








ORIGINAL PAPER

Mentat[®] ameliorates hypoxia-induced attention deficit hyperactivity disorder like behavior in rats

Sai Sindhura ¹, Gollapalle Lakshminarayanashastry Viswanatha ²,
Chirag Jain ¹, Onkaramurthy Mallappa ³, Mohammed Mukhram Azeemuddin ³,
Mohamed Rafiq ³, S.N. Manjula ¹

¹ JSS College of Pharmacy, Mysore, Karnataka, India

² Toxicology Division, Clinchoice Private Limited, Bangalore, Karnataka, India

³ Research and Development, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

ABSTRACT

Introduction and aim. The objective of the study was to evaluate the effect of Mentat[®] an herbal formulation in experimental models of hypoxia-induced attention deficit hyperactive disorder (ADHD) like behavior in rats.

Material and methods. Mentat[®] was evaluated at the dose of 100 and 200 mg/kg body weight. per oral., in two experimental models of hypoxia in Wistar rats. In the first model, after parturition, on a postnatal day 2 (PND-2), the pups were subjected to hypoxic exposure for 10 minutes to induce neonatal hypoxia. Pups were weaned from dams on PND-21 and subjected to drug treatments for 10 days. In the second model, phenytoin 150 mg/kg. b.wt. p.o. was administered orally to all pregnant animals throughout gestation to induce intrauterine hypoxia. Pups were subjected to assigned treatments after weaning. Behavioral and biochemical parameters relevant to ADHD were assessed.

Results. In the positive control group, hypoxic exposure resulted in significant changes in cognitive and neurologic skills compared to normal control. Open field test, elevated plus maze test, and Acetylcholine esterase levels showed a significant increase in positive control compared to normal control. In treatment groups, there was a dose-dependent decrease in all the above parameters compared to positive control. Dopamine and Nor-epinephrine levels in brain homogenate were decreased in positive control which subsequently increased with Mentat[®] treatment.

Conclusion. Mentat[®] showed a neuroprotective effect in different experimental models of ADHD. It may be recommended for the effective/preventive management of ADHD, especially associated with memory impairment and neurologic conditions.

Keyword. ADHD, herbal, Mentat[®], neonatal hypoxia, neuroprotective

Introduction

Attention-Deficit Hyperactivity Condition (ADHD) is a neurobehavioral and developmental disorder that is chronic and clinically diverse. Prefrontal dopamine insufficiency and insufficient central dopaminergic activity are the main causes of ADHD. ADHD is one of the most common childhood psychiatric diseases, affecting 3 to 5%

of all children in the United States and the Netherlands. Majority of youngsters are diagnosed with ADHD when they start school for the first time. 2 to 16 percent of all school-aged children have been diagnosed with ADHD, with roughly 75 percent of these children being male.^{1,2}

CNS stimulants such as methylphenidate and amphetamine, non-stimulants such as Atomoxetine, Cat-

Corresponding author: S.N. Manjula, e-mail: snmanjula@jssuni.edu.in

Received: 22.01.2022 / Revised: 16.04.2022 / Accepted: 8.05.2022 / Published: 30.09.2022

Sindhura S, Viswanatha GL, Jain C, Mallappa O, Azeemuddin MM, Rafiq M, Manjula SN. *Mentat[®] ameliorates hypoxia-induced attention deficit hyperactivity disorder like behavior in rats.* Eur J Clin Exp Med. 2022;20(3):298–305. doi: 10.15584/ejcem.2022.3.7.



echolaminergic antidepressants, alpha-agonists, and more recently reported drugs such as Mondaflinil and nicotinic agonists are the most often used pharmaceuticals for treating ADHD. The most common side effects with these medicines include nausea, dry mouth, lack of appetite, sleep difficulties, dizziness, irritability and mood swings, and headache.³ The current study is meant to evaluate Mentat® a well-known neuroprotective proprietary herbal formulation of the Himalaya Wellness Company, Bangalore, India, in experimental models of hypoxia-induced ADHD-like behaviour in rats, according to the safety profile.

There are a variety of environmental and genetic factors that may play a role in ADHD such as Cigarette smoking, alcohol use, infection and stress during pregnancy, brain injury, and heavy metal (lead).⁴ Variants of the dopamine D4 receptor gene, the dopamine D5 receptor gene, the dopamine transporter gene (DAT), have all been found to be strongly related with ADHD in molecular genetics investigations. Nor epinephrine transporter (NET), monoamine oxidase-A (MAO-A) and catechol-o-methyltransferase (COMT), serotonin receptor (HTR1B), serotonin transporter (5-HTT), are some of the other genes linked to an elevated risk of ADHD (SNAP-25).⁵ Dopamine is a key neurotransmitter in neuropharmacology because it has a role in a variety of brain illnesses, including Parkinson's disease, schizophrenia, and attention deficit disorder, as well as drug addiction and endocrine disorders. According to new studies, raising dopamine levels in youngsters with ADHD improves their conduct.⁶ Mentat® was clinically evaluated in children for the improvement in cognition, learning capacity, mental fatigue and neuroprotective effect. As Mentat® was found to be promising in these conditions, a preclinical study was performed on rodents to evaluate its effect in ADHD condition.⁷⁻⁹ By modulating multiple neurotransmitters, Mentat® shown neuroprotective efficacy in diverse experimental paradigms.

