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Appraisal of Nootropic Activity of *Morus Alba* Extracts

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Abstract- Mulberry (*Morus alba* L) species is native to northern China and is widely cultivated various Asian countries such as Japan, Iran, etc. Morus alba was traditionally used in Chinese medicinal remedy for ailments of bronchitis, insomnia, constipation and inflammatory. The research was planned to appraisal nootropic activity of aqueous and ethanol extracts of *Morus alba* using conditioned avoidance response in rat and estimation of acetyl cholinesterase activity by Ellman's method in rats. Conditioned avoidance response was evaluated by using the Perspex chamber apparatus. Animals were treated with scopolamine butyl bromide (1mg/kg bw, i.p) thirty minutes before foot shock to produce amnesia. Animals were trained to jump on the pole to avoid shock with receiving daily oral dose of aqueous and ethanol extracts of Morus alba at dose of 200 and 400 mg/kg body weight one hour before the induction of foot shock. The esterase activity was measured by providing an acetyl thiocholine which cause to release thiocholine as result of cleaving by AChE. Thiocholine reduced 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to thionitrobenzoic acid which absorbed light at 412 nm. The groups treated with aqueous and ethanolic extracts of *Morus alba* were found to nootropic activity by reversing the scopolamine induced amnesia. Acetyl cholinesterase inhibitory activity of extracts of Morus alba were performed the supportive nootropic activity by enhancing the cognitive function.

Keywords: *morus alba, nootropic activity, acetyl cholinesterase.*

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I. Introduction

Nootropic compounds exhibit a novel class of psychotropic agents with selective facilitating neural activity on integrative function on the central nervous system, especially on intellectual performance, memory and learning capacity (Giurgea, 1973). Indian herbal remedies have been used in the treatment of epilepsy, cognitive dysfunction and insomnia (Bhanumathy et al., 2010) such as *Baccopa monniera* and *Centella asiatica* (Mohan et al., 2005). There is crucial evidence that stress can modify cognitive functions which can lead to various neurodegenerative disorders such as Parkinson' disease or Alzheimer' disease (Koppula and Choi, 2011).

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The central cholinergic system is surveyed to be main neurotransmitter involved in the modulation of cognitive functions. Acetyl cholinesterase has closed interrelation activity with cholinergic function and cognition. Consequently assessment of AChE activity can supply a vital correlation of cognitive function and cholinergic activity (Srikumar et al., 2004). The Indian system of medicine emphasizes use of herbs for neurodegenerative disorders (Jakka Al, 2016). Accordingly the current research was investigated for nootropic activity of aqueous and ethanol extracts of *Morus alba*.

II. Materials and Methods

a) Plant material and Preparation of extracts

The fruits of *Morus alba* were collected from Chennai, Tamil Nadu, India and authenticated by Green Chem, Bangalore, Karnataka, India, a voucher specimen (MAT-SIP-501) were preserved for future references. The fruits materials (1kg) were dried, powdered and extracted with water and ethanol (60-80°C) using soxhlet methods. The filtrate was evaporated at 70 °C in a vacuum dryer to give final yield 40.5g.

b) Chemicals

Scopolamine butyl bromide and piracetam procured from Stride acrolabs Ltd, Bangalore, India. Other chemicals were analytical up grade and acquired from local store of Visveswarapura Institute of Pharmaceutical Sciences.

c) Animals

Male albino wistar rats (180-200gm) acquired from the NIMHANS animal house, Bengalore. The animals were kept under standard conditions in an animal house as per the guidelines of "Committee for the Purpose of Control and Supervision on Experiments on Animals" (CPCSEA) for at least one week prior to use. The rats had free access to standard rat chow and water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. (Registration No: 152/1999, renewed in 2012).

d) Conditioned avoidance response (Perspex chamber apparatus)

Animals were subjected to a training schedule individually by placing inside the perspex chamber of

the apparatus 60 minutes after oral administration. Buzzer was given followed by a shock through the grid floor. The rat had to jump on the pole to avoid foot shock. Jumping prior to the onset of the shock was considered as avoidance. The session was terminated after completion of 30 trials with an interval of 20–30 seconds given for each trial. This procedure was repeated at 24 h intervals until all groups reach 95 to 99% avoidance. After attaining complete training of a particular group, the animals were treated with a single dose of scopolamine butyl bromide (1 mg/kg body weight, i.p.), thirty minutes before the next day dosing. The training schedule was continued further with the daily doses of the aqueous and ethanol extracts of *Morus alba* until they returned to normal level from scopolamine induced amnesia (Cook and Weidley, 1957).

