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RESEARCH ARTICLE

NEUROPROTECTIVE EFFECT OF METHANOLIC LEAF EXTRACT FRACTIONS OF *BUTEA MONOSPERMA* ON B-AMYLOID PEPTIDE INDUCED AMNESIA IN MICE

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ABSTRACT

The objective of this investigation was to evaluate the neuroprotective activity of methanolic leaf extract fractions (ethyl acetate, n-hexane, chloroform and aqueous) of *Butea monosperma* (BM) on β-amyloid peptide induced amnesia in mice. From previous studies, the methanolic leaf extract showed neuroprotecive activity in amnesia mice. Further, the leaf extract is subjected to fractionization by using ethyl acetate, n-Hexane, chloroform and water as solvents. The fractions are subjected to jumping test and Y-maze test, rectangular maze test and also for biochemical parameters such as acetyl cholinesterase enzyme (AChE), peroxidase, MDA and DPPH radical scavenging activity. From the results, it was found that the fractions of extract possess neuroprotecive activity and ethyl acetate fraction having significant anti-amnesic activity and anti oxidant activity compared with other three fractions. By identifying and isolating the chemical constituents responsible for anti-amnesic and anti-oxidant activities, we may find a breakthrough in the treatment of other neurodegenerative disorders which occur due to oxidative stress.

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INTRODUCTION

Alzheimer's disease (AD), is a devastating neurodegenerative disease with progressive loss in memory (Ahmed and Gilani, 2009) and it is associated with a shrinkage of brain tissue, with localized loss of neurons mainly in the hippocampus and basal forebrain (Rang *et al*., 2001). AD is characterized by the deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles (composed of paired helical filaments) in the brain of AD patients (Reddy, 1997). The loss of cholinergic neurons in the hippocampus and frontal cortex is a feature of the disease (Selkoe, 2002). A wide variety of medicinal plants had been implicated to possess AChE inhibitory effect and may be appropriate to treat the neurodegenerative disease such as AD (Rao *et al*., 2005). To validate their folklore use for the treatment of different diseases. As CNS active agents, identification of *Rawolfia serpentina*, *Mucuna pruriens*, *Ocimum santum Withania somnifera*, *Centella asiatica* and *Bacopa monneria* indicated the importance for treatment of brain related disease and disorders (Bhattacharya *et al*., 2000). Especially *Centella asiatica* and *Bacopa monneria, Curcuma longa and Gingo biloba* are used to treat loss of memory including Alzheimer's types of dementia (Gertz and Kiefer, 2004). There were significant evidences to sustain the conception that abnormal production of reactive oxygen species (ROS) called as free radicals along with amyloid beta (Aβ) protein causes neuronal vulnerability and leads to neuropathological disease, such as Alzheimer's disease. Apart from the production of ROS and Aβ, the involvement of acetylcholinesterase, monamine oxidase enzymes, neurotransmitters and neurohormonal changes were consistent during the neuropathology of AD. One of the folklore claims of the plant *Butea monosperma* is used as rejuvenator in the treatment of neurodegeneration and in memory. Experimentally the present study was designed to determine the folklore effect of the selected plant extracts and the bioactive molecule in the involvement of AChE, ROS (reactive oxygen species), neuroimmune and neuroendocrine pathways for the learning and memory process (Veena and Kasture, 2002). (Siman *et al*., 200) evaluated the effect of leaf extract on stress, cognition, anxiety in rats. Main objective of the study is to assess the pharmacological activity of *B. monosperma* leaf extract on β-amyloid peptide induced amnesia in mice. Currently available drugs to treat AD like AChE inhibitors exert symptomatic relief but do not reduce progression of disease and have side effects. Previously, Thirupathi et al., 2016 reported that the methanolic leaf extract of *B. Monosperma* possess anti-amnesic activity. But, the fractionization activity is not reported. Hence, we attempted to

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study the role of fractions of methanolic leaf extracts on neurodegenaration disorder.