Aim

The study's goal was to see how Mentat® affected different psychological markers of ADHD, such as dopamine, acetylcholine esterase, and norepinephrine, as well as behavioral parameters associated with ADHD.

Material and methods

Phenytoin (Eptoin) manufactured by Abbott India Ltd. India, was procured from retail pharmacy store of Makali, Bengaluru, India. Mentat® tablets were obtained from Himalaya Wellness Company, Bengaluru, India, Atomoxetine (Attentrol) manufactured by Sun Pharmaceutical Industries, Ltd., India. was procured from retail pharmacy store of Makali, Bengaluru, India all other chemicals were of analytical grade and were procured from HiMedia Laboratories GmbH, Germany. The rat

feed was procured from VRK Nutritional Solutions, MIDC Miraj, Pune, India. The diet which was provided to the rats was the standard pellet diet which contains protein (19%), Total fat (4.6%), total carbohydrate (60%), total minerals (6.8%), crude fiber (2.5%) with all essential amino acids and minerals.

Experimental animals

Inbred Wistar rats, weighing 200–250 g were procured from central animal facility, R&D Center, Himalaya Wellness Company, Makali, Bangalore-562162, India. and housed at the temperature of $25 \pm 1^\circ \text{C}$, relative humidity of 45 to 55% and 12:12 h light–dark cycle. The Himalaya Wellness Company's Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (protocol no 127/13) and the experiments were carried out in accordance with the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

Procedure

Monoamine oxidase inhibitor (MAO-B) inhibitory activity (in-vitro)

Brain homogenate was prepared in 0.25 M sucrose-0.1 M Tris-0.02 M EDTA buffer (pH 7.4). Centrifugation was carried out in three steps; first step homogenate was centrifuged at 3000rpm for 20 mins. Supernatant was collected and centrifuged at 10000 rpm for 20 min, pellets were collected and washed twice with 0.25 M sucrose-0.1 M Tris-0.02 M EDTA buffer and pellets were resuspended in 10 mM sodium phosphate buffer (pH 7.4) containing 320 mM sucrose and centrifuged at 12000 rpm for 30 mins. The whole procedure was carried out at 4°C . Pellets collected and resuspended in ice cold 100 mM sodium phosphate buffers (pH 7.4). 2.5 ml 100 mM sodium phosphate buffer (pH 7.4) was taken in test tube, to which 100 μl test drug (Mentat®) and 150 μl brain homogenate was added and incubated at 37°C for 10 min, followed by the addition of 100 μl Benzylamine (substrate), absorbance was recorded in spectrophotometer at 249 nm. Blank (without substrate and test drug) and control (without test drug) readings were recorded.¹⁰

Neonatal hypoxia induced ADHD in rats

Sixteen female Wistar rats of 180–200 g were kept for cohabitation with male rats at 2:1 ratio. To confirm the mating vaginal smear was examined from the day-1 to day-5, once the mating is confirmed females were separated from the males and checked for abdominal enlargement on day-10. The pregnant dams will be isolated and housed individually. After the parturition, on PND2 male pups were divided into 5 groups consists of 8 animals each and they were subjected to hypoxic ex-

posure on PND2, PND3, PND4 and PND8 for 10 minutes. In brief, the pups were placed in an air tight glass chamber and made deprived of oxygen, depletion of oxygen within the chamber was confirmed based on the combustion principle i.e., by placing a burning candle in an air tight chamber and covering it with the lid, candle turns off after few seconds indicating that the oxygen within the chamber has been completely used, Animals show cyanosis which indicates that there was oxygen insufficiency. After the hypoxic exposure all pups were shifted to respective dams, after periodic hypoxia exposure the pups were weaned from the dams on PND 21 and subjected to following drug treatments for 10 days. Group-I and group-II served as control (normal control) and positive control (ADHD control), respectively and received only vehicle (demineralized water, 10 mL/kg, p.o.). Group-III served as reference standard and received Atomoxetine 50 mg/kg, p. o, Group-IV and group-V animals received Mentat[®] at the dose of 100 and 200 mg/kg, b. wt, respectively. All the group of animals were treated for a period of 10 days. At the end of assigned drug treatments, the animals were evaluated for hyperactivity and learning impairment and subjected to Actophotometer test, open field test and elevated plus maze test.¹¹⁻¹⁴ These tests were performed to evaluate the locomotor activity,

Following euthanasia with excess of 5% Isoflurane inhalation anesthesia, a brain homogenate was produced with ice cold 0.15 M KCl (10%) and centrifuged at 10,000 g for 10 minutes at 4°C, with the post-mitochondrial supernatant (PMS) utilised for total protein estimate and lipid peroxidation assay. The amount of protein in the brain homogenate was determined using the Bradford technique and a standard of bovine serum albumin.¹⁵ The amount of malondialdehyde in the brain homogenate was determined quantitatively using the Wills method to determine the degree of lipid peroxidation.¹⁶ Sedlak and Lindsay's method was used to calculate reduced glutathione in the brain homogenate.¹⁷ Ellman's approach was used to calculate acetyl cholinesterase in brain homogenate.¹⁸