e) *Estimation of acetyl cholinesterase activity by Ellman's method*

Rats were decapitated; brains were removed rapidly and kept in ice-cold saline. Frontal cortex, hippocampus and septum were quickly dissected out on a petri dish chilled on crushed ice. The tissues were homogenized in 0.1m Phosphate buffer. Added 0.4 ml of the homogenates to 2.6 ml phosphate buffer and 100 µl of DTNB. Absorbance was measured at 412 nm in a UV spectrophotometer. When absorbance reaches stable value, it was recorded as the basal reading. Added 20µl of acetyl thiocholine iodide and recorded the change in the absorbance for a period of 10 minutes. Change in the absorbance per minute was determined. The enzyme activity is calculated using the following formula (Srikumar et al., 2004):

$R = 5.74 \times 10^{-4} \times A/CO$

R= Rate in mole of substrate hydrolysed/minute/gm tissue

A= Change in absorbance/ minute

CO= Original concentration of the tissue (mg/ml)

f) *Treatment schedule*

For conditioned avoidance response in rat, wistar albino rats were divided into 5 groups consisting of 6 animals in each group. Group 1 rats served as normal control and received 2ml/100g bw distilled water, group 2,3 rats received aqueous extracts *Morus alba* orally at dose of 200 mg/kg and 400 mg/kg respectively. Group 4,5 rats were administrated orally with ethanol extracts of *Morus alba* at dose of 200 mg/kg and 400 mg/kg respectively and for estimation of acetyl cholinesterase activity by Ellman's method in rats, wistar albino rats were divided into 6 groups consisting of 6 animals in each group. Group 1 was vehicle group (Distilled water 2ml/100g bw). Group 2 received standard drug piracetam 200mg/kg i.p. Group 3,4 rats were administrated orally with aqueous extracts of *Morus alba* at dose of 200 mg/kg and 400 mg/kg respectively. Group 5,6 rats received ethanol extracts of *Morus alba* orally at dose of 200 mg/kg and 400 mg/kg respectively.

g) *Statistical analysis*

The data were expressed as mean ± S.E.M. Results were statistically analysed by using one way ANOVA followed by Dunnett's test and $p < 0.05$ was considered as statistically significant.

III. Results

a) *Evaluation of the nootropic activity of aqueous and ethanol extracts of Morus alba using conditioned avoidance response (CAR) in rats*

Figure 1 exhibits the effects of aqueous and ethanol extracts of *Morus alba* on mean percentage of conditioned avoidance response after oral administration in rats. The CAR of rats treated with the aqueous and ethanol extract of *Morus alba* and vehicle increased gradually to 95% over eight to ten days. The percentage avoidance was higher in the groups administered with aqueous and ethanol extract of *Morus alba* compared to vehicle treated control group. The acquisition (time to achieve 95% CAR) for the groups treated with aqueous and ethanol extracts of *Morus alba* was quicker and found to be dose dependent. Animal in group II and III administered with aqueous extract of *Morus alba* at a dose of 200 mg/kg p.o and 400 mg/kg p.o have taken ten days and nine days respectively to reach the point of acquisition. Whereas animals in group IV and V administered with ethanol extract of *Morus alba* at a dose of 200 mg/kg p.o and 400 mg/kg p.o have taken nine days and eight days respectively to reach the point of acquisition. Administration of scopolamine produced amnesia as seen from reduction in the observed CAR. The amnesia was found to be greater in control group compared with the groups treated with aqueous and ethanol extract of *Morus alba* and was also found to be dose dependent. Animals treated with aqueous extract of *Morus alba* at a dose of 200 mg/kg and 400 mg/kg had taken five and four days whereas, group treated with ethanol extract of *Morus alba* at a dose of 200 mg/kg and 400 mg/kg had taken three days each to reach the point of acquisition after administration of scopolamine butylbromide. The control group had taken eleven days for retention and recovery from scopolamine induced amnesia.

