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Pharmacological potential of acamprosate in epilepsy: A study in *Drosophila Melanogaster*

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Abstract
Epilepsy is the tendency to have seizures that start in the brain. The brain uses electrical signals to pass messages between brain cells. If these signals are disrupted, this can lead to a seizure. Epilepsy models have been developed to appraise the pathophysiology of epileptic seizures and to search for new effective anti-epileptic drugs. Around 50 million people worldwide have epilepsy. Establishment of bang induced seizure model of epilepsy in *Drosophila melanogaster* in laboratory. When fed to seizure-sensitive *Drosophila,* In particular, for drug treatments, the recovery time from seizure and paralysis is greatly reduced compared with untreated flies. Intriguingly we find that drug treatments result in a reduction in seizure sensitivity recovery time**.** Evaluation of pharmacological potential of acamprosate in bang induced seizure model of epilepsy in *Drosophila melanogaster.* Acamprosate is a synthetic amino acid and a neurotransmitter analogue that is used as an alcohol deterrent in management of alcohol dependence and abuse. Behavior parameters using in this protocol is mean recovery time (MRT), bang sensitivity (BS) and climbing assay. Biochemical parameter using in this protocol is estimation of total protein content, malondialdehyde (MDA) estimation, catalase (CAT) estimation, superoxide dismutase (SOD) estimation and nitric oxide (NO) estimation. Correlation of data observed in *Drosophila* model.

Keywords: Acamprosate, Drosophila, model of epilepsy, oxidative stress

Introduction
Epilepsy is a neurological disease arising from abnormal and uncontrollable electrical firings of a group of neurons appearing in the central nervous system. Which is involves the occurrence of spontaneous and recurrent seizures that alter the performance of the brain and affect several sensory and behavioral functions. Most seizures are classified into two groups, partial (Seizure activity starts in one area of the brain) and generalized (Seizure involves whole brain, consciousness is lost at the onset). Epilepsy models have been developed to appraise the pathophysiology of epileptic seizures and to search for new effective anti-epileptic drugs [1, 2]. India is home to about 10 million people with epilepsy (prevalence of about 1%) this being higher in the rural (1.9%) as compared with the urban counterpart (0.6%). Around 50 million people worldwide have epilepsy. Each year, approximately ¼ million new cases are added to this population [3]. According to literature survey acamprosate was shown effective results in various CNS disorders. Acamprosate (N - acetyl homotaurine) is NMDA receceptor partial co-agonist; they are derivative of essential amino acid taurine and structural resemblance to γ- Amino Butyric Acid (GABA). It was approved by USFDA (United state food and drug administration) in 2004 for treatment of alcoholic patients to decrease alcohol craving after alcohol detoxification [4, 5]. It inhibit nitric oxide synthesis by decreasing the NMDA receptor activation-induced nitric oxide release, exhibit anti-inflammatory activity by reducing the release of lipopolysaccharide induced Tumour necrosis factor-α (TNF-α) in whole blood, voltage- gated calcium channels in the inhibition by acamprosate [6, 7, 8]. Fundamental analysis of human disease-associated gene sequences in *Drosophila melanogaster* has acknowledged that about 75% of the human disease genes have a *Drosophila* ortholog. On the basis of its genetic similarity to humans, the use of *Drosophila* from the study of development to the modeling of human neurodegenerative diseases. A powerful approach to considering disease mechanisms is the development of transgenic rodent models, most notably in the rodent. However, development and analysis of such models can be costly and time consuming [9, 10]. So here we hypothesized acamprosate inhibit different steps involved in pathophysiology of epilepsy through above describe mechanism.

Materials and Methods
Fly strain: Oregon R+ strains will be used for the proposed studies which have obtained from *Drosophila* stock center, Mysore. The flies will be reared on a standard food medium containing cornmeal, yeast, agar, sugar and added propanoic acid as antimouldant (Standard Drosophila Medium = SDM) and maintained at 25 °C on natural light/dark cycle in glass bottles. Flies of either sex will be used for studies.

Bang induced Seizure Model: Bang-sensitive seizure induced by clean glass vials (9.3 cm × 2.4 cm.) and using a vortex mixer. Vials were mechanically stimulated by placement in a bench-top vortex for 13 second at the maximum speed. The time for each fly to right itself after vortexing was recorded [11].

Drug treatment: Three days old flies were placed in empty vial for 24 hours starvation before drug treatment then flies were placed in 20 ml of standard food medium supplement with acamprosate. Flies will be allowed to take food containing different concentration of drug for 24 hours before bang (Vortex mechanical stimulation). The control group flies were fed on standard food medium.

