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Autistic Effects of Nootropic Drug *Brahmi* against Propionic Acid-induced Behavior and Memory Impairment in Rat Model

P. K. Anamika[1] and P. Muralidharan[1*]

[1]Department of Pharmacology, C L Baid Metha College of Pharmacy, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author PM guided author PKA in planning and scheming the investigation. Author PM also helped in spacing the whole facilities for the research and supervised the whole research study. Author PKA led the entire laboratory works imparted in study design and interpreted the outcomes tapping efforts into statistical analysis with the guidance. Authors PM and PKA participated in the manuscript draft and have thoroughly checked and revised the manuscript for necessary changes in format, grammar and English standard. Both authors read and agreed on the final version of the manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the hydro alcoholic effect of *Brahmi* against propionic acid-induced behavior and memory impairment in rat model.
Study Design: This includes preclinical study on Sprague Dawley rats in which Propionic acid induced and evaluation of *in vivo* and *in vitro* models were performed.
Place and Duration of Study: Department of Pharmacology, C L Baid Metha College of Pharmacy Jan 2019 to June 2021.
Methodology: We include a total of 27 adult Sprague Dawley Rats and induced propionic acid intracerebroventricular route to induce autism. Drug treatment using hydro alcoholic extract of *Bacopa monnieri* was given in 250 mg/kg and 500 mg/kg was compared with negative group rats and control groups. *In vivo* parameters like Actophotometer and marble burying test was done, *In vitro* analysis of Serotonin and Glutamate was estimated in the above treated groups.

E-mail: anamikapk2191@gmail.com;

Results: The locomotor activity of rat was recorded individually for each animal using Actophotometer. HAEBM (250 mg/kg and 500 mg/kg) treated rat produced an increase in the level of significance (P<0.0001) on day one. In marble burying test Rats were located for thirty minutes in a standard cage covered with 5 cm depth of wood chip bedding with ten marbles evenly spaced. HAEBM (250 mg/kg and 500 mg/kg) showed significant (P<0.001) level of burying when compared to group-II rats (P<0.01). In this research study 5HT level showed a significant (P<0.001) increase in Group III, Group IV when compared with group-II (P<0.01). Glutamate is an excitatory Neurotransmitter. Group II showed significant increase (P<0.001) in the level of Glutamate but on drug treated groups III and IV shows decrease in concentration of glutamate.
Conclusion: The present study findings showed that the hydro alcoholic root extract of *brahmi* possesses neuroprotective activity with significant nootropic effects. The hydro alcoholic root extract of *Bacopa monnieri. L* showed the presence of various Gabapentin and flavonoids phenols may be the reason for its neuroprotection and memory improvement effects.

Keywords: Autism; Bacopa monnieri; actophotometer; neuro transmitter; serotonin.

1. INTRODUCTION

The Latin word *autismus* (*autism*) was invented by the Swiss psychiatrist Eugen Bleuler in the year 1910 as he was defining the symptoms of schizophrenia. He derived it from the Greek word *autos* meaning "self", and used it to mean morbid self-admiration, referring to "autistic drawing of the patient to his imaginations, against which any influence from outside becomes an intolerable disturbance [1]."About 18 months, half of parents of children with ASD notice their child's unusual behaviors, and about four-fifths notice by age two year [2]. Delay in referral for ASD testing may delay early diagnosis and treatment and affect the long-term outcome" [3]. No reply to name (or eye-to-vision gaze) by 180 days. No sign of babbling by 12 months. Not pointing and waving by one years. Not speaking a single word by 16 months. Not speaking two-word phrases by 24 months. Loss of communal skills, at any age.

The human cerebellum is one of the key regions of brain affected by Autism. Cerebellum and Brainstem located under the cerebrum [4]. Its function is to coordinate muscle movements, maintain the posture, and balance resulting in smooth and balanced muscular activity [5]. It is also important for learning motor behaviors. The Brain consists of cerebellum, cerebrum and brain stem [6].

It is a serious disorder in developing children which impact on nervous system that impairs the ability to affect overall health of the affected person emotionally, socially and physically [7]. The prevalence of autism spectrum disorder in 2021 people of all races and socio-economic groups are impacted by ASD. Globally 1 in 160 people is thought to have autism.