Phenytoin induced intrauterine hypoxia model

Phenytoin when administered to pregnant rats, phenytoin reduces uteroplacental blood flow, resulting in foetal hypoxia, leading to neurological abnormalities such as hyperactivity, learning difficulties, mental retardation, epilepsy, cerebral palsy, dystonia, and more. Phenytoin was produced in deionized water (pH 11.5) and given orally to pregnant rats from the 7th to the 20th day of pregnancy in this study. Following weaning, the pups of treated mothers were evaluated for behavioral measures. Fourteen female Wistar rats (200–250 g) were kept for cohabitation with seven male Wistar rats (200–250 g) at 2:1 ratio for five days. To confirm the mating vaginal smear

was examined from the day-1 to day-5, once the mating is confirmed females were separated from the males and checked for abdominal enlargement on day-10. The pregnant dams were isolated and housed individually and they were further divided in to 5 groups and consisting of two mothers.¹⁹ Group-I and group-II served as control (normal control) and positive control (ADHD control), respectively and received only vehicle (demineralized water, 10 mL/kg, p.o.). Group-III served as reference standard and received Atomoxetine 50 mg/kg, p. o. Group-IV and group-V animals received Mentat[®] at the dose of 100 and 200 mg/kg, b. wt, respectively. All the groups of animals were treated for a period of 10 days. Phenytoin (150 mg/kg, p.o.) was administered orally to all pregnant animals throughout the gestation except group-I. At the end of assigned drug treatments, the animals were subjected to the evaluation of hyperactivity and learning impairment by Actophotometer test, open field test and Elevated Plus maze test. The treatments and evaluation were completed between PND30–PND36.

The behavioral parameters pertaining to ADHD like hyperactivity and learning impairment were evaluated by similar procedures as mentioned earlier through Actophotometer test, open field test and elevated plus maze test.

Dopamine and nor-epinephrine was estimated using high performance liquid chromatography (HPLC) and based on the standard calibration curve of dopamine and nor-epinephrine: Briefly the method is explained as, the composition of mobile phase was prepared from filtered and degassed mixture of Acetonitrile and 1-Octane sulfonic acid in the ratio of 70:30. Pharmaceutical grade of dopamine and nor-epinephrine were procured from Sigma Aldrich. Stock solution of DA and NE were prepared by dissolving 1 mg in 1 mL of methanol in 10 mL volumetric flask separately and volume was made up to 10 mL using the diluents to get a standard stock solution of concentration 0.1 mg/mL (1000 ng/mL). the solutions were filtered using 0.2 μ syringe filter and this solution was used for analysis. Brain tissue samples (1 mm thick slice of brain) were homogenized using individual 1.5 mL centrifuge tubes which contain 400 μ L of tissue homogenizing solution (0.1 M perchloric acid, 1×10^{-7} M ascorbic acid; chilled on ice), the samples were centrifuged at 10,000 rpm for fifteen minutes. The supernatant samples were filtered using 0.2 μ m nylon disposable syringe filters and used for the analysis.

Statistical analysis

The values were expressed as mean \pm SEM. The results were analyzed statistically using one-way ANOVA followed by Dunnett's multiple comparison test using Prism GraphPad 6.07 (GraphPad Software Inc, San Diego, CA, USA) software. A p value < 0.05 was considered as statistically significant.

Results

Mentat® was evaluated in experimental models of neonatal hypoxia and phenytoin induced intrauterine hypoxia-brain injury in rats. Neurologic, biochemical, morphologic, and histopathologic data were used to examine Mentat's protective impact. Mentat's potential free radical scavenging and antioxidant activities were further investigated using in vitro antioxidant tests. In the present study IC50 values of MOA-B inhibitory activity of Mentat® and Amitriptyline was found to be 9.70 mg/mL and 0.45 mg/mL respectively. In the experimental model of neonatal hypoxia. Mentat® at 200 mg/kg showed a significant improvement in locomotor activity (Figure 1, Table 1) and associated parameters when compared to positive control, in elevated plus maze Mentat® at 200 mg/kg showed significant improvement in exploratory score ($p < 0.05$) and transfer latency at dose 100 mg ($p < 0.001$), 200 mg ($p < 0.05$) (Table 2)

Table 1. Behavioural parameters of the rats treated with Mentat® in open field test (neonatal hypoxia)

Groups	Time spent with movement (seconds)	No. of squares crossed	Time spent in periphery (seconds)	No. of rearing
Control	8.25 ± 1.95	2.57 ± 0.37	15 ± 3.45	4.429 ± 0.57
Positive control	16.8 ± 1.79 [#]	4.75 ± 0.8 [#]	18.22 ± 3.37 ^{##}	10 ± 1.59 ^{##}
Atomoxetine (50 mg/kg)	9.83 ± 3.72	2.83 ± 0.54 [*]	10.60 ± 2.68 ^{***}	3.5 ± 1.18 ^{***}
Mentat® (100 mg/kg)	2.83 ± 0.66 ^{****}	2.25 ± 0.25 ^{***}	1.50 ± 0.34 ^{****}	1.125 ± 0.64 ^{****}
Mentat® (200 mg/kg)	3 ± 1.38 ^{***}	2.43 ± 0.2 ^{**}	3.6 ± 1.6 ^{**}	1.857 ± 0.8 ^{****}