Chemicals: Laboratory grade chemicals were used for experimental procedure and and present studies. Sodium carbonate (Na_2CO_3), Copper (II) sulfate ($CuSO_4$), Potassium sodium tartrate, Sodium hydroxide, FC reagent, Bovine serum albumin (BSA), Sodium dodecyl sulfate (SDS or NaDS), Acetic acid, Thiobarbituric acid (TBA), n-butanol, Pyridine, Hydrogen peroxide, Potassium bicarbonate, Glacial acetic acid, Nitro blue tetrazolium (NBT), Triton X-100, Hydroxylamine hydrochloride, 1,1,3,3-Tetramethoxypropane. Purchase through proper channel by M. D. University from Loba Chemie Pvt. Ltd. P. Box No. 6139, Mumbai – 400005 India and CDH (Central Drug House) Ltd. Post Box No. 7/28 Vardaan House, Ansari Road Daryaganj, New Delhi-110002 (INDIA)

Experimental detail: Sexually active male flies were grouped separately and divided in to five groups; each group consists of 30 flies (3 flies per vial for behavior parameter). The divided groups were received standard drug with food medium by orally as a given below.

Table 1: Experimental detail

S. No	Groups	Treatments	Drug concentration
1.	Control	----------	Standard food medium
2.	Bang + Vehicle	Drug vehicle	Standard food medium with drug vehicle
3.	Bang+ Acamprosate	Acamprosate	20 µg/ml in food medium
4.	Bang+ Acamprosate	Acamprosate	100 µg/ml in food medium
5.	Bang+ Acamprosate	Acamprosate	200 µg/ml in food medium

Behavioral testing: Flies 3 days of age will be tested for bang-sensitive seizure behavioral phenotypes. Intact 3 flies will be placed in a clean glass vials (9.3 cm × 2.4 cm.) and given a 13 sec vibration using a vortex mixer at the highest speed. Sequence of seizure-and-paralysis responses observe in a bang-sensitive flies following mechanical shock (vortexing). The behavioral of flies is shown here as an example. Initially, seizure and paralysis occurs (a) Uncoordinated leg movement (b) After a period of quiescence, wing buzzing may occur (c) Sometimes even causing the fly to spin (d) The fly may resume the contorted posture (e) Walk away [11, 12].

Mean recovery time (MRT): The mean recovery time was average time taken for a fly illustrating bang sensitive (BS) behavior after mechanical stimulation by vortexing to recover in a group. After bang (vortexing) flies undergo seizure like behavior exhibits by initial seizure, temporary paralysis and recovery seizure and then record time was measured for each fly to stand up and start moving like normal flies behavior. [11]

Bang sensitivity (BS): This method use to only identification for BS of flies after mechanical vortex. Although generally obeying the BS exemplar of hyperactivity-paralysis-hyperactivity during seizure cycle, when observed under the magnifying glass. In contrast, the BS flies have significantly longer recovery times of 37, 52, and 198 s, respectively; they show 100% penetrance of the BS phenotype so we will be observe bang sensitivity of flies after BS (Vortexing). In this procedure only justify to BS is considered through yes or no.

Climbing assay: The climbing assay allocate on startle induced negative geotactic behavior, an innate escape response in flies. Single flies were placed in empty glass vials and gently tapped to the bottom of glass tube. Time taken for the knocked – down flies to climb 8 cm height of the glass tube wall was recorded. Individually fly was tested two times at one minute intervals, all flies were seen to reach the 8 cm target height within one minute, which the climbing cut - off time. Flies were observed during each climbing and their climbing time noted [13].

Biochemical testing

Homogenate preparation: After 6 hr. of bang sensitivity, legs and wings of all flies were separated from the body with sharp edge of blade and then homogenized in sodium phosphate buffer (0.1 M, pH 8.0) and was centrifuged at 2500g for 10 min. at 40 °C. The supernatant was filtered through nylon mesh and was used for following biochemical parameters.

Estimation of total protein content: The total protein content was determined by the Lowry method. There are two distinct steps which lead to final color with protein, reactivity of peptide nitrogen with copper ions in alkaline condition and reduction of Folin ciocalteau phosphomolybdic phosphotungstic reagent by the copper- treated protein. The Lowry method is sensitive to pH change and therefore the pH of assay solution maintain at 10 – 10.5. Blue purple color developed by using Folin Phenol regent and absorbance was read at 660 nm, using spectrophotometer. The levels of protein were expressed as mg/ml [14].

Malondialdehyde (MDA) estimation: Lipid peroxidation is a free radical mediated phenomenon. Primary products of such destruction are a complex mixture of peroxides which then breakdown to produce carbonyl compound. The malondialdehyde (MDA) is one of carbonyl compound, which forms a chromogenic adducts with two molecule of thiobarbituric acid (TBA). A secondary product of lipid peroxidation has been widely recognized for measuring lipid peroxidation. The absorbance of supernatant was read at 540 nm at room temperature against appropriate black [15].