Autism spectrum disorder (ASD) is one of the neuro developmental disability that impairs the ability to communicate and interact with others including restricted repetitive behaviors, interests and activities [8]. Impairments of the growth and development of the brain or central nervous system. Genetic or acquired biological brain disorder or condition is responsible for childhood-onset brain dysfunction [9]. Affects emotion, memory, learning ability, self-control and unfolds as individual grows. The disorder alarms its prevalence all over the world [10].

Bacopa monnieri L. is a Perennial creeping herb used in ayurvedic treatments. Also known as water hyssop which comes under the family Plantaginaceae native to countries such as Southern & East India, Europe, Africa North & South America. Pharmacological activities are Epilepsy, Asthma, Leprosy, Anemia, Tamil name of the above is Neerbrahmi [11,12].

2. MATERIALS AND METHODS

2.1 Materials

Chemicals-Propionic acid and neurotransmitters estimation kits (neuro transmitter Assay Colorimetric kit, Serotonin ELISA kit) were purchased from Sigma-Aldrich–Merck, Bengaluru, India, whereas all analytical grade chemicals and reagent were used.

2.2 Collection and Authentication

The Plant *Bacopa Monnieri* were collected from SSP Herbs Marthandam, Tamilnadu in the month of December 2018. The plant material was identified and authenticated by: Professor P. Jayaraman., PhD, Director Herbal Plant Anatomy

Research Centre, Tambaram, Chennai-600045 through morphology, taxonomy and microscopical method as: *Bacopa monnieri* (L.) Authentication Certificate No: PARC/2019/4049.

2.3 Methods of Preparation and Extraction [13,14]

The fresh plant of *Bacopa monnieri. L* was collected and removed all organic particles and earthy matters present in it. It was shade dried at room temperature. The dried plant material was made into coarse powder. The powder was extracted with (80:20) of ethanol and water in Soxhlet extractor.

The grounded powder was extracted by Soxhlet apparatus with ethanol and water (80:20) in water bath at room temperature for 24 hours. The solvent was then removed by filtration and fresh solvent was added to the plant material. The extract process was twice repeated, the combined filtrates were then evaporated to give dark green viscous mass and subsequently the extract was stored at 0-4^{0}c.

Total of twenty-seven rats were used for the study. All the rats were implanted by a cannula by surgical method prior to the procedure of study. On 15th day post-surgery, the rats were stratified in to four distinct groups containing six *Sprague Dawley* rats each group.

Group I: Control (Saline Treated 0.9%NaCl)

Group II: Negative group/Untreated +PPA

Group III: Received hydro alcoholic extract of *Bacopa monnieri (*HAEBM) at dose of 250 mg/kg -suspended in 0.9%NaCl +PPA

Group IV: Received hydro alcoholic extract of *Bacopa monnieri (*HAEBM) at dose of 500 mg/kg -suspended in 0.9%NaCl +PPA.

The vehicles and the extract were administered orally using an intragastric tube daily for 28 days. After 21 days of extract administration, each group of animals except Group I received intracerebroventricular (ICV) infusions of propionic acid (PPA) (4.0 µl of a 0.26 M solution PPA was dissolved in phosphate-buffered saline (PBS) vehicle) daily for 7 consecutive days (between 22nd and 28th day). Group I rat received ICV infusions PBS (4.0 µl of 0.1 M PBS) between 22nd and 28th day.

Before infusion of the propionic acid (PPA) ICV [15], it was dissolved in suitable vehicle. Phosphate-buffered saline (PBS) was used as vehicle which was buffered to pH 7.5 by means of concentrated hydrochloric acid and sodium hydroxide. All the animals received ICV infusions of PPA (4.0 µl of a 0.26 M solution) and the Group I rat received ICV infusions PBS (4.0 µl of 0.1 M PBS).

The infusions were given twice daily for 7 successive days (between 22nd and 28th days). The infusion duration was about 60 sec, but an additional 60 sec was allowed for the infusion cannula to persist in the place before being detached. On day 22 to day 28 assessment of habituation behavior and memory assessments were conducted following the infusion of PPA.

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Test [15,16,17]

Preliminary phytochemical screening of Hydro alcoholic extract of *Bacopa monnieri* were conducted and found in HAEBM absence of terpenoids, quinones and glycosides were confirmed.

