Assessing the effect of developed ayurvedic food for ADHD children on animal models

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Abstract

As stated by World Health Organization (WHO), a cerebral disarray are to amplify by 50 per cent in the year of 2020, is the fore most finding root of morbidity in kids. The kids represent on 40 per cent in India and the rate of psychopathology amid childhood is 5 to 15 per cent (The Global Economic burden of non-communicable diseases, 2011). ADHD is a multi factorial, highly heritable disorder, estimating a mean heritability of 76 percent (Farone and Mick, 2010**)**. The spontaneously hypertensive rat (SHR) has been used as a genetic animal model of attention deficit/hyperactivity disorder (ADHD). The open field test for loco motor activity, Y-maze test for inattention and Electro-foot shock aversive water drinking test **(**EFSD) for impulsivity were analyzed using one-way analysis of variance (ANOVA). However, impulsive behavior in the Y-maze tests, which is designed to detect anxiety-related behavior but also reflects impulsivity for novelty seeking, was comparable to Hyperactivity and inattention. ADHD like behaviors in SHR were ameliorated with methylphenidate at a low dose (0.05 mg/kg, i.p.). Rats were acclimated to their home cages for at least 7days before testing and all the rats were provided with commercially available normal diet. The animals were given fresh ayurvedic based health mix diet with 3 per cent, 6 per cent and 12 per cent incorporation of medhya rasayana (*Centella asiatica* and *Bacopa monneri*) daily for 100 g for each rat and leftover food was collected on next day measured and discarded. The body weights of each rat were measured every week. All the protocol and experiments were conducted in compliance with the ethical principles and guidelines provided by the committee. The experimental groups, consisting of 6 animals per drug and dose, were chosen by means of a randomized schedule and animal study has been carried out for three months. All tests took place between 9:00 am and 6.00 pm. Animal treatment and maintenance were carried out in accordance with the Principles of Laboratory Animal Care.

Key words: ADHD, *Bacopa monnier*i, *Centella asiatica*, Medya rasayana, SHR.

Introduction

Attention deficit/hyperactivity disorder (ADHD) is a neuro developmental disorder of childhood onset that is characterized by behavioral symptoms, including inattention, motor hyperactivity and impulsivity (American Psychiatric Association, 2013; Taylor, 1998; Giulia, 2021). ADHD is a heterogeneous and heritable disorder resulting from complex gene-gene and gene-environmental interactions (Russell, 2011). To simplify and understand the complex features of ADHD, animal models of ADHD can provide insights.

Mental disorder is now foremost thing a cause of disability in childhood (Castello *et al*., 1998). Attention deficit hyperactivity disorder (ADHD) is an unbearable mental confusion that slows down the children's growth (American Psychiatric Association, 2000). It is among the commonest childhood stage neurobehavioral disorderliness and is known to intensely impact the scholastic performance, happiness and communal relationship of kids (American Academy of Pediatrics, 2011). ADHD portray by persistent sign such as inattention, hyperactivity, and impulsivity. It has a universal occurrence with an estimated 6 per cent incidence and is commonly identified in the age group of 2-7 years that is in preschool aged children (Tripathi *et al*., 2017). For example, when children have learning difficulties together with behavioral and attentional deficits, they exhibit symptoms that could indicate a learning disability and/or ADHD, raising issues in their diagnosis and treatment. The main challenge in this research field is to understand why these two disorders occur together, how they interact, and whether this comorbidity coincides with particular neuropsychological profiles (Giulia, 2021).

The hereditary and the atmospheric surrounding are fore most position in etiology of ADHD and earlier research findings revealed about ADHD amongst identical twins (8%), fraternal twins (32%) and first degree relatives (25%). Environmental consequences such as maternal stress and smoking habit among family members during pregnancy, improper early care giving, prenatal complications and prematurity also plays a leading part (Vaidya *et al*., 2020). Although the neuropsychological profile of ADHD is heterogeneous, numerous studies indicate that it involves impairments in various executive function (EF) domains (Barkley *et al*., 1992; Pennington and Ozonoff, 1996; Sergeant *et al*., 2002).

The dimension of the brain construction for ADHD affected pupil is reduced around 5 to 10 per cent and magnetic renounce imaging screened reduced blood level in striatum and shortage in functional networks (Kliegman *et al*., 2016). The ADHD prime uniqueness are lack of concentration, tendency to act on a whim and restless and these personality will lead to lack of communal bond, acquisition of knowledge in the school and disturb other person as well (Bulut, 2007). The secondary personalities are difficulty to create bond with the peer group. In that case, this kind of personality kids may keep away from peer group and communal pupil (Hinshaw and Melnick, 1995; Cooper and Ideus, 1996).

Health benefits of ayurvedic plants

"Ayurveda" which is more than five thousand years older and it has a beneficial effect on nerve function (CNS) and increase the mental capability. Nootropic plants are used to increase mental function, example of ayurvedic plant with nootropic activity such as *Ginkgo*

biloba, *B. monnieri*, *C. asiatica*, *Withania somnifera* and *Convolvulus Pluricaulis*. The bramhi (*C. asiatica*, *B. monnieri*) which contain large amount of secondary metabolites providing active compounds stimulating cell upgrading, enhance physical and mental health. Both plants (*Bacopa monnieri* and *Centella asiatica*) possess neuroprotective properties, neurotropic action with beneficial application (Shinomol, 2011).