^a All the values are expressed as mean ± SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant. # $p < 0.05$, ## $p < 0.01$ compare to control, **** $p < 0.0001$, *** $p < 0.001$ and ** $p < 0.01$ compare to positive control

when compared to positive control. In ADHD induced group there was a free radical generation which is indicated by increased MDA and decreased GSH levels with respect to normal control. Mentat® at 200 mg/kg significantly ($p < 0.05$) decreased the malonaldehyde (MDA) in lipid peroxidation assay and significantly increased the GSH levels at dose 100 and 200 mg/kg ($p < 0.05$) which showed its protective effect against free radical generation and offered significant protection against hypoxia induced lipid peroxidation. The findings are provided in (Table 3), where the concentration of MDA was expressed as Nmoles MDA/mg

of protein in the brain homogenate AChE activity in positive control was more than normal control and in the treatment groups there was a significant decrease at dose 100mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.01$) (Figure 2) with respect to positive control. Dopamine (Figure 3) and nor-epinephrine (Figure 4) levels which are the hallmarks in ADHD were increased with the treatment of Mentat®.

Table 2. Behavioral parameters of rats treated with Mentat® in elevated plus maze test (neonatal hypoxia)^a

Groups	Exploratory score	Transfer Latency
Control	2.25 ± 0.25	73.63 ± 6.77
Positive control	4.25 ± 0.45 [#]	35.71 ± 2.37 ^{##}
Atomoxetine (50 mg/kg)	2 ± 0.03 ^{***}	90 ± 0.01 ^{**}
Mentat® (100 mg/kg)	2 ± 0.04	57.25 ± 13.15 ^{***}
Mentat® (200 mg/kg)	2.571 ± 0.36 [*]	78 ± 12.01 [*]

^a All the values are expressed as mean ± SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant. # $p < 0.05$, ## $p < 0.01$ compared to control, **** $p < 0.001$, *** $p < 0.01$ and * $p < 0.05$ compared to positive control

In the experimental model of phenytoin induced hypoxia. Mentat® at 100 mg/kg showed a significant improvement in locomotor activity ($p < 0.05$) (Figure 5, Table 4) and associated parameters when compared to positive control, in elevated plus maze Mentat® showed significant improvement in exploratory score and transfer latency at dose 100 and 200mg/kg ($p < 0.01$) (Table 5) when compared to positive control.

Table 3. Effect of Mentat® on glutathione, lipid peroxidation, dopamine and norepinephrine levels^a

Groups	Parameters			
	GSH $\mu\text{mol/L}$	LPO nmol MDA/ μg proteins	Dopamine mg/g protein	Norepinephrine mg/g protein
Control	0.7	350	0.0025	0.0048
Positive	0.25 [#]	450	0.0017	0.0036
Atomoxetine	0.7 ^{**}	375	0.0022	0.0047
Mentat® (100 mg/kg)	0.4 [*]	325	0.0025	0.0039
Mentat® (200 mg/kg)	0.3 [*]	300 [*]	0.0024	0.0047

^a The mean values of all the groups were compared by one way ANOVA followed by Dunnett's test using Graph pad Prism software version 6.07 for windows, "p" value less than 0.05 was considered to be statistically significant. # $p < 0.05$ compare to control, * $p < 0.05$ and ** $p < 0.01$ compare to positive control

Table 4. Locomotor activity of animals treated with Mentat® (phenytoin induced hypoxia)^a

Group	Time spent with movement (seconds)	No of squares in centre	No of squares crossed in periphery (seconds)	No of rearing
Control	32.83 ± 4.191	2.8 ± 0.374	25 ± 2.076	4.667 ± 0.989
Positive control	59.33 ± 9.656 [#]	7 ± 0.7303 [#]	46 ± 5.468 [*]	18.33 ± 2.155 ^{##}
Atomoxetine 50mg/kg	22.6 ± 5.115 ^{***}	2.5 ± 0.289 [*]	20 ± 3.467 ^{***}	7.2 ± 2.709
Mentat® 100mg/kg	36.8 ± 4.954	5 ± 1.225	23 ± 6.807	22.4 ± 2.293
Mentat® 200mg/kg	36.5 ± 10.53	4.333 ± 2.333	29 ± 9.452	20.25 ± 2.016

^a All the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett’s test using Graph pad Prism software version 6.07 for windows, “p” value less than 0.05 was considered to be statistically significant #p<0.05 compared to control, *p<0.05 and **p<0.01 compared to positive control

Table 5. Effect of Mentat® on the behaviour of the rats in EPM (Phenytoin induced hypoxia)^a