3.2 Acute Toxicity [18]

The acute oral toxicity study was done according to the OECD guidelines 423. A single administration of starting dose of 2000mg/kg of HAEBM to rats were observed for 14 days. No significant changes were observed for two weeks.

3.3 HR-LCMS Analysis [19,20,21]

Chromatogram of HR-LCMS analysis of Hydro alcoholic extract of *Bacopa monnieri* at positive ESI and negative ESI. The HR-LCMS study was performed for both the positive and negative mode of ionization to detect the various phytochemical constituents present in the above extracts.

In the chromatograms the positive and negative ESI of Hydro alcoholic extract of *Bacopa monnieri (L.)* was obtained and the finger prints found was interpreted. The positive ionization ESI study was detected the 57 compounds present in the Hydro alcoholic extract of *Bacopa monnieri (L.)* from the starting retention of 0.747

and ending retention time of 27.112. Among 57 compounds, 32 compounds have produced various biological activities based on literature review was given in Fig. 2.

3.4 *In vivo* Assessment of Habituation Behaviour

3.4.1 Effect of HAEBM on actophotometer [22,23]

The locomotor activity of rat was recorded individually for each animal using Actophotometer. PBS treated rat showed significant (P<0.0001) level of locomotion response when compared to negative group rat (P<0.05). HAEBM (250 mg/kg and 500 mg/kg) treated rat produced an increase in the level of significance (P<0.0001) on day one.

The HAEBM (250mg/kg and 500mg/kg) treated rat significantly (P<0.0001 and P<0.0001) increased the locomotion at day 2 and day 3 but in day 1 it did not produce significant difference in locomotion when compared with negative group. Locomotor activity of animals in all groups were recorded. The PPA induced animals developed Autism after treatment. Values plotted on the mean ±SEM (n=6) significant level were analyzed by using two-way ANOVA followed by Dunnet's multiple comparison test.