MATERIALS

Acetylthiocholine iodide, dithiobisnitro benzoic acid, reduced glutathione, β-amyloid peptide are purchased from Sigma Aldrich, Hyderabad, India. Other reagent solvents including ethyl acetate, methanol used were analytical grade purchased from Himedia laboratories, Hyderabad, India. DPPH, ascorbic acid, thiobarbituric acid, sodiumdodecyl sulphate, tetra ethoxy propane, trichloro acetic acid, donepezil were purchased from Hi media Labs, Hyderanad, India.

METHODS

Fractinization of methanolic leaf extract

The methanolic leaf extract of *Butea Monosperma* was collected as per the procedure of Thirupathi *et al*., 2016. The leaf extract was fractionated by using ethyl acetate, n-Hexane, chloroform and water as solvents of fractionization. The yield of each fraction was calculated and reported.

Animals

All the animals handling and experimentation were conducted in accordance with the prior approved guidelines from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi and the experimental protocol was approved by Institutional Animal Ethical Committee (IAEC/05/UCPSc/KU/2016). Swiss albino mice (male) weighing 22-25 g at the age of 5-6 weeks, obtained from Sainath agencies, Hyderabad was used for the pharmacological studies. The animals were kept under standard conditions maintained at 23-25^{o}C, 12h light /dark cycle and given standard pellet diet (Vyas Labs, Hyderabad) and water *ad libitum*. The animals were acclimatized to the laboratory conditions for a week prior to the experimentation and randomly divided into eleven groups of six animals each.

Grouping and induction of neurotoxicity

Neurotoxicity was induced by intra cerebroventricular (i.c.v.) injection of $A\beta_{(25-35)}$ peptide by identifying bregma point in the skull using stereotaxic apparatus (INCO, Ambala, India). Each animal was injected with 10µl which contain 10µg of β-amyloid peptide (Laursen and Belknap, 1986). Except group 1-vechicle control, received with p.o of phosphate buffered saline (PBS), group 2-negative control received only with i.c.v. injection of β-amyloid peptide, group 3- animals injected with β-amyloid peptide and treated with Donepezil (p.o.) 5mg/kg which is used as standard drug, remaining all group animals are injected with β-amyloid peptide and treated with 200 mg/kg and 400 mg/kg of ethyl acetate, n-hexane, chloroform and aqueous fractions of methanolic leaf extract, respectively.
Drug treatment started on 14th day of the β-amyloid peptide treatment and continued for six days. On the 7th day of the drug treatment or 21st day after β-amyloid peptide treatment behavioral studies and biochemical parameters are estimated (Hanish *et al*., 2011).

Jumping box (conditioned avoidance test)

It is done by using medicraft jumping box. Box divided into 2 equal chambers by plexiglass partition, with a gate providing access to adjacent compartment through 14×17 cm space. In each trial animal is subjected to light for 30 seconds followed by a sound stimulus for 10 seconds. Immediately after sound stimulus, mice receive a single low intensity foot shock (0.5mA, 3 sec). Each animal received a daily session of 15 trials with an inter trial duration of 15 seconds for 5 days (Barros *et al*., 2000).

Maze test

Assessment of memory was done using medicraft rectangular maze. The apparatus consisted of three interconnected chambers A, Band C. Chamber B constituted the maze. Food deprived mice were placed in chamber A and challenged to learn and remember the location of C, after travelling through B. Their presence in chamber C was indicated by a pilot light. Chamber C contained the reward which was food for the hungry animal. The animals were trained for consecutive daily sessions, and the time required to traverse the maze was noted. They were considered trained when the maze completion time for 3 consecutive days was more or less constant. Maze traverse time was then recorded for each animal before and after drug treatment (Parle *et al.,* 2004).