Catalase (CAT) estimation: The method is based on the fact that dichromate in acetic acid is diminished to chromic acetate when heated in the presence of H_2O_2 (hydrogen peroxide), with the formation of per chromic acid as an unstable intermediate. The reaction mixture (1.5 ml, vol.) contained 1.0 ml of tissue homogenate (supernatant), & 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate – acetic acid reagent (5% potassium dichromate and glacial acetic acid will be mixed in 1:3 ratio).

Catalase (CAT) will be assayed colorometrically at 620 nm [16].

Superoxide dismutase (SOD) estimation: SOD activity was defined as its concentration required to decreasing the rate of reaction by 50% in 1 min under the assay conditions. SOD was detected on the basis of its ability to inhibit superoxide mediated reduction. SOD, an antioxidant enzyme, reduces superoxide radicals to H_2O_2. Where in the reduction of nitazoblue tetrazolium (NBT) was inhibited by the superoxide dismutase is measured at 560 nm UV/visible spectrophotometer. Briefly, the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and tissue homogenate. The results were expressed as units/mg protein [17].

Nitric oxide (NO) estimation: Concentration of nitrite in the tissues was measured as an index for NO production. Tissue homogenized in sterile PBS (1 ml), supernatants were collected, and analyzed for NO production by modified Greiss method. Nitrate was converted to nitrites with nicotinamide adenine dinucleotide phosphate (NADPH; 1.25 mg/ml) and nitrate reductase followed by addition of Griess reagent. The reaction mixture was incubated at room temperature for 10 minutes followed by addition of TCA (Trichloroacetic acid). Samples were centrifuged, clear supernatants were collected, and optical density was recorded at 550 nm against suitably prepared blank solution (100 µL of distilled water was be used. The amounts of NO produced were determined by calibrating a standard curve using sodium nitrite [18].

Statistical analysis: The obtained data expressed as Mean ± SEM. Data was analysed by one way ANOVA followed by tukey test. The parametric data subjected to one way analysis of variation (ANOVA) followed by the Graphpad InState – [DATASET1.ISD].

