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Novel research on Neuroprotective effect of *Bacopa monnieri* L. against propionic acid induced investigation on autism in Sprague Dawley rats

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***Abstract*---**Autism spectrum disorder (ASD) is characterized by complex behavioral and memory impairments resulting from abnormal brain development. The research objective is to identify the lack of understanding of the etiology of autism. The Ayurvedic system of medicine recognizes Bacopa monnieri for their potential nootropic activity to maximize brain and neuronal processes such as learning and memory. Brahmi, Bacopa monnieri has been demonstrated in Ayurvedic preparation as memory booster medicine. The neuroprotective properties of hydroalcoholic extract of Bacopa monnieri against propionic acid-induced neuroprotective effect in autistic rat models were investigated in the present study. The animal species used in the study are adult Sprague Dawley rats of both sexes were divided into four groups and administered the vehicle/extract for 28 days. Between the 22nd and 28th days of the trial, autism was caused by an intracerebroventricular infusion of propionic acid. Rats were treated to the fundamental methods such as different in vivo behavior and memory evaluation procedures during this infusion period, including the actophotometer test and the Morris water maze test. Animals were euthanized on the 29th day of the investigation to obtain brain material. Extracted brain tissues were used to calculate levels of neuroinflammatory markers (TNF-α) and histological examination. The principle finding of this work is to identify the behavioural changes in normal and PPA induced rat models. Preclinical Investigation on Sprague Dawley rats included a preclinical study on Sprague Dawley rats in which Propionic acid was induced

and *invivo* and *invitro* models were evaluated. Pre-treatment of rats findings with extract at two dose levels (250 mg/kg and 500 mg/kg) shows significant reduction in the neuroinflammation, memory and cognitive impairment formed by the propionic acid-induced model in a dose-dependent manner. The findings revealed that Bacopa monnieri L has considerable neuroprotective and anti-inflammatory properties against propionic caused autism. Social impairment, communication and language struggles, and a limited set of interests and activities that are both unique to the individual and oriented predictably constitute ASD. Other neurological disorders that as intellectual impairment (ID), global development delay (GDD), and epilepsy can frequently be comorbid with autism spectrum disorder (ASD). ASD does have a multifaceted aetiology.

***Keywords*---**Autism Spectrum disorders, *Bacopa monnieri*, Brahmi, Memory impairment, Neuroinflammation, Dysregulation, Immunologic.

1. Introduction

Autistic (or autism spectrum disorders, ASD) is characterized by social and communication issues and repetitive and restricted behaviors that vary in severity amongst individuals (Kargas N *et al.*, 2015). Autism can be recognized as early as 18–24 months of age, whenever the hallmark symptoms of autism can be distinguished from typical development and other delays or developmental conditions (Baker-Ericzén MJ et al., 2021). Immune dysregulation has been identified as a characteristic of ASD. The strongest information for this criticism comes from several researches conducted over the last four decades that have discovered a common compromised immunological response in people with ASD (Haapakoski, R *et al.*, 2015). This review aims to find immunological biomarkers that can be used to investigate immune dysfunction in ASD diagnosis and treatment (Rossignol DA *et al.* 2012). As a result, a unique concept addressing probable intercommunication between the brain, immune system, stomach, and commensals has recently developed (Moradi K *et al.*, 2021). In this paper, we give an overview of how gut bacteria and their metabolites are linked to ASD neurobehavioral characteristics via multiple immunologic processes (Liu F *et al.*, 2019). It is clear from this review assessment that a thorough description must be provided to understand the process of the induced autistic model. The various approaches are described, the pathophysiology is explained, and numerous therapy strategies were investigated in the propionic-induced animal model (Ghosh A *et al.*, 2013). ICV administered PPA in rats' cause's considerable alterations in body weight, motor behavior, neuromuscular coordination, and immobility duration, according to recent results (Sharma R *et al.,* 2019).

On the other hand, chronic PUR therapy demonstrated a significant change in the dose-dependent behaviour of PPA mediated alteration, with the higher dose of PUR having more significant effects than the lower dose. During injection days, large doses of PUR. The significant body weight changes, locomotion, and

decreased number of slips, enhanced mobility time, and reduced immobility in rats more successfully than low doses (Gupta R *et al.,* 2022). This review combines behavioural research with neuromolecular mechanisms that may be involved in Bacopa monnieri (BM), a medicinal Ayurvedic herbal toxicity with cognition boosting activity (Nandy S *et al.*, 2020).