Y-maze test

The Y-maze task was used to measure the spatial working memory in mice. The maze is made of gray plastic. Each arm is 40 cm long, 13 cm high, 3 cmwide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze for 8 min. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know which arm they have already visited. The series of arm entries, including possible returns into the same arm, is recorded by a video-tracking system (VJ Instruments, Washim, Maharastra, India). Alteration is defined as the successive entries into the three arms, on overlapping triplet sets. The percentage of alteration is calculated as the ratio of actual alterations to possible alterations, defined as the total number of arm entries minus two, and multiplied by hundred. Typically, mice exhibit an alteration percentage of 60–70%, and perform 25–35 arm entries within the 8 min session (Reddy, 1997).

Biochemical estimations

Acetylcholinesterase (AChE) enzyme determination (Ellman *et al*., 1961)

In a Potter-Elvehjem homogenizer 20 mg of braintissue/ml with Phosphate buffer (pH 8, 0.1 M) was homogenized. 0.4 ml aliquot was added to cuvette contain 2.6 ml of 0.1M phosphate buffer (pH 8). To the photocell 100 µl DTNB solution was added and absorbance was read at 412 nm. Then change of absorbance was recorded after adding 20 µl of the acetylthiocholine iodide and change in absorbance/minute was calculated and activity of enzyme is expressed as µmoles/min/g tissue

Assay of glutathione peroxidase

GSH was determined by its reaction with 5,5-dithiobis(2-nitrobenzoic acid) DTNB to yield a yellow chromophore which was measured spectrophotometrically (Ellman,1959). The brain homogenate was mixed with an equal amount of 10% trichloro acetic acid and centrifuged at 2000 g for 10 minutes. The supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (ph 8.4), 0.5ml of DTNB and 0.4 ml of double distilled water were added and the mixture was shaken vigorously. The intensity of color developed was read at 412 nm immediately in spectrophotometer. The activity of GPx is expressed as µmoles / minutes / mg protein (Lawrence and Burk, 1976).

Estimation of MDA

MDA which is a measure of lipid peroxidation was described by Ohkawa *et al*., 1979. Briefly, brain tissues were homogenised with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. Mixture was then heated at 100^{o} C for 60 minutes. Mixture was then cooled with tap water and 5 ml of n-butanol:pyridine (15:1 % v/v), 1ml of distilled water was added. Mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, organic layer was withdrawn and absorbance was measured at 532 nm using spectrophotometer. 1,1,3,3-tetra ethoxy propane is used as standard.

DPPH radical scavenging activity assay

The free radical scavenging activity of different fractions on 2, 2-di-phenyl-2-picrylhydrazyl (DPPH) radical was measured by reduction of DPPH to DPPHH (Di-phenyl picryl hydrazine) [Sanchez-Moreno et al., 1999]. IC50 value was determined as the inhibitory concentration of extract that could scavenge 50% of the DPPH radicals. Ascorbic acid was used as positive reference.

RESULTS AND DISCUSSION

After fractionization of methanolic leaf extract, yield is noted in terms of w/w of dry material and was found to be 6.5, 5.3, 6.1 and 3.3% for ethyl acetate, n-hexane, chloroform and aqueous fraction, respectively. The fractions were considered as nontoxic, because it did not showed any toxic signs or symptoms and mortality in the oral dose of 2000 mg/kg of the methanol extracts of *Butea Monosperma* fractions in mice. According to OECD-423 guidelines, the LD_{50} of 2000 mg/kg and above is mentioned as unclassified. So further pharmacological screening is carried out. The fractions are subjected to various behavioral and biochemical parameters estimation in β-amyloid peptide induced amnesia mice.