Results

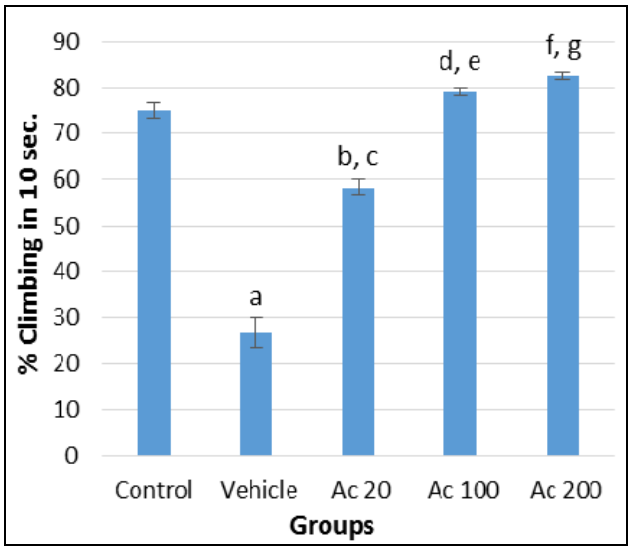

Fig 1.1: Percentage climbing in 10 sec. at after 2 hour

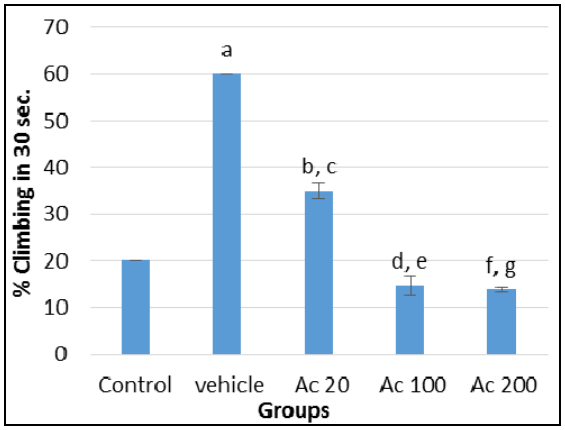

Fig 1.2: Percentage climbing in 30 sec. at after 2 hour

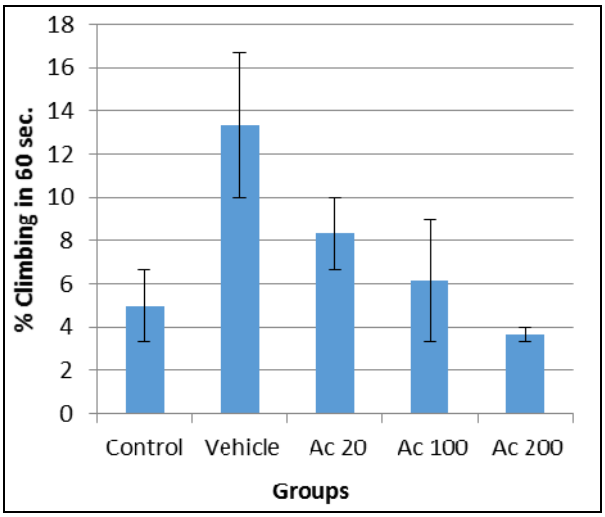

Fig 1.3: Percentage climbing in 60 sec. at after 2 hour

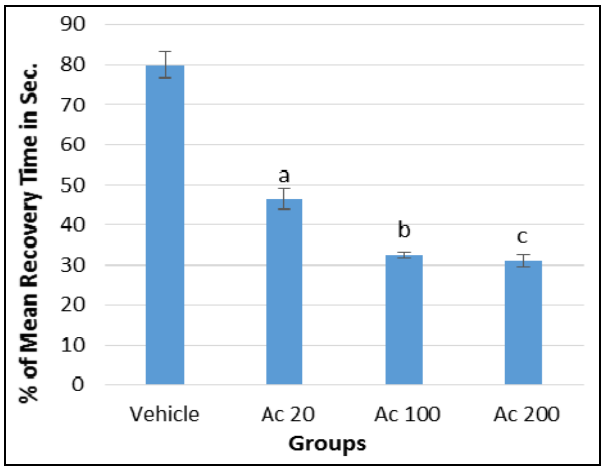

Fig 2.1: Percentage mean recovery time (MRT) in second.

The above figures showing effect of acamprosate on percentage climbing response of flies in 10, 30, and 60 second and percentage mean recovery time (MRT) in second. Values were expressed as Mean ± SEM. Data was analyzed by one way ANOVA followed by tukey test (Statistically significance: $p< 0.001$, $p< 0.01$). Figure 1.1: a. $p< 0.001$ vs control, b. $p< 0.01$ vs control, c. $p< 0.001$ vs vehicle, d. $p< 0.001$ vs vehicle, e. $p< 0.001$ vs Ac 20, f. $p< 0.001$ vs vehicle,

g. p< 0.01 vs Ac 20. The F- value was found to be F (4, 5) = 146.48. Figure 1.2: a. p< 0.001 vs control, b. p< 0.01 vs control, c. p< 0.001 vs vehicle, d. p< 0.001 vs vehicle, e. p< 0.001 vs Ac 20, f. p< 0.001 vs vehicle, g. p< 0.001 vs Ac 20. The F- value was found to be F (4, 5) = 269.37. Figure 1.3: Considered not significant, The F- value was found to be F (4, 5) = 2.885. Figure 2.1: a. p< 0.01 vs vehicle, b. p< 0.001 vs vehicle, c. p< 0.001 vs vehicle. The F value was found to be F (3, 4) = 103.55

Table: 2 Percentage of climbing time

Experimental groups									
Control		**Vehicle**		**Ac 20**		**Ac 100**		**Ac 200**	
Climbing time	% of climbing	Climbing time	% of climbing	Climbing time	% of climbing	Climbing time	% of climbing	Climbing time	% of climbing
10 sec.	74.99 %	10 sec.	26.66 %	10 sec.	58.33 %	10 sec.	79.16 %	10 sec.	82.495%
30 sec.	20 %	30 sec.	60 %	30 sec.	34.99 %	30 sec.	14.66 %	30 sec.	13.83 %
60 sec.	4.99 %	60 sec.	13.33 %	60 sec.	8.33 %	60 sec.	6.165 %	60 sec.	3.66 %

Table 3: Percentage Bang Sensitivity and mean recovery time (MRT)

S. No.	Groups	Drug concentration	% bang sensitivity	Mean recovery time (In %)
1.	Vehicle Group	Standard food medium with drug vehicle	100 % sensitive	79.95 sec.
2.	Bang +Acamprosate	20 µg / ml in food medium	100 % sensitive	43.83 sec.
3.	Bang +Acamprosate	100 µg / ml in food medium	100 % sensitive	32.48 sec.
4.	Bang +Acamprosate	200 µg / ml in food medium	100 % sensitive	31 sec.