The group of plant therapeutic called medhya rasayana are well known to boost mental health, intellect and memory (medhya) and are believed to prop up longevity and rejuvenation (rasayana). The brain, being the creative centre of humans is expected to be influenced by the brahmi as sanskrit name brahmi stems from Brahma the creative aspect of God. *B. monnieri* is recognized for use over centuries as a brain tonic, memory enhancer, revitalize sensory organs. It is also further used as a medicine in antianxiety, as a cardio tonic, diuretic, antidepressant and anticonvulsant agent. It is very effectively used as anti inflammatory, analgesic, antipyretic, epilepsy, anticancer therapeutics and is a good antioxidant (Tripathi *et al*., 1996; Sinha and Saxena, 2006).

The bioactive composites (Bacoside A&B) and amino acids in these ayurvedic are increase the protein kinase action to produce protein and these proteins may repair the damaged neurons. The numerous study noticed that two main saponin (Bacoside A&B) enhance the learning ability in rats. In adding up, Bacoside A fraction is more faster than the Bacoside B fraction and it plays foremost position to increase positive effect on mental function and this bioactive compound have antidepressant property as well (Singh and Dhawan, 1997; Zhou *et al*., 2007).

Various study revealed that the presence of different tetra cyclic triterpenoid saponin is accelerating the cognition activity. Numerous studies concluded that the bacosides are reported as a scavenger of free radicals (Singh *et al*., 2006; Govindarajan *et al*., 2005).

C. asiatica has a wider health benefits and therefore, its use in food processing is found increasing over the years. It is registered in medicinal use as a potential antioxidant, known for antimicrobial, cytotoxic, neuroprotective properties as well and other activities too. It possesses bioactive constituents *viz*., triterpenic acid (asiatic acid and madecassoside acid), triterpenic saponin (madecassoside and asiaticoside), flavonoids, and further phenolic compounds. The asiaticoside is the most plentiful triterpene glycoside, which, it enhance the antioxidant level in wound healing process (Joshi and Chaturvedi, 2013).

Measure of selected medhya rasayanas

Bacopa can be taken in the form of non-standardized powder (5-10g), mixture (8-16ml), and syrup (30ml) in each day (Vohora *et al*., 2000). Dosages of a 1:2 solution extort are in the range of 5-12 ml/day for adults whereas it was 2.5-6 ml/day for kids ages 6-12. A *Bacopa* syrup preparation, equal to 1gm dehydrated *Bacopa* leaf at the daily basis for 3 months among 40 children in the ages of 6-8 years, showed strengthening, reminiscence perception, and response performance and no side effects were reported (Sharma *et al*., 1987). Fifty four people (65 years) administrated 300mg of *Bacopa monnieri* extract for twelve weeks and none of the participants had any sign of dementia or severe memory loss (Stough *et al*., 2008). An additional study,

researchers gave participants aged 55 and above (person with memory loss) 125mg/day of *Bacopa monnieri* extract for 12 weeks, noticeably better mental control, logical memory function, and paired association learning (Raghav *et al*., 2006).

A usual each day quantity of *C. asiatica* approximately at 600mg of dehydrated leaf or mixture, single dose tablet, a 10mg strong extort accessible in the form of tablet was found very effective (Anonymous and Physicians, 1999). Dehydrated leaves as a tea, by addition of 5-10g in bubbling hot water (150ml) and allowed to sheer for 15 minutes and 3 cups/day was advisable. The systemized *Centella* extort have up to 100 per cent total saponins (triterpenoids), 60 milligram of extort 1 or 2/day, are regularly utilized in recent herbaceous medication. A few side effects such as skin allergy and burning sensations (with external use), headache, stomach upset, nausea, dizziness, and drowsiness were reported, however, for a recommended dose no known toxicity was found (Brinkhaus *et al*., 2000; Kashmira *et al.,* 2010).

Drugs are used in ADHD are psycho stimulants, tricyclic antidepressants and tranquilizers. These drugs may be enhancing the possibility of heart related problem, liver injury and other health issues (Behsnanh *et al*., 2011). Today the need came to find out effective treatment without hampering the development of health and psychology of children. Therefore, actual need of our natural medicine and several traditional based food products. In that case, the need for exploration of new food stuff from naturally available source may enhance the cognition ability and earlier studies noticed that the ayurvedic plant such as *Bacopa monnieri* (BA) and *Centella asiacitica* (CA) has potency to increase the mental function.

Materials and Methods

Biochemical estimation

- Moisture and protein content of the fresh, dried leaf powder and standardized products were determined by adopting the methods of AOAC (1995). Moisture was estimated by hot air oven method and protein was analyzed by kelplus method.
- The FTIR method was performed on aspectrophotometer system, which was used to detect the characteristic peak values and their functional groups (Ashokkumar and Ramaswamy, 2014).
- Fibre was analyzed by the (acid and alkaline digestion) by fibra plus method suggested by Sadasivam and Manickam (1996).
- The fat content of the sample was determined by the sacs plus.
- The Beta carotene content of the sample was determined by the spectrophotometer method.
- Antioxidant activity (Free radical scavenging activity by DPPH method).