Brahmi Ghrita (BG) is a polyhedral composition comprising Bacopa monnieri that's still mentioned in Ayurveda (an Indian system of medicine). Bacopa monnieri (8 g), Acorus calamus (4 g), Evolvulus alsinoids (4 g), Saussurea lappa (4 g) and cow's ghee (80 g) (Sriranjini *et al.*, 2015).

This formulation has traditionally been used as a memory enhancer and for anticonvulsant characteristics 12 tested the effect of BG (30, 50 and 100mg/kg, p.o.) on learning and memory processes. The effects of BG on memory acquisition and retention in rats were investigated (Gohil KJ *et al.*, 2010). The elevated plus maze paradigm (EPM) and the Morris water maze model (MWM) (Achliya GS *et al.* 2004). Brahmi is used to improve memory, cognition, mood, and other mental issues. Brahmi contains saponins, which are the main chemicals responsible for improving nerve impulse transmission (Devendra P *et al.*, 2018). Bacopa monnieri is an antioxidant that can be used in conjunction with other suggested and established medicinal remedies such as nervine tonic, cardio tonic, cognitive enhancer, and alterative (Bui TT *et al.*, 2017). Autism prevalence estimates, taking into account the impact of geographic, ethnic, and socioeconomic characteristics. Around the world, 1 in 100 children is diagnosed with autism spectrum disorder (Zhou H et al., 2020). Estimates of prevalence grew over time and varied significantly inside and across sociodemographic groups (Satoh M *et al.* 2012). These findings reflect variations in the definition of autism as well as variances in prevalence study methods and circumstances. This study hypothesis looked at the neuroprotective impacts of Hydroalcoholic extract of Bacopa monnieri (HAEBM) against PPA induced neuroinflammation in an autistic rat model (Anamika K & Muralidharan, P, 2015).

2. Materials and Methods

2.1. Collection and extraction of Bacopa monnieri

2.1.1. Plant authentication approval

Bacopa monnieri was collected from SSP herbs Marthandam, Tamilnadu, India. *Bacopa monnieri* was authenticated by Indian taxonomist Prof. P. Jayaraman, Ph.D., Director, Plant Anatomy & Research Centre, West Tambaram, Chennai. The Department of Pharmacognosy C.L.Baid Metha College of Pharmacy holds a voucher specimen (SES.CLBM.NO. 1460) Herbarium at Chennai, India .Authenticated certificate number: PARC/2019/4048

Preparation of Bacopa monnieri extract

It was shade dried, Coarsely Powdered using ethanol and a Soxhlet device, the powdered components were subjected to a hot continuous extraction (Tamboli FA *et al.* 2018). The extract was collected and condensed using a rotarat vacuum evaporator after mild heating. The concentrated extract was then weighed, the percent-age yield determined, and the extract was stored. According to OECD

guidelines animal studies done. A variety of preliminary phytochemical studies were performed on the extract (Moriasi GA, *et al.*, 2020). HR LCMS were performed on the plant extract Chemicals such Propionic acid, Sigma Aldrich–Merck, Bengaluru, India, provided estimate kits (TNF–ELISA Kit), as well as other chemicals and reagents, which were transported by Southern India Scientific Corporation, Kandanchavadi, Chennai, India. All of the chemicals and reagents used in this experiment were of analytical purity.

2.1.2. Ethics approval of research

A total of 27 adult healthy Sprague Dawley Rats of 200-250g were used in the study was procured from Tamilnadu Veterinary and Animal Sciences University, Chennai. All animal studies were performed after getting approval from the Institutional animal ethical committee with an approved reference IAEC NO: 05/321/PO/Re/s/01/CPCSEA, also possible measures were taken to minimize the suffering of animals and housed in animal house of C.L.Baid Metha College of Pharmacy and kept under light dark cycle for 24 hours with ad libitum.

Experimental design

Rats used an intracerebroventricular method to generate autism. Negative group rats and control groups were compared to drug treatment using hydro alcoholic extract of Bacopa monnieri in doses of 250mg/kg and 500mg/kg. Invivo parameters such as the actophotometer and the marble burying test were performed, as well as an in vitro investigation. The experiment used a total of twenty-four rats and three rats were used for acute toxicity studies.