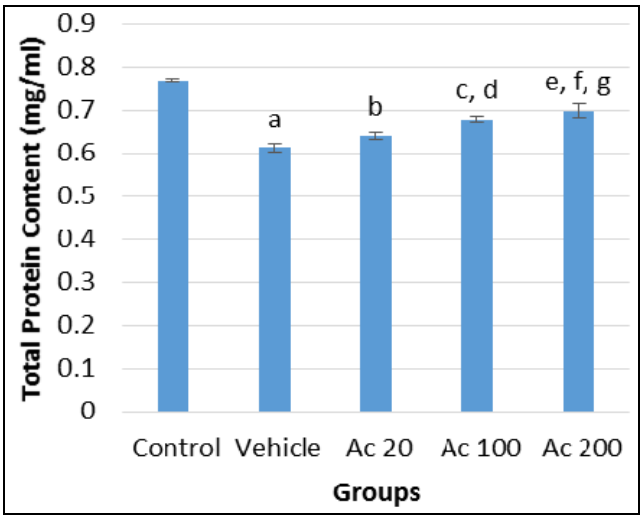

Fig 3.1: Total protein content

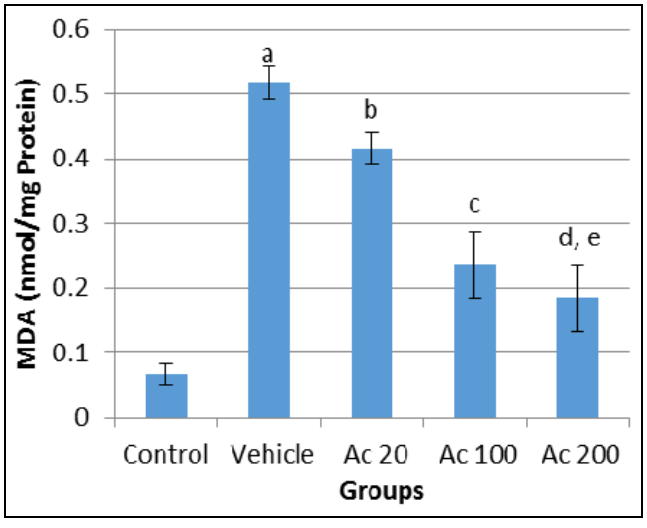

Fig 3.2: Malondialdehyde (MDA) estimation

The above figures show effect of acamprosate on level of protein content and malondialdehyde (MDA). Values were expressed as Mean ± SEM. Data was analyzed by one way ANOVA followed by tukey test (Statistically significance: p< 0.001, p< 0.01, and p < 0.05). Figure 3.1: a. p< 0.001 vs Control, b. p< 0.01 vs control, c. p< 0.01 vs control, d. p< 0.01 vs vehicle, e. p< 0.01 vs control, f. p< 0.01 vs vehicle, g. p< 0.05 vs Ac 20. The F- value was found to be F (4, 5) = 71.235. Figure 3.2: a. p< 0.01 vs control, b. p< 0.01 vs control, c. p< 0.05 vs vehicle, d. p< 0.01 vs vehicle, e. p< 0.05 vs Ac 20. The F- value was found to be F (4, 5) = 24.111.