Processing of brahmi leaf powder incorporated health drink mix

Fresh, green vallarai and neer brahmi leaves were separated from the thin branches. The good quality of leaves were chosen by discarding the discolored, decayed and wilted leaves since the decayed and wilted leaves gives a bad flavor.

The residual moisture was evaporated at a room temperature (32^0C, 2-3 hours), by scattering the leaves on the filter paper with regular turning over to avoid the fungal growth. The brahmi leaf was steam blanched for 2 to 3 minutes to inactivate the enzymes before drying. The cabinet dryer was preheated to 60^0 C and then the loaded trays with leaves were kept in an oven. The temperature was maintained at 60^0C for 6-7 hours. The research study revealed that the drying of leaves at 60^oC temperature to get a first grade of dried leaves.

Generally cleaned grains have standard moisture level is twelve per cent. Rinse the grain in the water and the grain has been died with muslin cloth around the aerobic environment for 1 to 3 days. Following the completion of sprouted grains was dried in cabinet dryer for 10 to 12 hours until a grain moisture content reach 5 per cent. After drying process, the rootlets and husk were removed.

In this study, the dehydrated brahmi leaf powder was incorporated at different levels (1, 2, 3, 4, 5 and 6 per cent) with 1:1 of *Bacopa monnieri* and *Centella asiatica* in health drink mix, with the intend to enhance the functional properties of selected food products.

Health drink mix was prepared with hot milk or water can be used as porridge. This drink mix was standardized with the incorporation of three, four and five per cent brahmi leaf powder. The ingredients and preparation of health drink were given in Table 1 and Fig.1.

Table 1. Ingredients used for health drink mix

Ingredients	**C**	**T_1**	**T_2**	**T_3**
Whole wheat flour (g)	50	50	50	50
Finger millet flour (g)	25	25	25	25
Green gram flour powder (g)	25	25	25	25
Vallarai and Neer brahmi powder (%)	-	3	4	5
Jaggery (g)	17	17	17	17
Milk (ml)	2000	2000	2000	2000
Water (ml)	500	500	500	500
Cardamom	2 pinch	2 pinch	2 pinch	2 pinch

C - Control

T_1 - 3 per cent (Indian pennywort and water hyssop)

T_2 - 4 per cent (Indian pennywort and water hyssop)

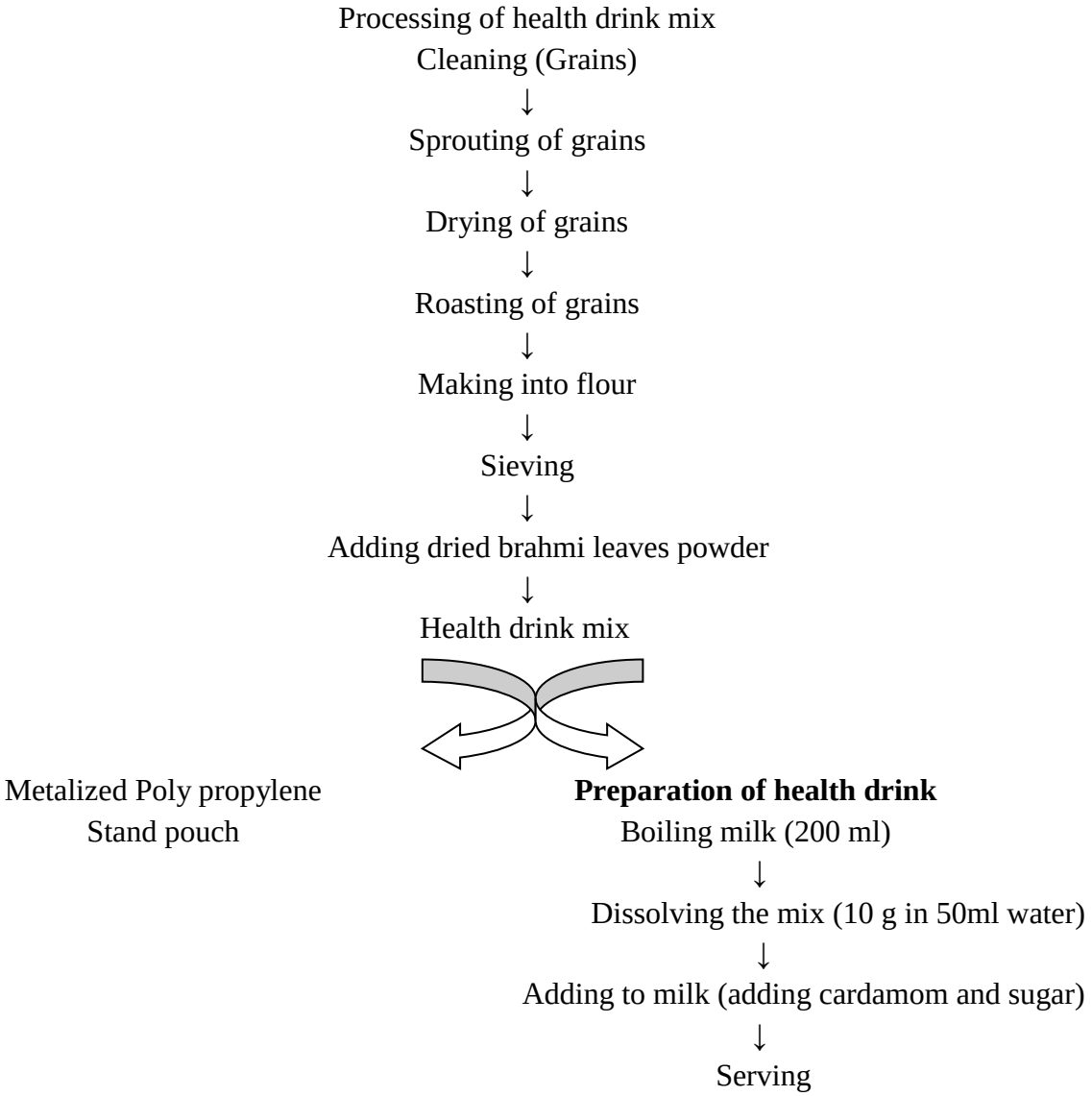
T_3 - 5 per cent (Indian pennywort and water hyssop)