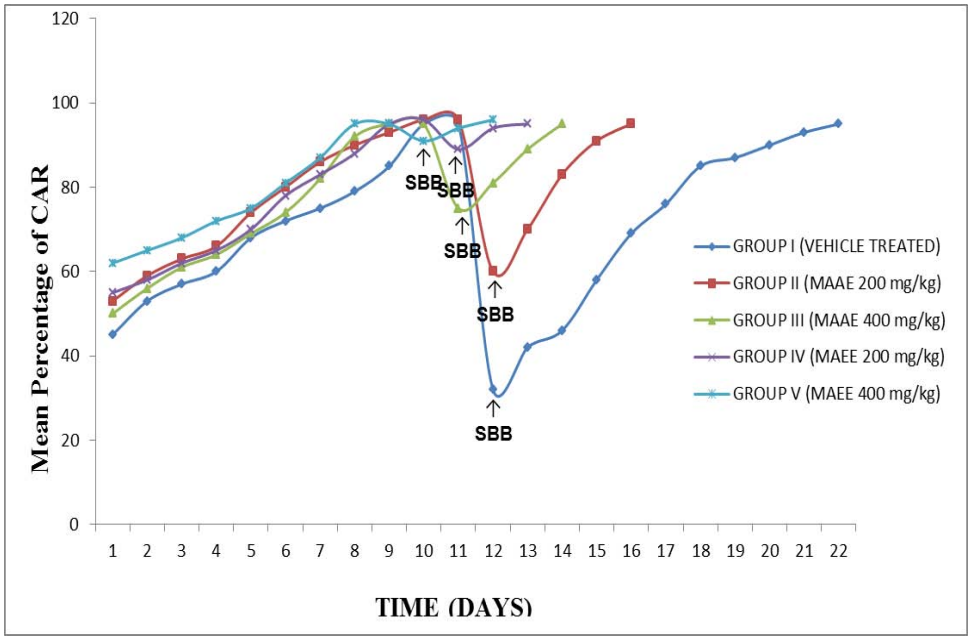

Figure 1: Effect of aqueous and ethanol extracts of *Morus alba* on mean percentage of conditioned avoidance response after oral administration in rats. Scopolamine butylbromide (SBB) was administered 30 minutes before next day dosing with the extracts after attaining complete acquisition. MAAE: aqueous extract of *Morus alba*, MAEE: ethanol extract of *Morus alba*.

b) *Estimation of acetyl cholinesterase activity by Ellman's method*

Figure 2 manifests the effects of aqueous and ethanol extracts of *Morus alba* on acetylcholinesterase (AChE) activity in rats' brain. The groups treated with aqueous and ethanol extract of *Morus alba* had indicated decrease in AChE activity as compared to control group. Control group had showed 7.460 X 10^{-7} μmol/min/g tissue of acetylcholinesterase activity in rat brain. Prior administration with Piracetam (standard) had showed a significant reduction in acetylcholinesterase activity 4.010 X 10^{-7} μmol/min/g. Prior administration of aqueous extract of *Morus alba* at dose of 200 mg/kg p.o and 400 mg/kg p.o have showed non-significant decrease in acetylcholinesterase activity 6.820 X 10^{-7} and 6.320 X 10^{-7} μmol/min/g respectively as compared to control group. However, significant decline was observed in groups treated with ethanol extract of *Morus alba* at dose of 200 mg/kg p.o and 400 mg/kg p.o with acetylcholinesterase activity 4.940 X 10^{-7} μmol/min/g ($P<0.01$) and 4.540 X 10^{-7} μmol/min/g ($P< 0.001$) respectively as compared to control group.