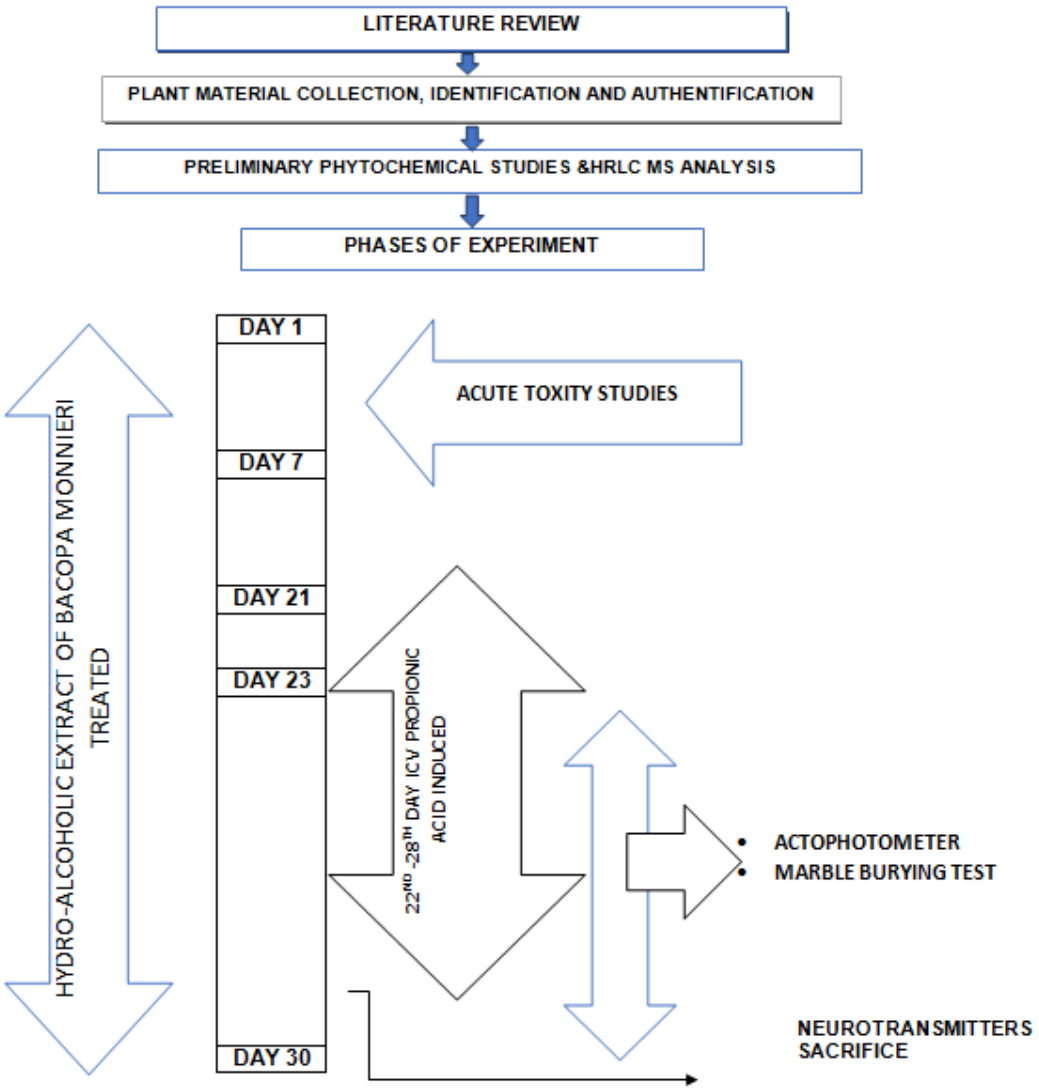

Fig. 1. Schematic diagram of the experimental design of Hydro Alcoholic extract of *Bacopa monnieri* on *Sprague Dawley* rats

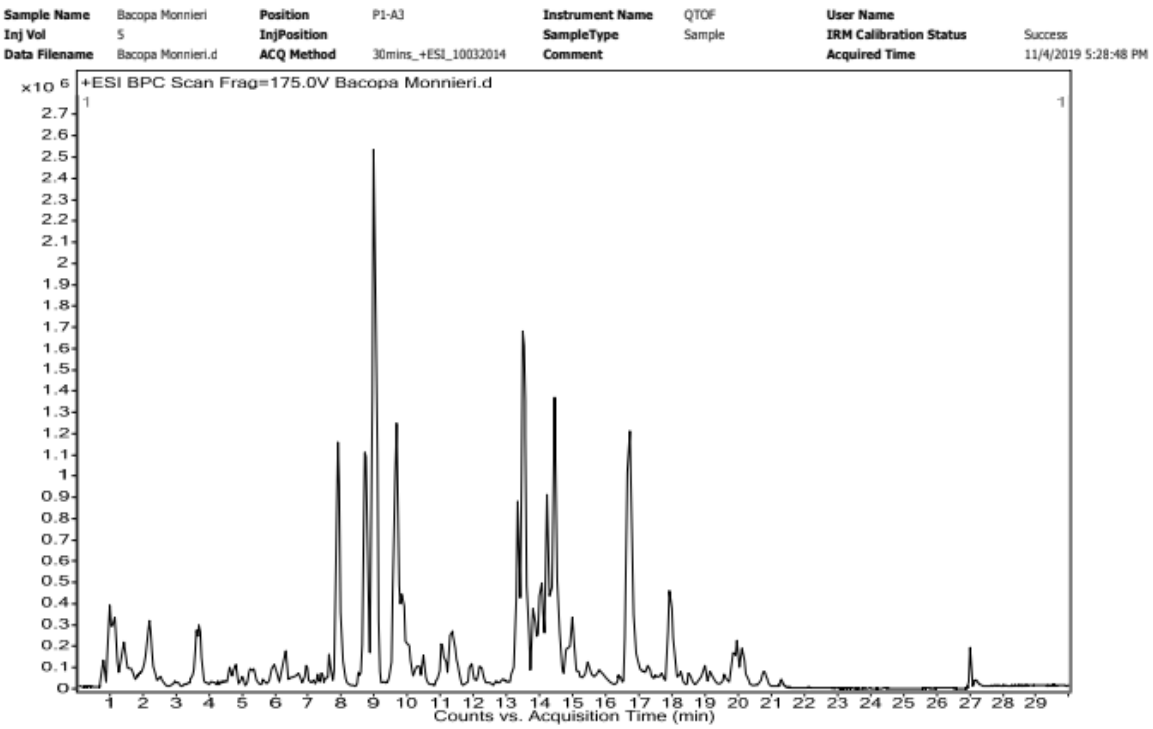

Fig. 2. High resolution Liquid Chromatography and Mass Spectrometry Chromatogram of the Hydro Alcoholic extract of *Bacopa monnieri* at positive ESI