Jumping box (conditioned avoidance test)

The conditioned avoidance test (memory) of amnesia mice were studied and the results are depicted in the Table 1. The activity was expressed in latency periods with sec time. In jumping box test, there was an in increase in latency period in

Table 1. Effect of *B. monosperma* fractions on different behavioral parameters in amnesia mice (Mean ± SD, n=6)

Group	Jumping box test (Sec)	Rectangular maze test (Sec)	Y-maze test (% alterations)
I (PBS)	12.5 ± 0.22	47.83 ± 2.37	47.61 ± 3.15
II (Aβ)	24.6 ± 2.07	144.66 ± 10.05	25.57 ± 2.87
III (Aβ + DPZ)	15.69 ± 1.21***	71.66 ± 2.15***	38.93 ± 2.38**
IV (Aβ + 200mg/kg BME)	17.4 ± 1.09**	81.16 ± 2.21**	38.21±2.14**
V (Aβ + 400mg/kg BME)	13.1 ± 1.17***	71.55 ± 3.84***	43.77±2.84***
VI (Aβ + 200 mg/kg BMH)	18.1±0.62**	85.76±2.81**	34.26±1.32*
VII (Aβ + 400 mg/kg BMH)	14.4±0.71**	73.79±2.54***	41.81±2.47**
VI III (Aβ + 200mg/kg BMQ)	20.8±1.24*	105.6±3.74*	29.54±1.08
IX(Aβ + 400mg/kg BMQ)	16.3±1.17**	96.7±2.86**	37.76±1.79**
X (Aβ + 200mg/kg BMC)	19.7±1.10*	97.4±2.48**	32.75±1.93*
XI (Aβ + 400mg/kg BMC)	15.3±0.75**	88.3±3.26**	39.88±2.05**

*p<0.05, **p<0.01, ***p<0.001 when compared to negative control group. ANOVA (one-way) followed by Bonferroni's test.

Table 2. Effect of *B. monosperma* fractions on different biochemical parameters in amnesia mice (Mean ± SD, n=6)

Group	AChE (µmole/min/g)	GSH (µmole/min/g protein)	MDA (µg/gm wet tissue)
I (PBS)	0.10 ± 0.0012	0.0298 ± 0.00014	23.46 ± 0.30
II (Aβ)	0.23 ± 0.0015	0.0159 ± 0.00023	51.63 ± 0.54
III (Aβ + Donepezil)	0.11 ± 0.0011***	0.0281 ± 0.0012***	28.66 ± 0.35***
IV (Aβ + 200mg/kg BME)	0.15 ± 0.0026**	0.0256 ± 0.0018**	39.98 ± 1.07**
V (Aβ + 400 mg/kg BME)	0.12 ± 0.0023***	0.0279 ± 0.0025***	31.26 ± 1.88***
VI(Aβ + 200mg/kg BMH)	0.17 ± 0.0021**	0.0247 ± 0.0008**	41.36 ± 1.16*
VII (Aβ + 400mg/kg BMH)	0.13 ± 0.0018***	0.0268 ± 0.0004***	34.78 ± 1.43**
VIII (Aβ + 200 mg/kg BMQ)	0.21 ± 0.0046	0.0227±0.0011*	47.72±1.86*
IX (Aβ + 400 mg/kg BMQ)	0.18 ± 0.0051*	0.0242±0.0024**	39.92±2.54**
X (Aβ + 200mg/kg BMC)	0.19 ± 0.0025*	0.0239±0.0013*	43.31 ± 1.62*
XI (Aβ + 400mg/kg BMC)	0.16 ± 0.007**	0.0253±0.0027**	33.67 ± 2.64**

*p<0.05,**p<0.001,***p<0.0001 when compared to negative control group, ANOVA (one-way) followed by Bonferroni's test.

negative control group (24.6±2.07) when compared to vehicle control (12.5 ± 0.22). In case of treated groups, there is a decrease in latency period in groups treated with BME (17.4±1.09 and 13.1±1.17 for 200 and 400 mg/kg), chloroform BMC (19.7±1.10 and 15.3±0.75 for 200 and 400 mg/kg), BMH (18.1±0.62 and 14.4±0.71 for 200 and 400 mg/kg) and aqueous extract BMQ (20.8±1.24 and 16.3±1.17 for 200 and 400 mg/kg). From the results, the BME fraction shows statistically significant more activity compared to BMC, BMH and BMQ fractions and also all the fractions exhibited dose dependent effect.