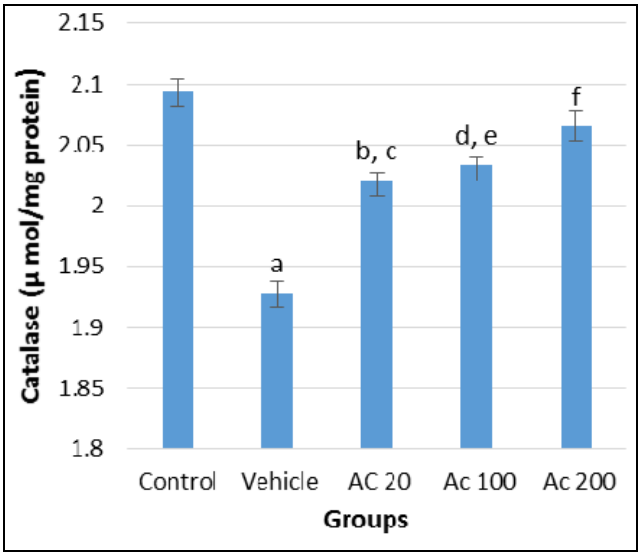

Fig 3.3: Catalase (CAT) estimation

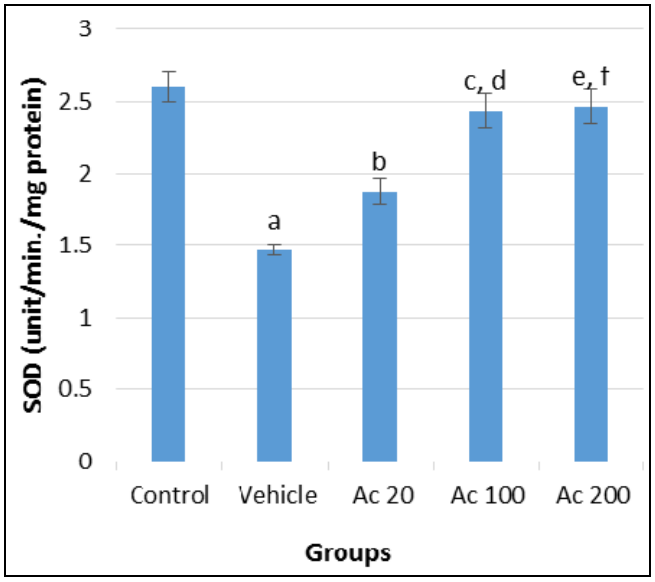

Fig 3.4: Superoxide dismutase (SOD) estimation

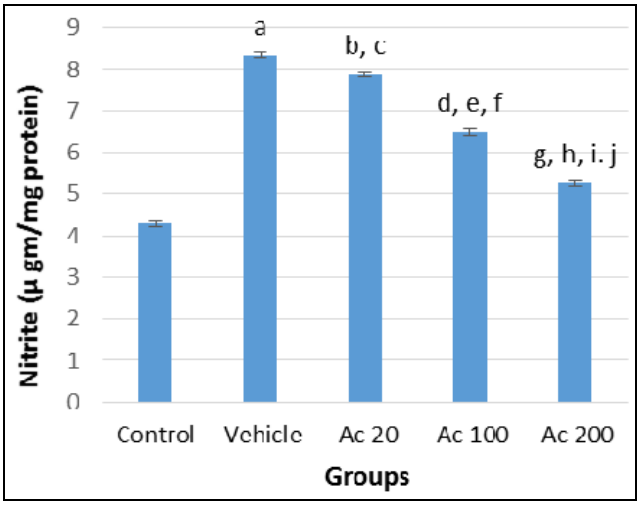

Fig 3.5: Nitric oxide (NO) estimation

The above figures show effect of acamprosate on level of catalase (CAT), superoxide dismutase (SOD) and Nitric oxide (NO). Values were expressed as Mean ± SEM. Data was analyzed by one way ANOVA followed by tukey test (Statistically significance: p< 0.001, p< 0.01, and p < 0.05). Figure 3.3: a. p< 0.001 vs control, b. p< 0.05 vs control, c. p< 0.01 vs vehicle, d. p< 0.05 vs control, e. p< 0.01 vs vehicle, f. p< 0.001 vs vehicle. The F- value was found to be F (4, 5) = 46.146. Figure 3.4: a. p< 0.01 vs control, b. p< 0.05 vs control, c. p< 0.01 vs vehicle, d. p< 0.05 vs Ac 20, e. p< 0.01 vs Ac 200, f. p< 0.05 vs Ac 100. The F- value was found to be F (4, 5) = 24.068. Figure 3.5: a. p< 0.05 vs vehicle, b. p< 0.001 vs control, c. p< 0.05 vs vehicle, e. p< 0.001 vs vehicle, f. p< 0.001 vs Ac 20, g. p< 0.01 vs control, h. p< 0.001 vs vehicle, i. p< 0.001 vs Ac 20, j. p< 0.001 vs Ac 100. The F-value was found to be F (4, 5) = 534.38.