All of the rats had a surgical procedure for cannula implantation prior to the start of the trial. Prior to the experimental procedure, all of the rats were surgically implanted with a cannula. On the 15th day after surgery, the rats were divided into four groups, each containing six Sprague Dawley rats. (21)The rats were divided into four groups, each including six Wistar rats, after 14 days of operation. The rats were assigned to the following groups of six rats each.

Control (Saline) Group I, 0.9 percent NaCl treatment), Group II: Untreated +PPA/Negative group, Group III was given a 250 mg/kg dosage of hydro alcoholic extract of Bacopa monnieri (HAEBM) suspended in 0.9 percent NaCl +PPA.Group IV was given a 500 mg/kg dosage of hydro alcoholic extract of Bacopa monnieri (HAEBM) suspended in 0.9 percent NaCl +PPA.

For 28 days, the vehicles and extract were given orally using an intragastric tube. After 21 days of extract administration, all groups of animals received daily intracerebroventricular (ICV) infusions of propionic acid (PPA) (4.0 l of a 0.26 M solution PPA dissolved in phosphate-buffered saline (PBS) vehicle) for 7 days (between 22nd and 28th day). ICV infusions in PBS (4.0 l of 0.1 M PBS) were given to Group I rats. Before infusion, the propionic acid (PPA) ICV was dissolved in a suitable medium. Phosphate-buffered saline (PBS) was used as the vehicle, which was pH 7.5 after being buffered with strong hydrochloric acid and sodium

hydroxide. All of the animals were administered PPA (4.0 l of a 0.26 M solution), while the Group I rat was given PBS (4.0 l of 0.1 M PBS).
For seven days in a succession, the infusions were given twice a day (between 22nd and 28th days). The infusion lasted 60 seconds, with the infusion cannula remaining in place for another 60 seconds before being removed. On days 22 to 28, following the infusion of PPA, assessments of habituation behaviour and memory were conducted.

2.2. In-Vivo Assessment Of Habituation BehaviouR

Assessment of the novel objects recognition test

The maze has four arms and is built of medium-density wooden board with a matte black acrylic top (two open without walls and two enclosed by 30 cm high walls) 50 cm in length and 10 cm in width. Although the open arms of the labyrinth we use do not have a railing, adding a 3–5 mm high railing to the plus maze's open arms has been shown to boost open arm exploration (Morris RG *et al.*, 1989).

The maze's arms are each fastened to robust plastic legs, allowing it to be elevated 50 cm off the ground. The maze's legs may be readily removed for storage when not in use, and they can also be adjusted to keep the maze level on uneven floors. The maze's base is made up of a white melamine platform with four casters that is only as big as the maze requires. This allows the maze to travel freely within and outside the testing room as needed. An independent contractor developed, built, and set up the maze and base in our laboratory, working within these restrictions.

Elevated Plus-Maze

Initially, the Elevated Plus-Maze (EPM) was used to assess anxiety in rats (Matovu D *et al.*, 2018). EPM is made up of two open arms (50 10 cm) with a 5-cm-high edge and two closed arms (50 10 cm) with 40-cm-high walls that extend from a shared center platform (5 5 cm). The whole thing is 50 cm off the ground. The EPM was steady on the floor and did not sway with the rat's locomotion. The rats were initially put on the central platform, facing the open arm. After then, a rat's activity for 5 minutes (Pahle J *et al.*, 1758). Finally, the animal actions were graded based on each animal's recorded footage. It was considered that the rat had entered the arm after placing all four limbs of each rat in the arm. When the animal was placed on the exterior side of the arm with at least two front limbs, it was assumed that the studied rat exited the arm. The percentages of time spent on the open arms and the entries associated with them are regarded as trustworthy anxiety indicators (Thorpe CM *et al.,* 2003). After each animal test, the process of cleaning the maze was completed. All of the behavioural tests were conducted one day before the animals were sacrificed, from 8:00 a.m. to 2:00 p.m., in a room with indirect lighting (Thomas RH *et al.*, 2010).

3. Results

Primary Phytochemical Examination

Preliminary phytochemical screening of Bacopa monnieri hydro alcoholic extract was carried out, and the lack of terpenoids, quinones, and glycosides in HAEBM was established.

Acute Toxicity

The OECD guidelines 423 were used to conduct the acute oral toxicity investigation. The rats were examined for 14 days after receiving a single dose of 2000mg/kg of HAEBM. For two weeks, no notable changes were detected.