Table 2. Sensory evaluations of health drink mix

Particulars	**C (%)**	**T_1 (%)**	**T_2 (%)**	**T_3 (%)**
Appearance	82	79	77	70
Flavour	79	78	77	74
Consistency	82	79	78	75
Taste	82	80	80	73
Overall acceptability	80	80	81	73

The health drink was given for sensory evaluation to 15 trained judges by using 9 hedonic scales (Table 2). The sensory attributes revealed that the T_2 (5% incorporation of brahmi leaf powder) secured maximum score in overall acceptability.

Fig 1. Processing and preparation of brahmi leaf based health drink mix



Grouping of animal

Group 1	Control diet + water 10ml/kg normal saline
Group 2	Control diet + water + Toxic DSP4 10mg/kg
Group 3	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 3 per cent (*Bacopa monnieri + centella asiatica*) (Medhya rasayana) incorporated diet + water
Group 4	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 6 per cent (*Bacopa monnieri + Centella asiatica*) (Medhya rasayana) incorporated diet + water

Group 5	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 12 per cent (*Bacopa monnieri* + *centella asiatica*) (Medhya rasayana) incorporated diet + water
Group 6	Standard control Methyl phenidate 2mg/kg

Materials

Animals	:	Wistar albino rats (SHR) (180-220gm)
Food	:	Brahmi based health drink mix
Chemical	:	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) (Fritschy and Grzanna, 1991) and at a dose of 10 mg/kg (Cheetham *et al*., 1996)

Selection of animal models

A number of animal models for ADHD have been proposed and characterized. Among them, the most used and validated are the spontaneously hypertensive rats (SHR) which mimic the behavioral characteristics observed in ADHD such as motor hyperactivity, motor and cognitive impulsivity, and decreased sustained attention (Sagvolden, 2000). Abnormalities both in the genetic and neurotransmitter functions seen in ADHD were also observed in SHR (Russell *et al*., 2007).

Male SHR (180-220g), obtained from KM College of Pharmacy, Madurai, Tamil Nadu were used in this study. All animals were housed in the animal house of KM College of Pharmacy, Madurai, Tamil Nadu and were maintained in a standard light dark cycle room with ambient temperature. Rats were acclimated to their home cages for at least 7days before testing and all the rats were provided with commercially available normal diet. The approval of the Institutional Animal Ethical Committee (Ethical committee approved no. IAEC/T.Amaravathi/TNAU/PDF/KMCP/68/2019). All the protocol and experiments were conducted in compliance with the ethical principles and guidelines provided by the committee.

The experimental groups, consisting of 6 animals per drug and dose, were chosen by means of a randomized schedule and animal study has been carried out for three months. All tests took place between 9:00 am and 6.00 pm. Animal treatment and maintenance were carried out in accordance with the principles of laboratory animal care.

The animals were given fresh ayurvedic based health mix diet with 3 per cent, 6 per cent and 12 per cent incorporation of medhya rasayana daily for 100g for each rat and leftover food was collected on next day measured and discarded. The body weights of each rat were measured every week. The animals were fasted overnight and the respective sample of the experimental group was elicited for blood sample collection. Next day, the animals were anesthetized using chloroform and the blood was collected through retro orbital punching.

Stimulants (Eg. methylphenidate and amphetamines) are the most widely used medications approved by the US Food and Drug Administration for the treatment of ADHD (Findling, 2008). In particular, methylphenidate, the most prescribed stimulant ADHD drug,

causes behavioral normalization by enhancing dopamine levels in the striatum and nucleus accumbens by targeting and blocking the actions of the DAT.

Statistical analysis

For the animal study all data were expressed as the mean+ S.E.M. Data obtained from the open field (loco-motor activity), Y-maze (inattention) and EFSD (impulsivity) tests were analyzed using one way analysis of variance (ANOVA). When statistically significant differences were found, Dunnett's test was used as a post hoc test. All statistical analyses were conducted using Graph Pad Prism Version 5 software.

Results

Nutrient content of brahmi leaves (*Bacopa monnieri* and *Centella asiatica*)

Nutrient content of the brahmi leaves described in Table 3. Herbs *Bacopa monnieri* (Neer brahmi) and *Centella asiatica* (Vallarai) are proved very powerful to direct inattention, hyperactivity and impulsivity and other (CNS) central nervous system related diseases. Therefore, there is a demand for new food products that could aim to recover the mental capabilities.