Table 1. The locomotion activity of rat using actophotometer

Grouping	Day-1	Day-2	Day-3
Control	127.6±11.240	112.4±6.34	129.4±2.72**
Negative control	148.6±6.34	141±1.472	149.8±0.64
Low Dose (200 mg/kg.)	176.80±10.18	169.8±4.45****	182.0±4.10***
High Dose (400 mg/kg.)	200.50±11.48*	195.80±3.19****	196.00±6.15****

**Comparison of locomotion activity using Sprague Dawley rats for three days*

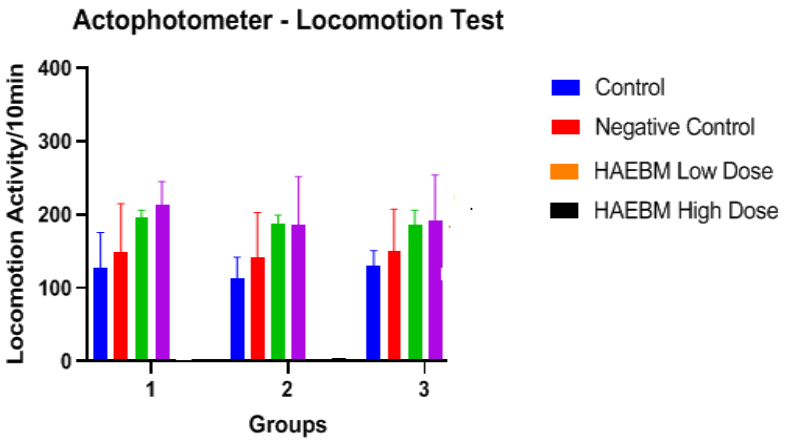

Fig. 3. Graph obtained by HAEBM on locomotion activity

**PPA induced ASD on Test drugs HAEBM 250mg/kg low dose and HAEBM 500mg/kg high dose.*

3.4.2 Effect of HAEBM on marble burying test [24,25]

Rats were placed for thirty minutes in a standard cage filled with 5 cm depth of wood chip bedding with 10 marbles evenly spaced. It was important to keep the laboratory quiet while performing this experiment to avoid unintended results. Younger rat buried marbles faster than the older ones. HAEBM (250mg/kg and 500mg/kg) showed significant (P<0.001) level of burying when compared to group-II rats (P<0.01).

3.4.3 Effects on various neurotransmitters levels

3.4.3.1 Effect of HAEBM on serotonin levels [26,27,28]

Altered 5-HT (5-Hydroxy tryptamine) system in brain is one of the key findings associated with Autism. The study results showed an increased concentration of serotonin in the brain tissue homogenates of Group II rats as compared with Group I animals. The HAEBM treatment at two dose levels 250 mg/kg and 500 mg/kg (Groups III and IV, respectively) exhibited a significant ($p < 0.05$ and $p < 0.0001$) decrease in the level of brain serotonin in comparison with Group II animals. Results obtained are picturized in Fig. 5.

Serotonin/5HT is an inhibitory neurotransmitter and a key hormone in modulation of mood and cognition. In this research study 5HT level showed a significant (P<0.001) increase in Group III and Group IV, when compared with group-II (P<0.01). High dose shows significantly decreased when compared to negative control and low dose shows significant decrease in the level of serotonin when compare to negative control.