HR-LCMS analysis

At positive ESI and negative ESI, chromatogram of HR-LCMS analysis of Bacopa monnieri hydro alcoholic extract. To detect the numerous phytochemical compounds contained in the above extracts, an HR-LCMS investigation was conducted in both the positive and negative modes. The positive and negative ESI of Bacopa monnieri (L.) hydro alcoholic extract were obtained in the chromatograms, and the finger prints detected were interpreted. The 57 chemicals found in the hydro ethanol extract of Bacopa monnieri (L.) were discovered using a positive ionization ESI study with a starting retention of 0.747 and an ending retention time of 27.112. Figure 2 shows that out of 57 chemicals, 32 have created varied biological activities based on a literature review.

Invivo Assessment of Habituation Behaviour

Novel Object Recognition Test

Assessment of the Novel Objects Recognition Test

The novel objects, three different small toys were placed at equidistance from each other approximately 10cm from the wall of the open-field arena. Group I rat showed increased level of recognition when compared to Group II rat. In recognition of black block Group III and Group IV rat produce equal level of significance increase in recognition when compared to Group II rat. In recognition of white block. The recognition of Group VI rats increased significantly ($p<0.01$), and the recognition of Toy was highly significant Values are represented in Mean±SEM (n=6) significant level were analysed by using two way ANOVA followed by Dunnet-s multiple comparison test**Comparison a-group II vs Group III, IV, V & VI