Group	Transfer latency	Exploratory score
Control	74.2 ± 7.14	3.667 ± 0.615
Positive control	27 ± 3.67	5.667 ± 0.615 [#]
Atomoxetine 50mg/kg	66 ± 15.68	2.4 ± 0.4 ^{**}
Mentat® 100mg/kg	59.4 ± 17.37	2.4 ± 0.4 ^{**}
Mentat® 200mg/kg	47 ± 24.83	2.5 ± 0.5 ^{**}

^a All the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett’s test using Graph pad Prism software version 6.07 for windows, “p” value less than 0.05 was considered to be statistically significant #p<0.05 compare to control, **P<0.01 compare to positive control

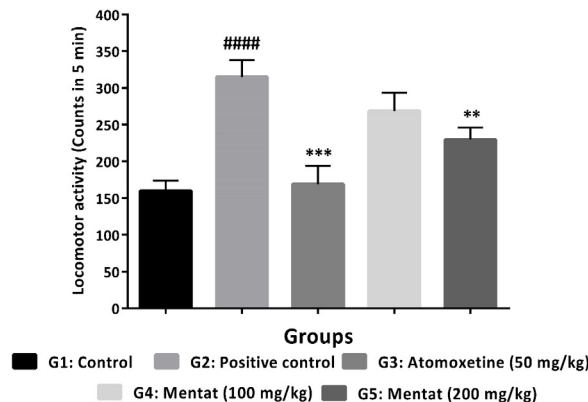


Fig. 1. Locomotor activity of the animals treated with Mentat® in Actophotometer (Neonatal hypoxia), all the values are expressed as mean ± SEM, mean of all the groups were compared by One-way ANOVA followed by Dunnett’s test, “p” value less than 0.05 was considered to be statistically significant. ####p<0.0001 compared to control, **p<0.01 and ***p<0.001 compared to positive control

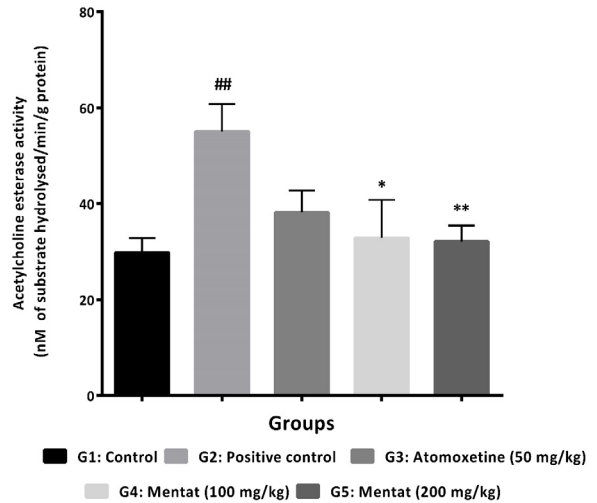


Fig. 2. Effect of Mentat® on acetylcholine esterase inhibitory activity, all the values are expressed as mean±SEM, mean of all the groups were compared by one way ANOVA followed by Dunnett’s test, “p” value less than 0.05 was considered to be statistically significant #p<0.05 compare to control, *p<0.05 and **p<0.01 compare to positive control