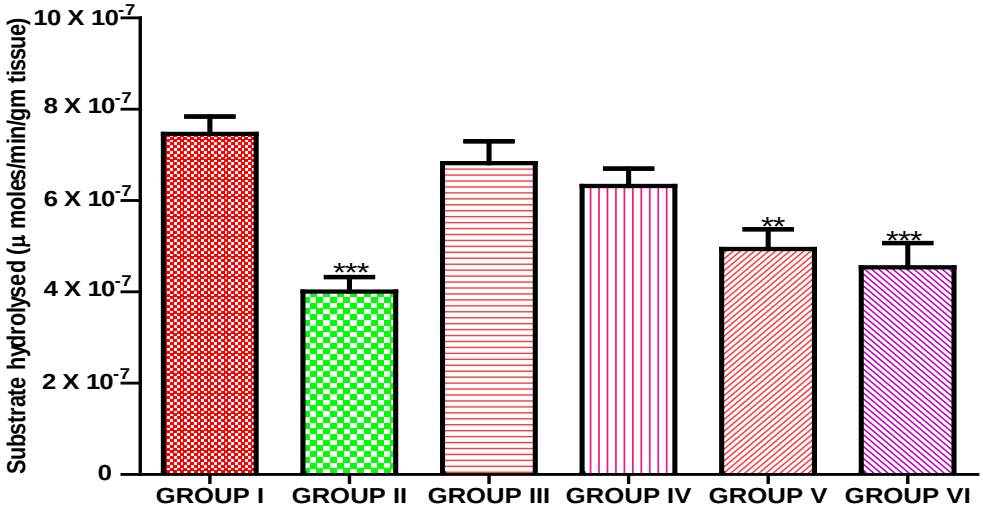

Figure 2: Effect of aqueous and ethanol extracts of *Morus alba* on acetylcholinesterase (AChE) activity in rat's brain. Data is expressed as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Dunnett's test. $^{**}P< 0.01$ and $^{***}P< 0.001$ were considered statistically significant.

IV. Discussion

The original definition for nootropics was laid out by Dr. Corneliu E. Giurgea in 1972 who also is the inventor behind Piracetam. The word itself is taken from the Greek language and is a combination of two words: "noos" (mind) and "tropein" (turning). It is literally translated as “towards the mind” or “affecting the mind”, it means enhancement of learning and memory (Shivkumar et al., 2011). A nootropic drug is distinguished by activating of the higher integrative brain mechanisms directly which lead to enhance cortical vigilance, a telencephalic functional selectivity, and a particular efficiency in restoring deficient higher nervous activity (Giurgea, 1973). These drugs have particularly the intellectual performance, learning capacity and memory. Nootropics drug can work out by mechanism of action of increasing the brain supply of neurochemicals, improving brain oxygen supply or by stimulating nerve growth. The learning and memory is closely allied with functional status of central cholinergic system (Shivkumar et al., 2011). In the current investigation, administration of aqueous and ethanol extracts of *Morus alba* in *wistar* rats exhibited significant improvement in memory functions by reversing the scopolamine butyl bromide induced amnesia in learning and memory task performed on perspex chamber of the apparatus. The scopolamine model recommends that the cognitive deficits that can be observed after scopolamine treatment are directly associated to a decline in central cholinergic functions (Ellis and Nathan, 2001). The memory and learning is tightly related to the functional status of the central cholinergic system, the basal forebrain provides the major source of cholinergic input to the neocoretx and hippocampus (Shivkumar et al., 2011). Pervious literatures have shown that scopolamine impairs retrieval memory in rats and such amnesia is associated with elevated MDA and reduced GSH levels (Koppula and Choi, 2011). Since oxidative stress has been implicated in the pathophysiology of dementia, and also scopolamine has been reported to elevate rat brain oxidative stress, scopolamine-induced amnesia in rats could be used as a valid model to screen drugs with potential therapeutic benefit in dementia (Shivkumar et al., 2011). Nalini et al, correlated the improvement in learning and memory to the reduction in the levels of NE, DA and 5-HT (Nalini et al., 1995). The previous phytochemical investigation of *Morus alba* manifested the presence of phenolic compounds such as flavonoids (Quercetin, rutin), tannin which could be responsible for nootropic activity (Srikumar et al., 2004). NE is synthesized by dopamine. Previous reports displayed that these phytochemicals can diminish dopamine level and also these bioactive compounds can prevent activity of tryptophan hydroxylase enzyme which is involved in the biosynthesis of 5-HT (Bharani et al., 2010). ACh has a crucial role in the enhancement of sensory perceptions and in sustaining attention. Damage to the cholinergic system has been exhibited to be possibly related to the memory deficits associated with alzheimer’s disease. Inhibition of ACh hydrolysis may be achieved through the use of AChE inhibitors (Jagetia et al., 2004). Aqueous and ethanol extracts of *Morus alba* showed significant decrease in AChE activity in a dose dependent manner, hence maintaining the acetylcholine level which is responsible for memory. Flavonoids may mimic the actions of estrogens in the brain (Jager and Saaby, 2011) or may influence the synthesis of acetylcholine and neurotropic factors such as BDNF and nerve growth factor in hippocampus and frontal cortex. *Morus alba* contains flavonoids (Ayoola et al., 2011) as one of its active constituent which expected to be responsible for acetylcholine synthesis and improvement of memory.

V. Conclusion

The current research evinces that aqueous and ethanol extracts of *Morus alba* have nootropic activity so it can be appraised worthwhile for supportive therapy in memory deficits associated with alzheimer’s disease or amnesia.

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