ns= non-significant*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

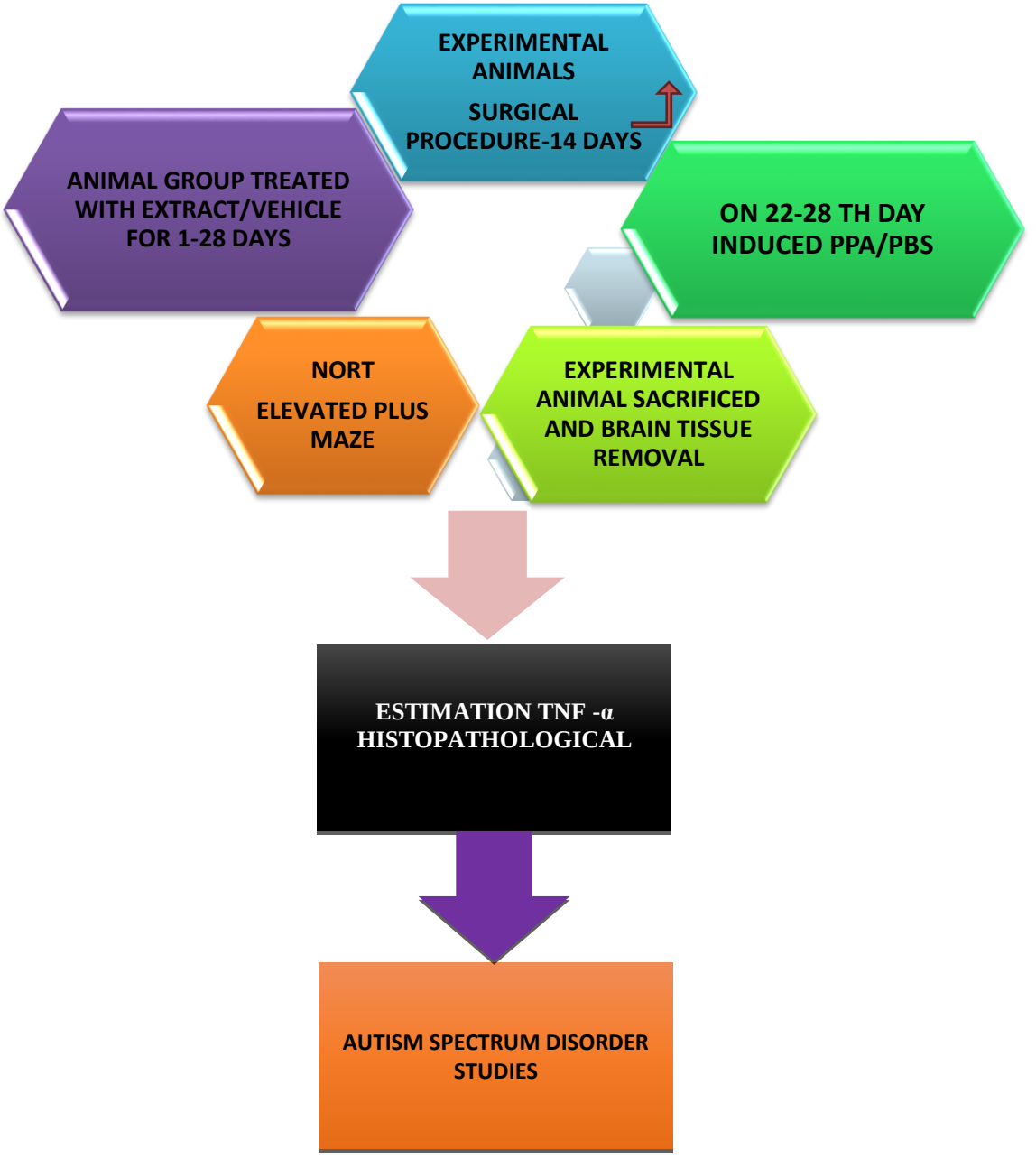


Figure 1. Schematic diagram of the Experimental design