Discussion

Still epilepsy affects over 50 million people around the world, a comprehensive understanding of the disease, due to its complexity and heterogeneity, is in the broad majority of cases lacking. The complicated nature of epilepsy is also noticeable by the variety of anticonvulsants used to treat the disorder, and even though drug therapy has proven effective in many cases, a significant number of patients do not feedback, or only partially respond, to the accessible drugs, although the emergence of second-generation anticonvulsants such as lamotrigine, vigabatrin, & gabapentin. The basis for this phenomenon is presently unclear, while it does demonstrate that new, more effective anticonvulsants are needed to conflict the disease. In this experiment, present the description of a method by which oral administration in *Drosophila* can test the ability of a compound to suppress seizures. Using a *Drosophila melanogaster* (fruit fly) for behavioral and biochemical study, were able to demonstrate its viability in assaying the anticonvulsant properties of acamprosate, an early this drug use for treatment of alcoholic patients to decrease alcohol craving after alcohol detoxification, still in use for humans [19]. In Percentage climbing time of flies in all groups control, Ac 20 µg/ml, Ac 100 µg/ml, and Ac 200 µg/ml at different concentration of acamprosate in food medium is more effective in 10 second but in vehicle group within disease condition they flies is more climbing to 30 second and they are Statistically significance: p< 0.001, p< 0.01. Acamprosate doesn't show any significant effect on % climbing in 60sec. The data showing a reduction in MRT (mean recovery time) with increasing concentration of acamprosate suggest that this drug acts at the level of neuronal excitability, they are statistically significance: p< 0.001, p< 0.01. The drug also ameliorated BS (bang sensitive) behavior in flies that still responded to the stimulus, with fewer flies exhibiting hyperactivity during the recovery phase and elimination of the recovery phase with increasing dose. Physiologically, it decreased the susceptibility of the flies to seizure by increasing their seizure threshold [20]. The BS behavioral phenotype of flies is completely penetrate, 100% of flies are paralyzed by mechanical stimulation at different concentration of drug. The total protein content was determined by the Lowry method. There are two distinct steps which lead to final color with protein, reactivity of peptide nitrogen with copper ions in alkaline condition and reduction of Folin ciocalteau phosphomolybdic phosphotungstic reagent by the copper- treated protein. After bang, tissue protein contains level showing decreasing in flies' homogenate in vehicle group as compared to control group.Change protein contains level in flies and its modulation by acamprosate was recorded. Statistically significance: p< 0.001, p< 0.01, and p < 0.05, show significant increasing compared to vehicle group while when compared to each other significant effect was observed between acamprosate treated groups. Reactive oxygen species and reactive nitrogen species associated lipid peroxidation, activation of matrix metalloproteinases are suggested to be the major contributor to the pathogenesis. Free radical and oxidative stress also appear to be a common mediator of apoptosis and necrosis, directly or via lipid peroxidation. Although the results are somewhat inconsistent in animal studies have established that ROS scavenging acamprosate may play an important role in the prevention and combat against ROS-mediated brain tissue abnormalities. It was reported earlier that reactive oxygen species generated after bang sensitive by mechanical mediated injury induces

lipid peroxidation. Which either triggers apoptosis or necrosis depending on its extent. In accordance with these reports, we have found a considerable increase in MDA level, the index of lipid peroxidation, in acamprosate treated flies post treatment was potentially effective in lowering this level. The possible mechanism involved in reducing lipid peroxidation by acamprosate in this model could be its strong free radical scavenging properties. Vehicle group compared to control group. Changes in MDA level in flies and its modulation by acamprosate was recorded. Acamposate statistically $p< 0.001$, $p< 0.01$, and $p < 0.05$ show significant reduction compared to vehicle group while when compared to each other significant effect was observed between acamprosate treated groups [21]. Catalase is an enzyme which catalyzes the decomposition of hydrogen peroxides to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species. In tramatic brain injury, there is depletion of this antioxidant enzyme. The catalase level showing decreases in fly's homogenate in vehicle group compared to control groups. Acamposate statistically significance $p< 0.001$, $p< 0.01$, and $p < 0.05$ show significant increase in catalase level compared to vehicle group effect was observed between acamprosate treated groups. After bang sensitive mechanical stress, SOD level showing decrease in flies compare to control group and level of SOD statistically significant effect of acamprosate treated groups. Acamposate statistically significance $p< 0.001$, $p< 0.01$, and $p < 0.05$ show significant increase in Superoxide Dismutase (SOD) level compared to vehicle group effect was observed between acamprosate treated groups. Under normal physiological conditions, the level of nitric oxide is low. Uncontrolled production of the inflammatory mediator NO is an important element in development of pathological conditions of the brain, such as those caused by infectious diseases, epilepsy, trauma, neurodegenerative diseases, and ischemia. NO overproduction in the brain is a sign of acute brain inflammation, and can be used to examine anti-inflammatory actions of administered compounds. The expression of iNOS would thereby be prevented, and the production of NO would become reduced. After bang stress, NO level showing increase in flies homogenate in vehicle group as compared to control group. Acamposate show significant decrease NO level compared to vehicle group. Acamposate statistically significance $p< 0.001$, $p< 0.01$, and $p < 0.05$ show significant decrease in NO level compared to vehicle group, effect was observed between acamprosate treated groups. In this paper we have presented evidence that a known anticonvulsant properties. Acamprosate can be effective in suppressing seizure in *Drosophila menanogaster*. The technique we have described here suggests that a simple feeding procedure can be used to assay anticonvulsant activity.

Conclusion

Different concentration of acamprosate is, ameliorate BS (bang sensitive) behavior like seizure in *Drosophila melanogaster*. The similarities between epilepsy and bs behavior, as well as the effects of these drugs in both systems, suggest some common underlying mechanisms. This and other studies of these fliess lay the groundwork for the use of BS (bang sensitive) behavior as a model for epilepsy. This system can be further utilized to understand the basis of hyperactivity in the nervous system, to explore the mechanism of AED (Antiepileptic drug) action, and possibly to test candidate AEDs (Antiepileptic drugs). We found that the drug (acamprosate) can significantly reduce the recovery period of the most "sensitive" of the BS (bang sensitive) flies.

