25;8(11):1789.
28. Carey RJ. Serotonin and basal sensory–motor control. InHandbook of Behavioral Neuroscience 2010 Jan 1 (Vol. 21, pp. 325-330). Elsevier.
29. Sedighi S, Nasiri B, Alipoor R, MoradiKor N. Modulation of 6-Gingerolin Antidepressant-like Effects in Mice Model. GMJ Medicine. 2023 May 10;3(2):59-63.
30. Kanova M, Kohout P. Serotonin—Its synthesis and roles in the healthy and the critically ill. International journal of molecular sciences. 2021 May 3;22(9):4837.
31. Esch T, Stefano GB. The neurobiology of pleasure, reward processes, addiction and their health implications. Neuroendocrinology letters. 2004 Aug 1;25(4):235-51.
32. Noble E. Alcoholism and the dopaminergic system: a review. Addiction biology. 1996 Oct;1(4):333-48.
33. Qian QH, Yue W, Wang YX, Yang ZH, Liu ZT, Chen WH. Gingerol inhibits cisplatin-induced vomiting by down regulating 5-hydroxytryptamine, dopamine and substance P expression in minks. Archives of pharmacal research. 2009 Apr;32:565-73.
34. Adefegha SA, Oboh G, Adefegha OM, Henle T. Alligator pepper/Grain of paradise (Aframomummelegueta) modulates Angiotensin-I converting enzyme activity, lipid profile and oxidative imbalances in a rat model of hypercholesterolemia. Pathophysiology. 2016 Sep 1;23(3):191-202.
35. Li D, Yu S, Long Y, Shi A, Deng J, Ma Y, Wen J, Li X, Liu S, Zhang Y, Wan J. Tryptophan metabolism: Mechanism-oriented therapy for neurological and psychiatric disorders. Frontiers in Immunology. 2022 Sep 8;13:985378.