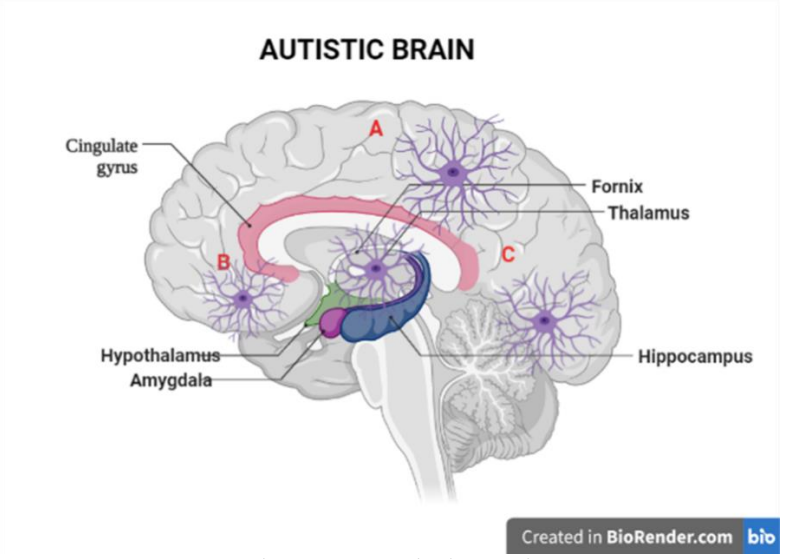


Figure 2. Autistic Brain

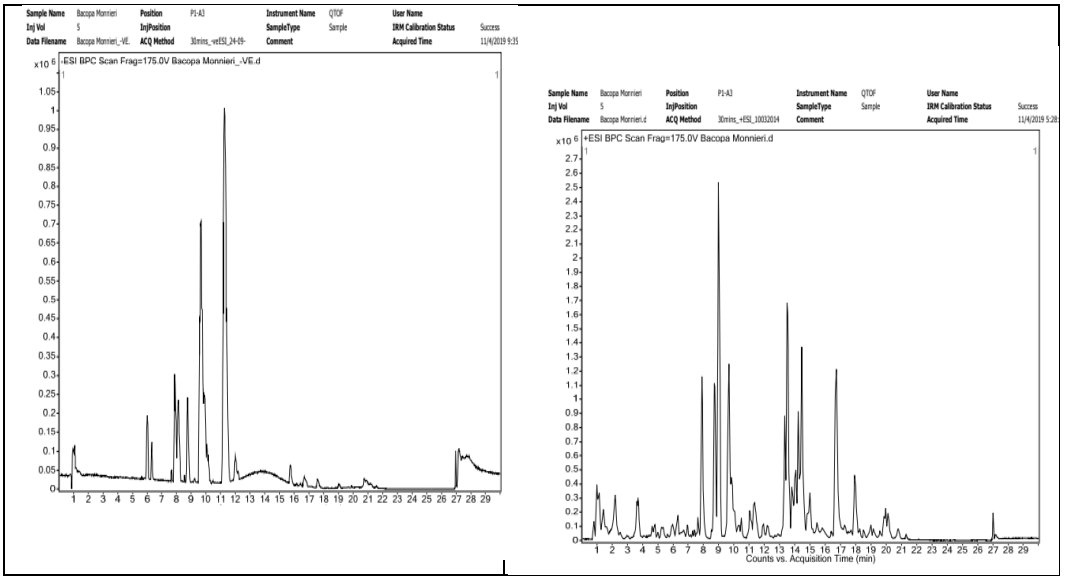
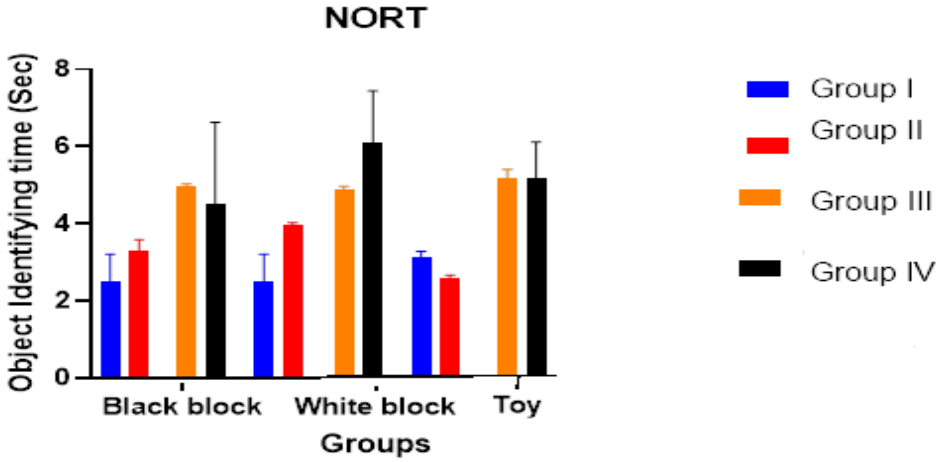