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References

1. Akdogan I, Adiguzel E, Yilmaz I, Ozdemir MB, Sahiner M, Tufan AC. Penicillin-induced epilepsy model in rats: dose- dependent effect on hippocampal volume and neuron number. Brain Res Bull. 2008; 77(4):172-177.
2. Dos Santos Branco C, Scola G, Rodrigues AD, Cesio V, Laprovitera M, Heinzen H, Salvador M. Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate (Ilex paraguariensis St. Hil.) on pentylenetetrazol-induced seizures in Wistar rats. Brain Res Bull. 2013; 92:60-68.
3. http://www.who.int/mediacentre/factsheets/fs999/en/. WHO Media centre and Media centre Epilepsy, Fact sheet N°999, October 2012 (Downloaded at 20/01/2015, PM 05:15).
4. Dannon PN, Rosenberg O, Schoenfeld N, Kotler M. Acamprosate and baclofen were not effective in the treatment of pathological gambling: preliminary blind rater comparison study. Front Psychiatry. 2011; 33(2):1-4.
5. Pierrefiche O, Daoust M, Naassila M. Biphasic effect of acamprosate on NMDA but not on GABA A receptors in spontaneous rhythmic activity from the isolated neonatal rat respiratory network. Neuropharmacol. 2004; 47(1):35-45.
6. Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. FASEB J. 2002; 16(1):27-35.
7. Sternberg Z, Cesario A, Rittenhouse-Olson K, Sobel RA, Pankewycz O, Zhu B. *et al.* Acamprosate modulates experimental autoimmune encephalomyelitis. Inflammopharmacol. 2012; 20(1):39-48.
8. Oka M, Hirouchi M, Tamura M, Sugahara S, Oyama T. Acamprosate {monocalcium bis (3-acetamidopropane-1-sulfonate)} reduces ethanol-drinking behavior in rats and glutamate-induced toxicity in ethanol-exposed primary rat cortical neuronal cultures. Eur J Pharmacol. 2013; 718(1):323-331.
9. Cho KS, Bang SM, Toh A. "Lipids and Lipid Signaling in Drosophila Models of Neurodegenerative Diseases". In: Watson RR, De Meester F. (eds.) Omega-3 Fatty Acids in Brain and Neurological Health. Elsevier; 2014, 327.

10. Sang TK, Jackson GR. Drosophila models of neurodegenerative disease. Neuro Rx. 2005; 2(3):438-446.
11. Burg MG, Wu CF. Mechanical and temperature stressor-induced seizure-and-paralysis behaviors in Drosophila bang-sensitive mutants. J Neurogenet. 2012; 26(2):189-197.
12. Song J, Parker L, Hormozi L, Tanouye MA. DNA topoisomerase I inhibitors ameliorate seizure-like behaviors and paralysis in a Drosophila model of epilepsy. J Neurosci. 2008; 156(3):722-728.
13. Bland ND, Robinson P, Thomas JE, Shirras AD, Turner AJ, Isaac RE. Locomotor and geotactic behavior of Drosophila melanogaster over-expressing neprilysin 2. Peptides. 2009; 30(3):571-574.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J biol Chem.1951; 193(1):265-275.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358.
16. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; (47):389-94.
17. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978; 186(1):189-195.
18. Mittal R, Gonzalez-Gomez I, Goth KA, Prasadarao NV. Inhibition of inducible nitric oxide controls pathogen load and brain damage by enhancing phagocytosis of Escherichia coli K1 in neonatal meningitis. Am J Pathol. 2010; 176(3):1292-1305.
19. Tan JS, Lin F, Tanouye MA. Potassium bromide, an anticonvulsant, is effective at alleviating seizures in the Drosophila bang-sensitive mutant bang senseless. Brain Res. 2004; 1020(1):45-52.
20. Reynolds ER, Stauffer EA, Feeney L, Rojahn E, Jacobs B, McKeever C. Treatment with the antiepileptic drugs phenytoin and gabapentin ameliorate seizure and paralysis of Drosophila bang-sensitive mutants. J neurobiol. 2004; 58(4):503-513.
21. Verma RK, Haque SE, & Pillai KK. Cactus grandiflorus, a homeopathic preparation has protective effect against doxorubicin induced cardiomyopathy in rats. International Journal of Phytopharmacology. 2012; 3(3):281-290.
22. Muceniece R, Zvejniece L, Kirjanova O, Liepinsh E, Krigere L, Baumane L, Dambrova M. β-and γ-melanocortins inhibit lipopolysaccharide induced nitric oxide production in mice brain. Brain Res. 2004; 995(1):7-13.