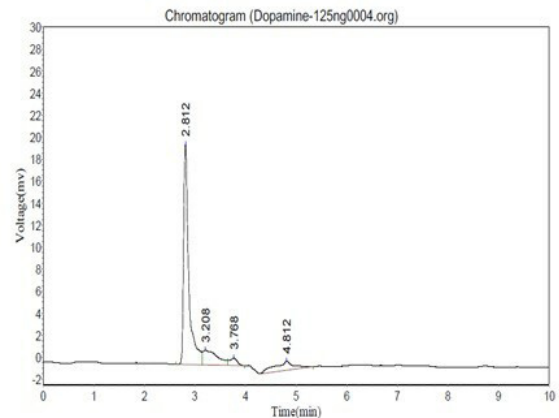


Fig. 3. HPLC Chromatogram of standard Dopamine

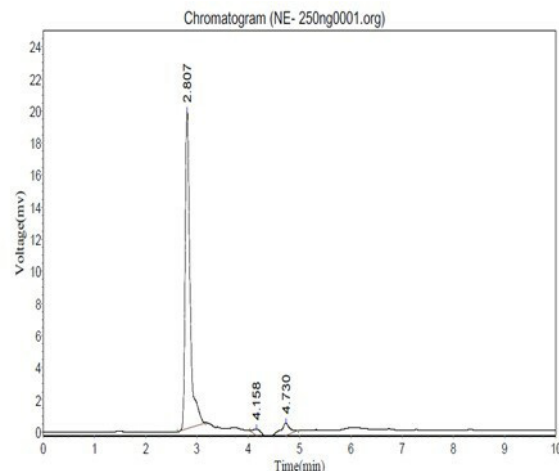


Fig. 4. HPLC Chromatogram of standard Nor-epinephrine

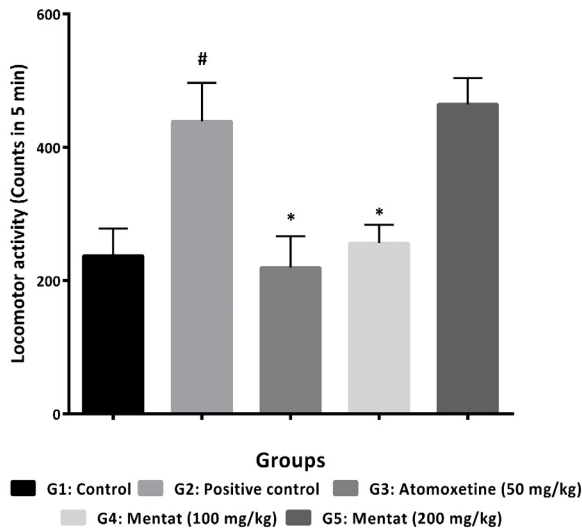


Fig. 5. Locomotor activity of animals treated with Mentat® (Phenytol induced hypoxia), All the values are expressed as mean±SEM, mean of all the groups were compared by one Way ANOVA followed by Dunnett's test, "p" value less than 0.05 was considered to be statistically significant #p<0.05 Compare to control, *p<0.05 compare to positive control

Discussion

ADHD is one of the most common childhood disorders, and it can last far into adulthood." Treatment for ADHD includes variety of stimulant drugs as well as more natural therapies. Many neurons are packed into distinct regions of brain when it comes to brain function. Each region performs a certain function in our body and is responsible for it. Neurotransmitters are created in minute amounts by neurons. Their job is to deliver messages. They excite the relevant cell in the brain, ensuring that the message is delivered to the correct brain region.²⁰

ADHD is caused by a shortage in certain neurotransmitters such as adrenaline and dopamine, according to brain scientists. Brain uses neurotransmitters to activate or inhibit activation in brain cells. The brain must be sufficiently aroused in order to pay attention. Areas of the brain must be effectively controlled, repressed, or slowed down in order to have proper control over our impulses. Both the stimulation and repression mechanisms are malfunctioning in ADHD children.²¹ The diagnosis of ADHD is a multi-step process that takes a lot of time. Parents, teachers, and other caregivers should all be involved in the child's evaluation.²²

Stimulant drugs work by inducing the brain to generate more nor epinephrine; non-stimulant medications work by reducing the pace at which nor epinephrine is broken down. The brain operates normally once the level has been corrected.²³ Dietary supplements are becoming the preferred choice as they are easily accessible and with relatively no side effects. Essential fatty acids are among the most popular. Essential fatty acids are need-

ed for proper cerebral functioning and may aid in the transmission of nerve impulses. Many children with ADHD cannot absorb essential fatty acids normally. There are evidences that herbal medications ameliorate the behavior of ADHD patients. Ginkgo Biloba is effective for neuronal disorders such as memory impairment. Lemon balm is known to help restore the balance and function of the brain and nerve cells

Mentat® which is a proprietary polyherbal formulation of Himalaya Wellness Company is used for neuro-protective activity. It has been found to be beneficial in rat models of transient global ischemia and reperfusion-induced brain injuries.²⁴ Mentat's® protective impact was assessed by evaluating its ability to alleviate cognitive, motor, and behavioral impairments caused by I/R-induced brain injury.²⁵ It improves memory and learning abilities. Mentat® contains natural substances that boost mental quotient, memory span, and attention, as well as treating neurological illnesses. Mentat® lowers tribulin levels, an endogenous monoamine oxidase inhibitor that rises during anxiety. Mentat® has relaxing properties that help with insomnia and seizures. Mentat® is useful as an adjuvant in the treatment of epilepsy and enuresis because of its anticholinesterase, dopaminergic-neuroprotective (important neurotransmitter in the brain), adaptogenic, and antioxidant qualities.^{26,27} Because there is no evidence of Mentat® effects on ADHD, experimental models of neonatal hypoxia and Phenytoin-induced hypoxia were chosen to assess the drug's efficacy. Rats subjected to neonatal hypoxia presented global brain atrophy ipsilateral to arterial occlusion in the regions analyzed: the total hemisphere, cerebral cortex, white matter, hippocampus and striatum. In addition, contralateral white matter was also affected by the hypoxia procedure.²⁸ Hypoxia in perinatal rats causes a malfunctioning nigrostriatal dopaminergic system, which is thought to produce ADHD behavior by increasing the expression of vesicular monoamine transporter 2 (VMAT2) and D1 receptor in the striatum. When phenytoin, a powerful anticonvulsant, is given to pregnant women, it reduces utero-placental blood flow, resulting in foetal hypoxia. Hypoxia during pregnancy might result in temporary or permanent brain damage. Through overstimulation of excitatory amino acid receptors, cellular calcium influx, and the production of free radicals and nitric oxide, the hypoxia/ischemia cascade causes neuronal cell death. Reactive oxygen species cause embryonic mortality or teratogenicity by oxidizing molecular targets such as DNA, protein, and lipid.²⁹