Table 2. The effect of HAEBM activity of rat using Marble Burying test

Grouping	Day-1	Day-2	Day-3
Control	273.6±11.24	292.4±6.34	272.4±2.72**
Negative control	255.6±6.34	296±1.472	249.8±0.64
Low Dose (200 mg/kg.)	236.80±10.18	239.8±4.45****	239.0±4.10***
High Dose (400 mg/kg.)	274.50±11.48*	258.80±3.19****	276.00±6.15****

**Test dose HAEBM 250mg/kg and 500mg/kg was compared with negative group II*

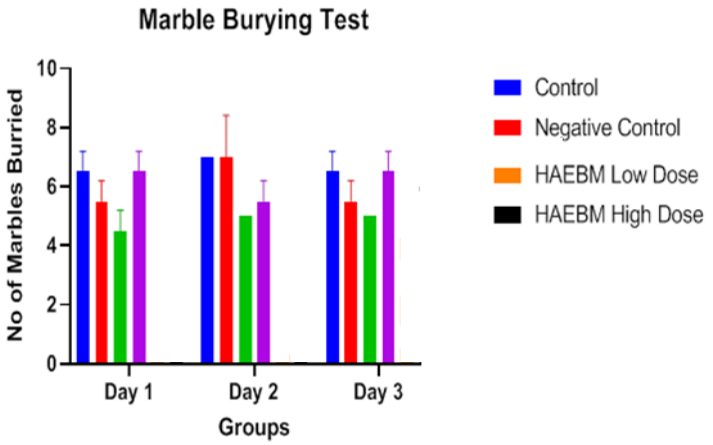

Fig. 4. Graph obtained by HAEBM on locomotion activity

**PPA induced ASD on Test drugs HAEBM 250mg/kg low dose and HAEBM 500mg/kg high dose.*

Table 3. The effect of HAEBM Serotonin

Group	Serotonin
Control	125.56±1.15
Negative control	200.25±2.05
Low Dose	180.15±0.48
High Dose	120.22±1.34

**Test dose HAEBM 250 mg/kg and 500mg/kg was compared with negative group II*

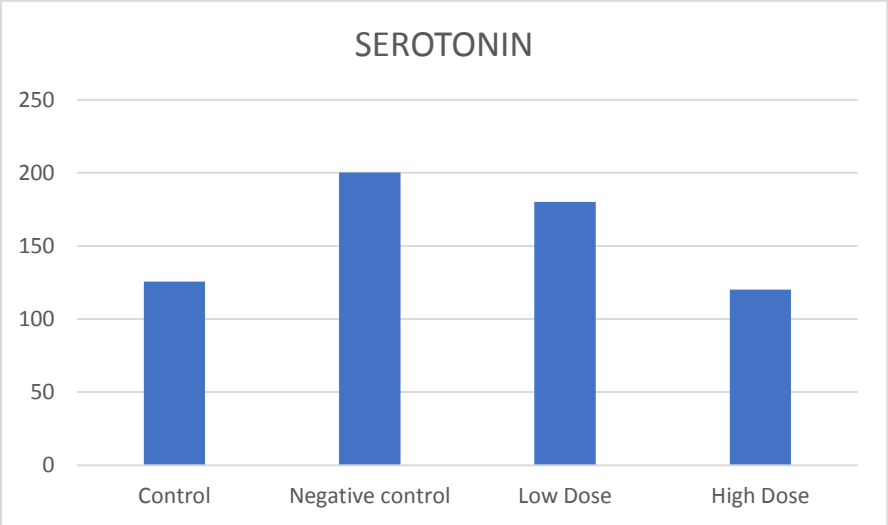

Fig. 5. Graph obtained by HAEBM serotonin

3.4.4 Glutamate [29,30,31]

Glutamate is an excitatory Neurotransmitter. Group II showed significant increase($P<0.001$) in the level of Glutamate but on drug treated groups III and IV shows decrease in concentration of glutamate.

3.4.5 Effect of HAEBM on GLUTAMATE levels

Glutamate is one of the excitatory neurotransmitters well known for its prominent role in memory and cognitive skill development in humans/animals. PPA infusion in High dose shows significantly increased when compared to negative control and low dose shows significant decrease in the level of Glutamate when compare to high dose ($P < 0.0001$ for both the groups) decrease in the level of brain. Glutamate in comparison with Group II animals. Results obtained are picturized in Fig. 6.