Table 3. Nutrient content of brahmi (*Bacopa monnieri* and *Centella asiatica*)

Particulars	*Bacopa monnieri*	*Centella asiatica*
Moisture content (%)	85.16±2.35	83.13±2.30
Vitamin C (mg)	63±0.51	114±3.80
Total anti-oxidant activity (%)	85.58±2.03	83.74±1.65
β-carotene (mg)	24.62±0.80	28.95±0.27
Protein (g)	2.1±0.007	4.76±0.01
Iron (mg)	7.8±0.16	19.56±0.31
Carbohydrate (g)	5.9±0.040	4.24±0.04
Ash (g)	1.9±0.01	6.41±0.17
Calcium (mg)	202±0.96	425±1.73

Nutrient content Sensory evaluation and storage stability of developed health mix

Table 4. Changes during storage of developed health drink mix

Months	**Moisture (%)**		**Protein (g/100g)**		**Fat (g/100g)**	
	SP	**MPP**	**SP**	**MPP**	**SP**	**MPP**
0 days	6.1 ± 0.12^{aA}	6.1 ± 0.14^{aA}	7.3 ± 0.11^{aA}	7.3 ± 0.15^{aA}	2.1 ± 0.030^{aA}	2.1 ± 0.025^{aA}
30 days	6.4 ± 0.16^{bB}	6.2 ± 0.19^{bA}	6.8 ± 0.14^{bA}	7.0 ± 0.21^{bA}	2.0 ± 0.038^{bA}	2.1 ± 0.03^{aB}
60 days	6.8 ± 0.18^{cB}	6.5 ± 0.09^{cA}	6.6 ± 0.12^{cA}	6.9 ± 0.17^{cB}	1.8 ± 0.012^{cA}	2.0 ± 0.05^{bB}
Months	**Fiber (g)**		**β carotene (mg)**		**Iron (mg)**	
	SP	**MPP**	**SP**	**MPP**	**SP**	**MPP**
0 days	3.7 ± 0.006^{aA}	3.7 ± 0.10^{aA}	1893 ± 34.57^{aA}	1893 ± 7.80^{aA}	4.0 ± 0.09^{aA}	4.0 ± 0.03^{aA}
30 days	3.5 ± 0.05^{bA}	3.6 ± 0.10^{aB}	1890 ± 61.72^{aA}	1892 ± 7.72^{aA}	3.7 ± 0.01^{bA}	3.9 ± 0.10^{aB}
60 days	3.4 ± 0.02^{cA}	3.5 ± 0.05^{bB}	1886 ± 56.46^{aA}	1889 ± 6.42^{aA}	3.4 ± 0.09^{cA}	3.6 ± 0.09^{bB}

Table 5. Sensory evaluations of health drink mix

Particulars	C (%)	T_1 (%)	T_2 (%)	T_3 (%)
Appearance	82	79	77	70
Flavour	79	78	77	74
Consistency	82	79	78	75
Taste	82	80	80	73
Overall acceptability	80	80	81	73

Values are Mean ± SE (n=3), Values with different superscript in the same row and column for the same parameter differ significantly ($p<0.05$)

SP - Stand pouch

MPP - Metalized poly propylene

FTIR Spectroscopic Analysis (Fourier-Transform Infrared Spectroscopy)

The brahmi leaves were placed directly on the germanium piece of the Infrared spectrometer with constant pressure applied and the data of infrared absorbance collected over the wave number ranged from 4000 cm–1 to 1000 cm–1 and computerized for analyses by using the IRPal (IR*Pal is a* program that may come in quite handy in interpreting *IR*-spectra of organic compounds). All spectra were collected with a resolution of 4^{-1} cm to improve the signal-to-noise ratio, 256 scans were co-added and averaged.

Table 6. Fourier-Transform Infrared Spectroscopy (FT-IR) functional group of *Centella asiatica*

S. No	Frequency	Group	Functional group
1	958.01	C=C bending	Alkene
2	1019.14	C=C bending	Alkene
3	1101.42	C-N stretching	Amine
4	1151.16	C-O stretching	Tertiary alcohol
5	1252.34	C-O stretching	Ether
6	1365.01	C-H bending	Alkane
7	1415.59	S=O stretching	Sulfate
8	1628.61	C=C bending	Alkene
9	1735.21	C=O stretching	δ-lactone
10	2848.12	C-H stretching	Carboxylic acids
11	2917.50	C-H stretching	Carboxylic acids
12	2165.20	S-CΞN stretching	Thiocyanate
13	3346.94	O-H stretching	Phenol

Table7. FT-IR functional group of *Bacopa monnerie*

S. No	Frequency	Groups	Functional groups
1	718.88	C=C bending	Alkene
2	955.71	C=C bending	Alkene
3	1029.49	C-N stretching	Amine
4	1107.47	C-N stretching	Amine
5	1162.62	C-O stretching	Ester
6	1250.04	C-O stretching	Ether
7	1392.60	S=O stretching	Sulfate
8	1358.11	O-H bending	Alcohol
9	1463.88	C-H bending	Alkane
10	1519.07	N-O stretching	Nitro compound
11	1638.64	C=C bending	Alkene
12	1732.83	C=O stretching	δ-lactone
13	2162.90	S-CΞN stretching	Thiocyanate
14	2849.76	C-H stretching	Carboxylic acids
15	2916.89	C-H stretching	Carboxylic acids
16	3371.50	O-H stretching	Phenol