Bacopa monnieri (Brahmi), *Withania somnifera* (Ashwagandha), *Centella asiatica* (Mandookaparni), *Valeriana wallichii* (Tagar), *Evolvulus alsinoides* (Shankhapuspi) are important herbal extracts present in Mentat® which in combination are responsible for showing the desired efficacy. *Bacopa monnieri* is most

studied nootropic plant for ADHD patients' Clinical trials have shown that it improves memory. *B. monnieri* revitalizes the nervous system, strengthens the mind, and promotes both energy and sleep; it is frequently used to treat insomnia. It is also used to aid in the recovery from fatigue and stress. It is prescribed for conditions such as Parkinson's disease, Alzheimer's disease, dementia, and ADHD, and *B. monnieri* demonstrated a 66% reduction in total ADHD score.³⁰ *Withania somnifera* roots contain active phytoconstituents, primarily withanolides; alkaloids used in treatment of a variety of brain disorders, and have a wide range of neuroprotective properties. Clinical research suggests that *W. somnifera* may help children with ADHD improve their attention and behavioral control by enhancing normal brain development. According to research, *W. somnifera* accomplishes this by inhibiting the activity of the enzyme that degrades acetylcholine, a neurotransmitter associated with cognition and memory, as well as stimulating neuronal growth.³¹ *Centella asiatica* is a brain tonic that improves memory and brain strength. It improves the ability to speak and the poetic imagination. It is an effective treatment for children who are mentally retarded or emotionally disturbed. It aids in the treatment of stress, insomnia, ADHD, depression, mental fatigue, and anxiety.³² *Valeriana wallichii* has primarily antioxidant properties and is a choline esterase inhibitor. *V. wallichii* roots aid in the reduction of anxiety and the improvement of sleep by relaxing the central nervous system due to their sedative and anxiolytic properties.³³ *Evolvulus alsinoides* balances neurotransmitter levels of noradrenaline, glutamate, and acetylcholine in children and provides neuroprotection against free oxidative radicals and amyloid-induced neurotoxicity with its high antioxidant compounds. *E. alsinoides* lowers cortisol levels and helps to combat stress. It relaxes the nervous system and is extremely effective against insomnia.³⁴ When these herbal extracts are combined, they may have a synergistic effect that will be beneficial in treating ADHD.

Conclusion

Mentat[®] showed improvement in the behavior of ADHD rats, which exerts its effect by increasing the dopaminergic and norepinephrine response, decreasing the acetylcholine esterase and inhibiting the monoaminoxidase activity. As Mentat[®] showed a neuroprotective effect in different experimental models of ADHD, it may be recommended for the effective/preventive management of ADHD, especially associated with memory impairment and neurologic conditions. It can also be recommended as an adjuvant along with modern medicine in the management of ADHD. However clinical trials are required to further support the claim.

Acknowledgements

The authors acknowledge Himalaya Wellness Company for providing Mentat[®] samples and support for conducting the study and Dr. Pramod, Professor and Principal, JSS College of pharmacy, Mysore, for providing technical insights and support during this research.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, M.R., and S.N.M.; Methodology, S.S., G.L.V., C.J., and M.M.A.; Software, O.M.; Validation, S.S., G.L.V., C.J., and M.M.A.; Formal Analysis, G.L.V., O.M.; Investigation, G.L.V., O.M., and M.M.A.; Resources, S.S., C.J., and M.M.A.; Data Curation, G.L.V., O.M., C.J., and M.M.A.; Writing – Original Draft Preparation, S.S., G.L.V., C.J.; Writing – Review & Editing, O.M., and M.M.A.; Visualization, M.R., and S.N.M.; Supervision, M.R., and S.N.M.; Project Administration, M.R., and S.N.M.; Funding Acquisition, M.R.

Conflicts of interest

Some of the authors were the employees of Himalaya Wellness Company during the course of research work; authors declare no other conflict of interest.

Data availability

The data have not been made public, but are kept with the authors and will be provided, if necessary.

Ethics approval

The study was approved by Himalaya Wellness Company's Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (protocol no 127/13) and the experiments were carried out in accordance with the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

References

1. van der Kooij MA, Glennon JC. Animal models concerning the role of dopamine in attention-deficit hyperactivity disorder. *Neurosci Biobehav Rev.* 2007;31(4):597-618.
2. Faraone SV, Banaschewski T, Coghill D, et al. The world federation of ADHD international consensus statement: 208 evidence-based conclusions about the disorder. *Neurosci Biobehav Rev.* 2021;128:789-818.
3. van Hienen FJ. Organophosphate based pesticides and ADHD 2010. <https://studenttheses.uu.nl/>. Accessed June 21, 2022.
4. Rigan S. RANG & DALES Pharmacology 9th edition (TRUE PDF). Elsevier; 2012.