Table 4. The effect of HAEBM on Glutamate

Group	Glutamate
Control	221.25±1.67
Negative control	66.12±0.94
Low Dose	99.14±1.03
High Dose	121.22±0.81

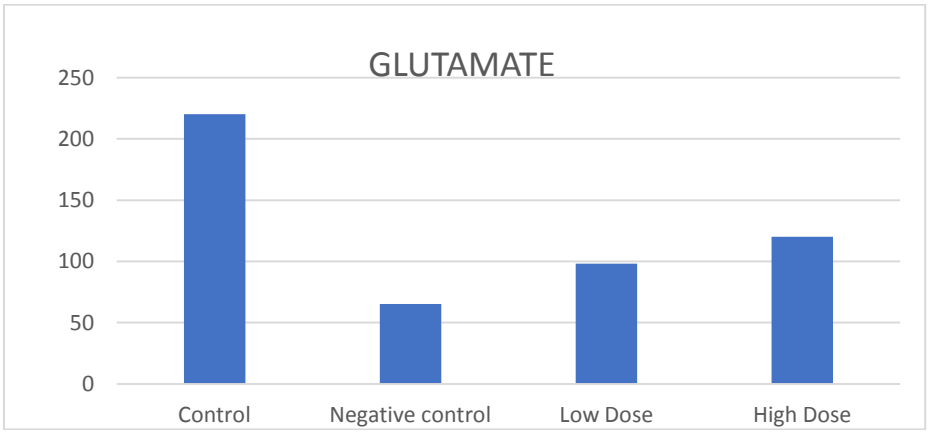

Fig. 6. Graph obtained by HAEBM on glutamate
**Effect of HAEBM on propionic acid induced ASD rats*

3.5 Statistical Analysis [32,33,34]

The statistical analysis was performed using Graph pad prism software 9.0. All the results were expressed as mean±SEM. The data were analyzed using one-way analysis and two-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test was used for comparison.

4. DISCUSSION

In conclusion the effect of *Bacopa monnieri* on its toxicological and pharmacological action on autism spectrum disorder in animal models" revealed more significant evidence-based data. Identification of components from plant extracts using HRLCMS technique showed neuro related activity and proved its neuroprotective effects. Treatment with low and high dose of *Bacopa monnieri* was effective in improving the memory including investigative Autism screening and learning memory in animals. This is further supported by the performance of animal belonging to treatment group in various memory and learning related task like Marble burying test, Actophotometer provided during *in-vivo* studies. The literature study observes the capability of *Bacopa monnieri* to hinder the release of pro-inflammatory cytokines, the protected cells of the brain that contribute in inflammation in the CNS. The effect of Bacopa on beckoning enzymes associated with CNS inflammatory pathways was also studied using the Pro inflammatory markers.

Data's collected from the *in vitro* neurotransmitters and neuroinflammation, Antioxidant Enzymes, Histopathological estimation further strengthen the efficacy of the drug. It is observed that treatment with the effect of *Bacopa monnieri* significantly improved the level of Serotonin and Glutamate. The present study findings showed that the ethanolic root extract of *Bacopa monnieri L.* possesses neuroprotective activity with significant nootropic effects. The levels of serotonin and glutamate in the autistic rat brain mainly shows the effects. Preliminary phytochemical analysis and HR LCMS (High-Resolution Liquid Chromatography–Mass Spectrometer) analysis of ethanolic root extract of *Bacopa monnieri L.* showed the presence of various anthocyanin and flavonoids polyphenol; these may be the reason for its neuroprotective and memory enhancement effects.

5. CONCLUSION

Hydroalcoholic extract of *Bacopa monnieri* for the propionic acid induced ASD had designed in this paper. Learning behavioural studies is a pre-clinical phase for adult Sprague Dawley rats was considered and Autistic rats need extra attention to improve the memory skills; the training has done to enhance its memory functioning. The research study design was striking for autistic animals, though the special training had given new aspects could be the future scope of the application. However, further research should be carried out in *Bacopa monnieri L.* to isolate the possible phytoconstituents and evaluate the same to find out their actual mechanism for neuropharmacological effects.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Before conducting the animal experiments and procedures hereby declare that the animal study was approved by the Institutional Animal Ethical Committee of C.L. Baid Metha College of Pharmacy, Chennai, India. Approval No: IAEC NO: 05/321/PO/Re/S/01/CPCSEA.All experiments have been examined and approved by the Ethical Committee.

RESEARCH SIGNIFICANCE

The study highlights the efficacy of "Ayurvedic" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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