Maze test

The hippocampal learning of Aβ induced group (negative control) was declined and shown a significant (p<0.001) increase in escape latency while comparing the control group. The readings were showed in Table 1. From the results, in rectangular maze test there was an increase in maze traverse period in negative control group (144.66±10.05 sec) when compared to vehicle control (47.83±2.37 sec), and there was a dose dependent decrease in traverse period in groups treated with BME (81.16 ± 2.21 and 71.55 ± 3.84), BMC (97.4±2.48 and 88.3±3.26), BMH (85.76±2.81 and 73.79±2.54) and BMQ (105.6±3.74 and 96.7±2.86) fractions.

Y-maze test

The Y-maze test were performed in amnesia mice and reported in % alterations in Table 1. As the dose increased from 200 to 400 mg/kg, % alteration was increased. From the results, in negative control and vehicle groups the % alteration was found to be 25.57 ± 2.87 and 47.61 ± 3.15, respectively. In case of BME, BMH, BMC and BMQ the % alteration were found to be 38.21±2.14 and 43.77±2.84, 34.26±1.32 and 41.81±2.47, 32.75±1.93 and 39.88±2.05; 29.54±1.08 and 37.76±1.79 for 200 and 400 mg/kg, respectively.

Biochemical estimations

The fractions were subjected to various biochemical parameters i.e., AChE, glutathione and MDA levels. The differences in biochemical parameters in treatment groups were summarized in the Table 2. The i.c.v injection of Aβ peptide in negative control animals an extremely significant (p<0.001) increase in brain AChE levels. The AChE enzyme levels were significantly decreased in the treatment groups of BME (0.15 ± 0.0026 and 0.12 ± 0.0023), BMC (0.19 ± 0.0025 and 0.16± 0.007), BMH (0.17 ± 0.0021 and 0.13 ± 0.0018)and BMQ (0.21 ± 0.0046 and 0.18± 0.0051) and indicated a difference with p<0.01, p<0.05, p<0.001 and p<0.05, respectively when compared with the amnesia induced group. Further, the fractions showed dose dependent activity in all the amnesia mice. From the previous reports, the decreased levels of AChE in treated animals are the indication of anti-amnesic activity. In case of MDA levels, the levels are increased in negative (51.63 ± 0.54) when compared to vehicle control (23.46 ± 0.30), and decreased levels are observed in BME (39.98 ± 1.07 and 31.26 ± 1.88), BMC (43.31 ± 1.62 and 33.67 ± 2.64), BMH (41.36 ± 1.16 and 34.78 ± 1.43) and BMQ (47.72±1.86 and 39.92±2.54) fractions. The decreased levels of MDA parameter in fractions treated group was the indication of anti-amnesic activity of BM fractions. Glutathione levels are decreased in negative (0.0159±0.00023) when compared to control (0.0298±0.00014), and levels are increased in BME (0.0256±0.0018 and 0.0279 ± 0.0025), BMC (0.0239±0.0013 and 0.0253±0.0027), BMH (0.0247 ± 0.0008 and 0.0268 ± 0.0004) and BMQ (0.0227±0.0011 and 0.0242±0.0024) fractions. The results are statistically significant compared with negative control (p<0.05). The antioxidant property of different fractions were evaluated by using DPPH free radical scavenging assay and these fractions exhibited dose dependent free radical scavenging activity. The IC_{50} value of BME, BMC, BMH and BMQ fractions were found to be 26.42, 33.77, 30.15 and 41.04 µg/ml, with respective to IC_{50} value of ascorbic acid and was found to be 12.92 µg/mL.