5. Kim HJ, Joo SH, Choi IH, et al. Effects of Red Ginseng on neonatal hypoxia-induced hyperactivity phenotype in rats. *J. Ginseng Re.* 2010;34(1):8-16.
6. Dogra P, Lata S. ADHD: Pathophysiology, Management, and Assessment Scales. *JMSCR.* 2018;6:341-347.
7. Saha A, Bhatia BD, Kulkarni KS. Evaluation of the efficacy of Mentat in children with learning disability: a placebo-controlled double-blind clinical trial. *Neurosci Today.* 2002;6(3):184-188.
8. Sharma AK, Agrawal A, Agrawal U, et al. Influence of mentat on memory and mental fatigue in cases of anxiety neurosis and depression. *Ind J Cancer Bio Res.* 1990;68(7.58):90-95.
9. Dave UP, Chauvan V, Dalvi J. Evaluation of br-16 a (mentat) in cognitive and behavioural dysfunction of mentally retarded children—a placebo-controlled study. *Ind J Paed.* 1993;60(3):423-428.
10. Dhingra D, Goyal PK. Inhibition of MAO and GABA: probable mechanisms for antidepressant-like activity of *Nardostachys jatamansi* DC. in mice. *Indian J Exp Biol.* 2008;46:212-218.
11. Pitchaiah G, Viswanatha GL, Srinath R, et al. Anxiolytic and anticonvulsant activity of aqueous extract of stem bark of *Erythrina variegata* in rodents. *Int J Pharm Tech Res.* 2010;2(1):40-48.
12. Naewla S, Sirichoat A, Pannangrong W, et al. Hesperidin alleviates methotrexate-induced memory deficits via hippocampal neurogenesis in adult rats. *Nutrients.* 2019;11(4):936.
13. National institute of mental health. U.S. <https://www.nih.gov/>. Accessed July 21, 2022.
14. Kulkarni SK, Singh K, Bishnoi M. Comparative behavioral profile of newer antianxiety drugs on different mazes. *Indian J Exp Biol.* 2008;46:633-638.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72(1-2):248-254.
16. Wills E. Mechanisms of lipid peroxide formation in animal tissues. *Biochem J.* 1966;99(3):667.
17. Kharbangar A, Khynriam D, Prasad SB. Effect of cisplatin on mitochondrial protein, glutathione, and succinate dehydrogenase in Dalton lymphoma-bearing mice. *Cell Biol Toxicol.* 2000;16(6):363-373.
18. Ellman GL, Courtney KD, Andres Jr V, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 1961;7(2):88-95.
19. Mach M, Dubovický M, Navarová J, et al. Experimental modeling of hypoxia in pregnancy and early postnatal life. *Interdiscip Toxicol.* 2009;2(1):28.
20. Romm AJ, Romm T. *ADHD alternatives: A natural approach to treating Attention-Deficit Hyperactivity Disorder.* Storey Publishing; 2000.
21. Feldman R. *Child development, 4th edition. Upper Saddle River, NJ: Pearson Prentice Hall. An overview of child development. Middle Childhood Years and Cognitive Development.* MD, Gelfand; 2008.
22. Allen KE, Allen CG. *The Exceptional Child.* Albany: Delmar Publishers; 2010.
23. ADDitude - ADD & ADHD Symptom Tests, Signs, Treatment, Support n.d. <https://www.additudemag.com>. Accessed December 21, 2021.
24. Viswanatha GL, Kumar LM, Rafiq M, et al. LC-MS/MS profiling and neuroprotective effects of Mentat® against transient global ischemia and reperfusion-induced brain injury in rats. *Nutrition.* 2015;1,31(7-8):1008-1017.
25. Hillard CJ. Role of cannabinoids and endocannabinoids in cerebral ischemia. *Curr Pharm Des.* 2008;14(23):2347-2361.
26. Himalaya Mentat Tablets - Enhances Memory – Himalaya Wellness (India) n.d. <https://himalayawellness.in/products/mentat>. Accessed December 21, 2021.
27. Scheuer C, Boot E, Carse N, et al. *Disentangling inclusion in physical education lessons: Developing a resource toolkit for teachers.* In: Physical Education and Sport for Children and Youth With Special Needs Researches–Best Practices–Situation. Slovakia; Slovak Scientific Society for Physical Education and Sport and FIEP: 2021;343-354.
28. Miguel PM, Schuch CP, Rojas JJ, et al. Neonatal hypoxia-ischemia induces attention-deficit hyperactivity disorder-like behavior in rats. *Behav Neurosci.* 2015;129(3):309.
29. Prince J. Catecholamine dysfunction in attention-deficit/hyperactivity disorder: an update. *J Clin Psychopharmacol.* 2008;28(3):39-45.
30. Katz M, Adar Levine A, Kol-Degani H, et al. A compound herbal preparation (CHP) in the treatment of children with ADHD: a randomized controlled trial. *J Atten Disord.* 2010;14(3):281-291.
31. Zahiruddin S, Basist P, Parveen A, et al. Ashwagandha in brain disorders: A review of recent developments. *J Ethnopharmacol.* 2020;257:112876.
32. Negi J. The ayurvedic pharmacopoeia of india the ayurvedic pharmacopoeia of India. Part - II. Government of India Ministry of Health and Family welfare department of Ayush. <http://www.ayurveda.hu/>. Accessed July 21, 2022.
33. Suhani S, Soubhik K. Effects and safety profile of commonly used herbal anti-diabetic preparations. *GSC Biol Pharm Sci.* 2018;4(2):66-79.
34. Sethiya NK, Nahata A, Mishra SH, et al. An update on Shankhpushpi, a cognition-boosting Ayurvedic medicine. *Chin J Integr Med.* 2009;7(11):1001-1022.