The FT-IR analysis of absorption spectrum (Fig. 4, 5, 6 &7) and functional groups of *Centella asiatica* and *Bacopa monneria* were given in the Tables 11&12 respectively. The FTIR analysis showed that the presence of bio active constituent group present in the plants *Centella asiatica* and *Bacopa monneria*. In *Centella* there are 13 functional groups and in *Bacopa* there are 16 functional groups. This 9 same functional groups present in the *Bacopa* are also present in the *Centella*. The presence of 9 common functional groups like phenol, carboxylic acids, ethers, alkenes, amine, alkanes, alcohol, δ-lactone and thiocyanate in both the plants and it indicates that the plants possess same active principles which are useful for memory enhancement activity.

Open field test: Hyperactivity

Adolescent SHR (6 weeks old) were injected intra peritoneally (i.p.) with saline (control), compound medhaya rasayana (3, 6, and 12% and methylphenidate 2mg/kg) and placed individually in the center of an activity box (measuring47X47cm) bordered by 42cm side walls. Spontaneous activity of each rat was measured for 20min using automated systems. The following indices of loco-motor activity were recorded by the computer program: moved distance, movement duration and frequency of rearing.

Table 8. Effect of medhya rasayana at different concentration on attention deficit/hyperactivity disorder like behaviours of SHR

Groups	Treatment	Distanced moved (CM)
Group 1	Control diet + water 10ml/kg normal saline	2000
Group 2	Control diet + water + Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) 10mg/kg	6690
Group 3	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 3 per cent (*Bacopa monnieri* + *Centella asiatica*) medhya rasayana incorporated diet + water	3340
Group 4	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 6 per cent (*Bacopa monnieri* + *Centella asiatica*) + Medhya rasayana incorporated diet + water	3130
Group 5	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 12 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	2150
Group 6	Standard control Methyl phenidate 2mg/kg	1830

Result showed that toxic DSP4 treated animals (Group 2) movement is higher than normal animals (Group 1). On the other hand, toxic DSP4 treated animals which received medhya rasayana simultaneously (Group 3) movement is less than comparing with the toxic DSP4 alone treated animals (Group 2).

Table 9. Effect of medhya rasayana at different concentration on movement duration

Groups	Treatment	Movement duration (sec)
Group 1	Control diet + water 10ml/kg normal saline	512
Group 2	Control diet + water + Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) 10mg/kg	860
Group 3	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 3 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	690
Group 4	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 6 per cent (*Bacopa monnieri* + *Centella asiatica*) DSP4 Medhya rasayana incorporated diet + water	585
Group 5	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 12 per cent (*Bacopa monnieri* + *Centella asiatica*) medhya rasayana incorporated diet + water	510

Group 6	Standard control Methyl phenidate 2mg/kg	435

The effects of medhya rasayana at different concentration in alleviating ADHD like behaviors in SHR were investigated. Tables 6 and 7 shows marked differences in locomotors activity (distance moved and movement duration) between toxic control and treatment control rats. In accordance with the findings of our previous studies (Yoon *et al*., 2013), drug treated SHR (Toxic control) exhibited pronounced hyperactivity compared with normal control. One way ANOVA showed significant differences in locomotors activity among treatment SHR groups. Comparisons indicated that treatment of medhya rasayana at different concentration at the 3 per cent, 6 per cent and 12 per cent reduced hyperactivity in SHR ($P<0.05$). Interestingly, methylphenidate at a dose of 2 mg/kg did not influence locomotors activity of the SHR.

Y-maze test: Attention

Spontaneous alternation behavior requires attention (Katz and Schmaltz, 1980) and working memory (Sarter *et al*., 1988). Y-maze test was used to identify the influence of compound medhaya rasayana on sustained in attention behavior of adolescent SHR. The methods used were similar to those described previously (Sarter *et al*., 1988) with some modifications. Each arm of the Y-maze was 45cm long, 10cm wide, and 20cm high and both arms were positioned at equal angles. Thirty minutes after saline or drug (medhya rasayana compound and methylphenidate) administration, rats were placed individually at the end of an arm and allowed to enter the maze freely for an 8min test session. An arm entry was defined as the entry of all four paws and the tail into one arm. The sequence of arm entries was recorded using automated systems. The alternation behavior (actual alternations) was defined as the consecutive entry into three arms, i.e., the combination of three different arms, with stepwise combinations in the sequence. The maximum number of alternations was thus the total number of arms entered minus 2, and the percentage of alternation behavior was calculated as

$$\text{Alternation behavior} = \frac{\text{Actual alternations}}{\text{Maximum alternations}} \text{ X } 100$$