Conclusion

The pharmacological evaluations on different fractions (ethyl acetate, chloroform, n-hexane and aqueous) of *Butea Monosperma* indicated the anti-amnesic effect. The neuroprotective and anti-amnesic effect of these fractions were evidently supported by decrement of neurotransmitter metabolic enzyme (AChE) with escalation in antioxidants. Thus, to conclude that the fractions of methonalic leaf extract especially, the ethyl acetate fraction expressed a prospective effect on learning and memory process. Therefore, the results demonstrated the anti-amnesic activity of methonalic leaf extract fractions of *B. monosperma*.

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Declaration of interest

The authors declare that no conflict of interest.

REFERENCES

Ahmed T, Gilani AH.2009. Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. *Pharmacol Biochem Behav.* 91: 554-559.

Barros DM, Izquierdo LA, Mello ST, Ardenghi P, Pereira P, Medina JH Izquierdo I. 2000. Molecular signaling pathways in the cerebral cortex are required for retrieval of one-trial inhibitory avoidance learning in rats. *Behav brain res.* 114: 183-192.

Bhattacharya S.K, Bhattacharya A, Kumar A, Ghosal S.2000. Antioxidant activity of Bacopa monniera in rat frontal cortex, striatum and Hippocampus, *Phytother Res,* 14(3):174-179.

Ellman GL, Courtney KD, Anders V, Featherstone RM.1961. A new and rapid colorimetric determination of acetyl cholinesterase activity. *Biochem pharmacol.* 7:88-95.

Gertz HJ. Kiefer M.2004. Review about Ginkgo Biloba Special Extract EGb 761 (Ginkgo), *Curr pharm des.* 10: 261-264.

Hanish Singh JC, Alagarasamy V, Prakash Diwan V, Sateesh Kumar S, Narsimha Reddy Y.2011. Neuroprotective effect of *Alpinia galanga* (L.) fractions on $A\beta_{(25-35)}$ induced amnesia in mice. *J Ethnopharmacol.* 138, 2011, 85- 91.

Laursen S.E., Belknap J.K.1986. Intra cerebro ventricular injections in mice. Some methodological refinements. *J Pharmacol Methods,* 16: 355–357.

Lawrence RA, Burk RF.1976.Glutathione peroxidase activity in selenium deficient rat liver. Biochem Biophy Res Communic. 71:952-958.

Ohkawa H, Ohishi N, Yagi K.1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analy Biochem.* 95(2): 351–8.

Parle M, Singh N.2004. Animal models for testing memory. *Asia Pac J Pharmacol* .16:101-120.

Rang HP, Dale MM, Ritter JM, Flower RJ.2001. The vascular System. Text book of Pharmacology, 6th Edn New Delhi: Churchill Livingstone, Harcourt publishers limited, 298-320.

Rao Sulochana B, Chetana M, UmaDevi P.2005. Centella asiatica treatment during postnatal period enhances learning andmemory in mice. *Physiol Behav*. 86: 449-457.

Reddy DS.1997. Assessment of nootropic and amnesic activity of centrally acting agents. *Ind J Pharmacol.* 29: 208-221.

Sanchez-Moreno C, Larrauri J, Saura-Calixto F.1999. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Res Int*., 32:407–12.

Selkoe DJ. 2002. Alzheimer's disease is a synaptic failure. *Science.* 298: 789-791.

Soman SAM, Kasture SB.2004. Effect of leaves of *butea frondosa* on stress, anxiety, cognition in rats. *Pharmacol Biochem Behav.* 79: 11-16.

Thirupathi G, Pavan Kumar A, Narsimha Reddy Y.2016. Effect of methanolic leaf extract and ethyl acetate fraction of *butea monosperma* on β-amyloid$_{(25-35)}$ induced amnesia in laboratory animals. *Int J Pharma Biolog Sci.,* 6(4): 78-84.

Veena SK, Kasture SB, Chopde CT.2002. Anti convulsive activity of *Butea monosperma* flowers in laboratory animals. *Pharmacol Biochem Behav.,* 72: 965-972.

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