Figure3.Chromatogram of the Hydro Alcoholic Extract of Bacopa Monnieri at positive ESI and negative ESI

The positive and negative ESI of Bacopa monnieri (L.) hydro alcoholic extract were obtained in the chromatograms, and the finger prints detected were interpreted. The 57 chemicals found in the hydro ethanol extract of Bacopa monnieri (L.) were discovered using a positive ionization ESI study with a starting retention of 0.747 and an ending retention time of 27.112. Graph.1 shows that out of 57 chemicals, 32 have created varied biological activities based on a literature review

Table 1. Effect of HAEBM on NORT

S. No	Grouping	TREATMENT	Recognition Time (Secs)		
			Black block	White block	Toy
1.	Group-I	Normal saline, p.o.	2.5± 0.70ns	2.5 ±0.70ns	3.1±0.14ns
2.	Group-II	Propionic acid (PPA) (4µl of 0.26M solution, icv)	3.3±0.28	3.95±0.50	2.55±0.50
3.	Group-III	HAEBM p.o. (250mg/kg) + PPA	4.95±0.70*	4.85±0.70*	5.15±0.212*
4.	Group-IV	HAEBM p.o. (500mg/kg) + PPA	4.5±2.12**	6.05±1.34**	5.15±0.91**

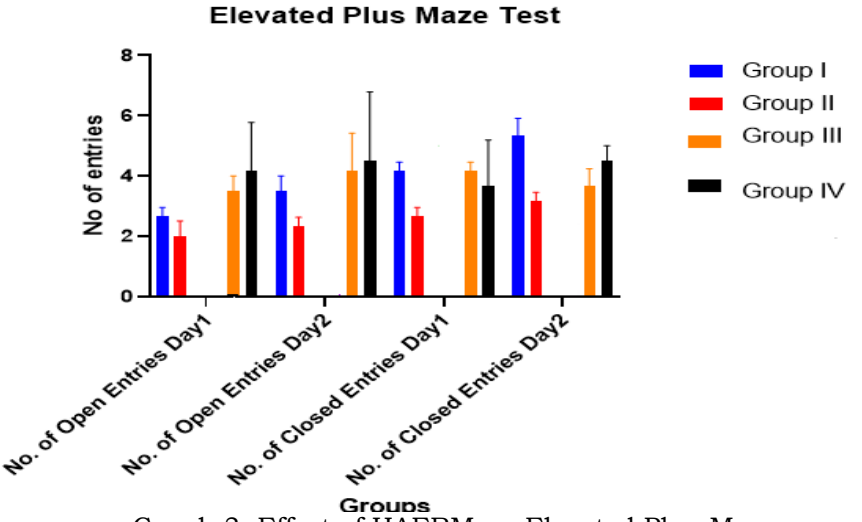


Graph 2. Effect of HAEBM on North

Table 2. Effect of HAEBM on Elevated Plus Maze

S. No	Grouping	TREATMENT	No of Entries (Secs)			
			No. of Open Entries		No. of Closed Entries	
			Day 1	Day 2	Day 1	Day 2
1	Group-I	Normal saline, p.o.	2.66±0.28	3.5±0.50	4.16±0.16	5.33±0.33
2	Group-II	Propionic acid (PPA) (4µl of 0.26M solution, icv)	2±0.5*	2.33±0.167*	2.66±0.16*	3.16±0.16*
3	Group-III	HAEBM (250mg/kg) + PPA	3.5±0.5*	4.16±0.72*	4.16±0.16*	3.67±0.33*
4	Group-IV	HAEBM(500mg/kg) + PPA	4.16±0.9**	4.5±1.3**	3.6±0.88 **	4.6±0.4**

Values are represented in Mean±SEM (n=4) significant level were analysed by using two way ANOVA followed by Dunnet-s multiple comparison test**

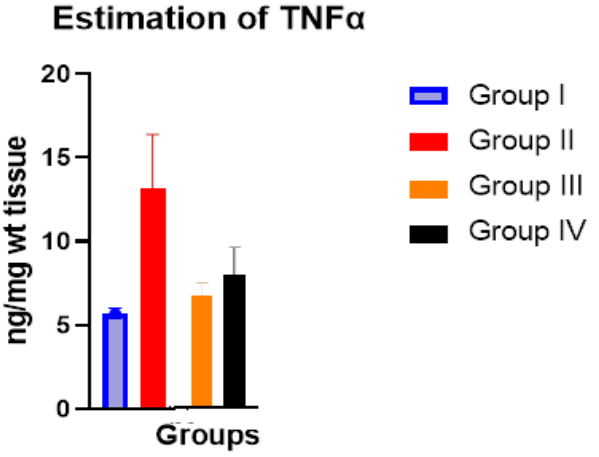


Graph 3. Effect of HAEBM on Elevated Plus Maze

Table 3. Effect of HAEBM on TNFα

S.No	Grouping	TREATMENT	ng/mg weight tissue
1	Group-I	Normal saline, p.o.	5.7± 0.3
2	Group-II	Propionic acid (PPA) (4µl of 0.26M solution, icv)	13.2±3.2
3	Group-III	HAEBM p.o.(250mg/kg) + PPA	7.9±1.10****
4	Group-IV	HAEBM p.o.(500mg/kg) + PPA	9.4±0.2****

Comparison a-group II vs Group III, IV, V & VI
ns= non-significant*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001



Graph 4. Effect of TNF-α

Values are represented in SEM± (n=4) significant level were analysed by using two way ANOVA followed by Dunnet-s multiple comparison test**
Comparison a-group II vs Group III, IV, V & VI
ns= non-significant*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

4. Discussion

PPA is a short-chain fatty acid that naturally arises in the human body as a fermentation byproduct of intestinal bacteria that are resistant to antibiotics, such as Clostridia which forms in stomach can reach brain and responsible for behavioural parameters (25).Propionic acid shows elevated level in autistic kids as per literature reviews and our study shows the extraction of hydro alcoholic extract of Bacopa monnieri shows the positive and negative esi which shows 57 components were isolated in which 32 components are having neuro action for example gabapentin. Indications of a widespread innate neuro-inflammatory response without a recognized aetiology were revealed in a recent neuropathological examination of corpse material from ASD patients of different ages. This response was characterized by reactive astrogliosis, activated microglia, and aberrant cytokine levels. Our study's findings indicated that PPA-treated rats in group II showed autistic effect and change of behavioural pattern when had compared to group I, there were no signs of neuronal death.

5. Conclusions

This research showed that *Bacopa monnieri* protected against intracerebroventricular PPA infusion from the biochemical, behavioural, and physiological perspectives. In order to determine the actual mechanism of action of the plant extract, more research should be done to isolate the potential active compounds.

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Conflict of Interest

"All the authors declare no conflict of interest."

Author Contributions

Planning and designing the research were assisted by PM. Planning and designing the research were assisted by PM. In addition, PM oversaw the entire research and assisted in setting up the facilities. APK carried out all laboratory tasks required for the study's design, analysed the findings, and worked with PM to undertake statistical analysis of the data. PM and APK took part in the writing of the manuscript and carefully reviewed and revised it to make any necessary grammatical, format, and English-standard corrections. The final draft of the work was read and approved by all writers. The final manuscript was approved by APK and PM.

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