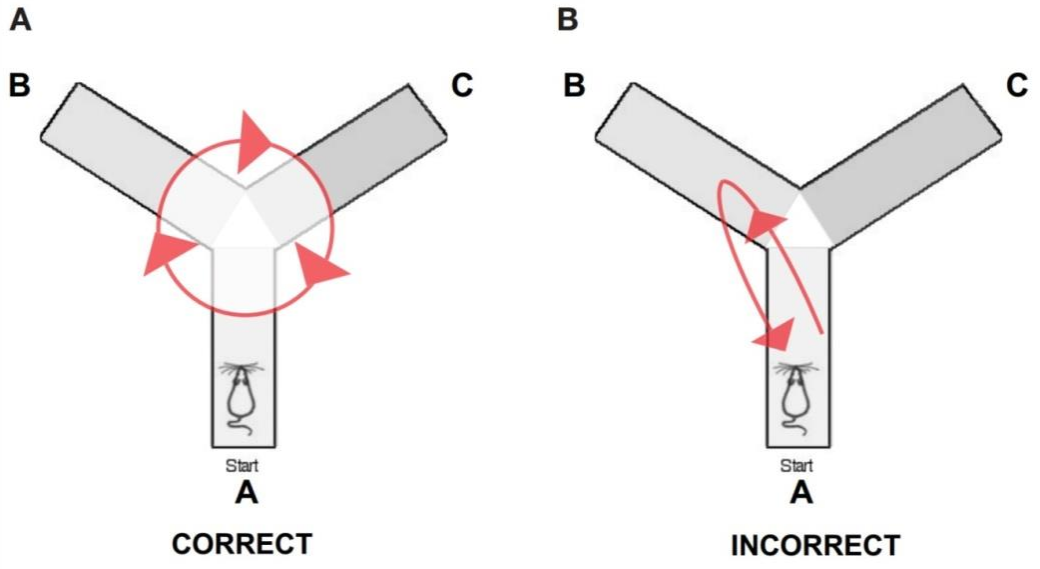


Table 10. Effect of medhya rasayana at different concentration on spontaneous alteration

Groups	Treatment	Spontaneous alteration %
Group 1	Control diet + water 10ml/kg normal saline	58
Group 2	Control diet + water + Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) 10mg/kg	96
Group 3	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 3 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	72
Group 4	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 6 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	67
Group 5	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 12 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	63
Group 6	Standard control Methyl phenidate 2mg/kg	60

The spontaneous alternation task is used to assess spatial working memory in rodents and is based on the innate tendency of rodents to explore a prior unexplored arm of a T or Y-maze. Thus, a rodent typically remembers which arm it has just visited. Results showed that (Table. 8) toxic DSP4 treated animals (Group 2) spent less time in open arm on comparing with normal animals (Group 1). On the other hand toxic DSP4 treated animals which received medhya rasayana simultaneously (Group 3) spent more time in open arm of EPM on comparing with the toxic DSP4 alone treated animals (Group 2). Treatment control groups (medhya rasayana) showed inattention as evidenced by lower spontaneous alternation behavior compared with toxic control groups. One-way ANOVA revealed significantly different behavioral responses among treatment control (medhya rasayana) and standard control groups. 2 mg/kg of methylphenidate ($P<0.05$) significantly increased spontaneous alternation in SHR. On the other hand, methylphenidate (2 mg/kg) did not affect spontaneous alternation behavior.

Electro foot shock aversive water drinking test (EFSDT): Impulsivity

We used a paradigm called the electro foot shock aversive water drinking test (EFSDT) to measure the effects of drugs on SHR impulsivity (Kim *et al*., 2012). In the EFSDT, impulsivity is manifested by the persistence of subjects to obtain a biological reward (water) despite the presentation of an aversive consequence (electroshock). Tests were conducted in a test box described in previous studies (Kim *et al*., 2012; Yoon *et al*., 2013). The methods of the impulsivity tests were patterned after those described by Kim *et al*. (2012) with some modifications. Tests consist of a training phase and test phase. During the test phase (30 min), saline or drug (medhaya rasayan and toxic drug) administered SHR rats were tested for

impulsive behaviors, i.e., the persistence to drink water despite presentation of an electroshock (50 pulses/s, 0.5ms of pulse width, 4mA) for every drink made or drinking attempt. The number of drinking attempts for each rat was recorded by an observer blinded to the experimental cohort.

Table 9. Effect of medhya rasayana at different concentration on drinking attempts

Groups	Treatment	Drinking attempts No. for 30 minutes
Group 1	Control diet + water 10ml/kg normal saline	20
Group 2	Control diet + water + Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) 0mg/kg I.P	33
Group 3	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 3 per cent (*Bacopa monnieri + Centella asiatica*) Medhya rasayana incorporated diet + water	30
Group 4	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 6 per cent (*Bacopa monnieri + Centella asiatica*) Medhya rasayana incorporated diet + water	27
Group 5	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 12 per cent (*Bacopa monnieri + Centella asiatica*) Medhya rasayana incorporated diet + water	25
Group 6	Standard control Methyl phenidate 2mg/kg	23

Results showed (Table 9) that toxic DSP4 treated animals (Group 2) more impulsive comparing with normal animals (Group 1). On the other hand toxic DSP4 treated animals which received medhya rasayana simultaneously (Group 3) less impulsive on comparing with the toxic DSP4 alone treated animals (Group 2). As shown in result toxic treated rats showed high levels of impulsivity as indicated by higher frequency of drinking attempts (frequency in the water area) compared with treatment control (medhya rasayana) rats. One way ANOVA also showed significant differences among toxic and treatment control groups. Methylphenidate (2 mg/kg) was also effective in improving impulsivity in SHR.

Medhya rasayana on dopamine level of an animal model

Table 10. Effect of medhya rasayana at different concentration on dopamine levels

Groups	Treatment	Dopamine (units/g)x10^3
Group 1	Control diet + water 10ml/kg normal saline	37.10±1.75
Group 2	Control diet + water + Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) 10mg/kg	20.60±0.96*a
Group 3	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine	31.20±1.35*b

	(DSP4) + 3 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	
Group 4	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 6 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	33.70±1.48*b
Group 5	Neurotoxin N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine (DSP4) + 12 per cent (*Bacopa monnieri* + *Centella asiatica*) Medhya rasayana incorporated diet + water	34.22±1.56*b
Group 6	Standard control Methyl phenidate 2mg/kg	36.20±1.64*b

Values are expressed as mean±SEM

*a Values are significantly different from normal control ($p<0.05$)

*b Values are significantly different from toxic control ($p<0.05$)

Results (Table 10) showed that dopamine levels in toxic DSP4 treated animals (Group II) were significantly less on comparing ($p<0.05$) with normal animals (Group I). On the other hand dopamine levels in medhya rasayana at different concentration at the 3 per cent, 6 per cent and 12 per cent treated animals (Group III, IV and V) were significantly increased on comparing ($p<0.05$) with the DSP4 alone treated animals (Group II). Methylphenidate (2 mg/kg) treated animals also significantly increased dopamine levels when compared to Group II.

In this study, we demonstrated the potentiality of the medhya rasayana at different concentration as an ADHD therapy, based on its in vivo, i.e. in alleviating ADHD like behaviors of the SHR. The property of the ayurvedic food is essential considering the abuse liability of current ADHD pharmacotherapy (eg. stimulant drugs such as methylphenidate and amphetamines).

The dynamic developmental theory of ADHD asserts that ADHD symptoms are caused by dysfunction in dopamine neurotransmission (Sagvolden *et al*., 2005) and that drugs that increase extra cellular dopamine levels (eg. pyschostimulants) effectively manage ADHD symptoms thereby normalizing the underlying dopamine dysfunction (Solanto, 1998; Volkow *et al*., 2001).

We examined the effects of medhya rasayana at different concentration in normalizing ADHD like behaviors of SHR. Our findings showed that medhya rasayana at different concentration effectively alleviated SHR hyperactivity, inattention and impulsivity. Interestingly, while all doses of medhya rasayana at different concentration significantly improved ADHD like behaviors of SHR, only the 2mg/kg of methylphenidate ameliorated sustained inattention and impulsiveness in this rat strain. Additionally, previous studies which employed the open field tests also found inability of methylphenidate treatment (at 2mg/kg doses) in reducing hyperactivity in SHR (Van Den Bergh *et al*., 2006; Wultz *et al*., 1990; Yang *et al*., 2011). On the other hand (Sagvolden *et al*., 2009) indicated a number of factors limiting the construct and predictive validity of the open field test. The psycho stimulant methylphenidate increases

extracellular dopamine concentration by blocking the DAT, and has been considered as the mainstay of treatment of ADHD (Heal *et al*., 2009). Considering that the effects of medhya rasayana at different concentration were mediated by enhancement of dopamine neurotransmission.

Furthermore, medhya rasayana at different concentration neither produced conditioned place preference response in subjects nor maintained self administration behavior. Our findings in the open field test showed that medhya rasayana at different concentration did not increase, but rather, decreased locomotors activity in SHR. Administration of DSP4 decreased the brain dopamine levels, thereby leading to neurodegenerative disorder. Current results also confirm, the decrease in levels of dopamine in DSP4 treated rats. The results of the present study as seen in pharmacological tests, showed medhya rasayana at various concentration increase significantly dopamine levels.

Cost analysis

The cost involved in the processing of the brahmi based health mix were raw materials, labour, fuel, electricity and packaging materials were added and presented as total cost of value added products. The cost analysis of the brahmi based health mix was given below.

Health drinks Rs.10/serve (150 ml)

Conclusion

This article move along the scientific substantiation on cognitive and neuropharmacological enhancement effects of *Bacopa monnieri and Centella asiatica* in animal models. The neuro psycho nutraceuticals producing companies have been endowed massive resources in the identification of natural nootropics, which could possibly alleviate debilitating disorders and slow the onset of mental retardation, though their full potential is yet to be determined and harnessed.In this study, the results illustrate that consuming of brahmi leaves incorporated food products (nutri balls mix, puttu mix, health drink mix, cookies and soup mix). Fresh leaves with minimal processing can be feed to children and provide as a good tonic and improve the memory function. However it may be hard for children to consume, as they are bitter. The present study clearly picture the development procedure of ayurvedic based food product which can be broadly accepted by the people of all age groups mainly due its health benefits. The study confirms that both the plants are having the bioactive constituents of the same functional groups. This bioactive component in medhya rasayana leaf powder had improved the nutrient content in developed food products. The medhya rasayana based value added product could be valuable for developing new functional foods with improved nutritional quality for ADHD affected children. *Bacopa monniera* Linn. (Brahmi) and *Centella asiatica* Linn. (Mandookparni) is evidenced to control inattention and hyperactivity and thus pave way to further research to set up in a scientific manner.

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