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Anti-Alzheimer Activity of Hydroalcoholic Extract of *Butea monosperma* leaves

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ABSTRACT

Objective: The key objective of the study is to evaluate Anti-Alzheimer activity of hydroalcholic extracts of *Butea monosperma* leaves (BMLE) by streptozotocin induced Alzhemier. *Butea monosperma* (Lam.) is medium size tree belonging to family Leguminoceae. It is reported to have numerous uses in the indigenous system of medicine in India.

Material and Methods: AD was induced by streptozotocin i.e. STZ (3 mg/kg, ICV) day 1st and 3rd day after surgery. Surgery was done on anesthetized rats by the help of stereotaxic apparatus. STZ induced AD rats were treated with ethanolic extracts of Butea monosperma (100 and 200 mg/kg, p.o.) for 14 days. Effect of BMLE in AD rats were assessed by estimating inflexion ratio in EPM and the alteration in the behavior (Y maze apparatus), biochemical parameter in the brain tissue acetylcholinesterase (AchE) .

Result: The preliminary phytochemical investigation of BMLE showned the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, saponins, fixed oil, vitamin C, proteins and amino acids.Extracts at dose levels of 100 mg/kg and 200 mg/ kg showed a significant elevation in inflexion ratio in elevated plus maze and elevation in percentage alternation in Y-maze model. A significant decline in brain AChE level was noted in animals treated with extracts in both models.

Conclusion: These results suggest that, the Anti-Alzheimer effect of BMLE might be due to enhancement of cholinergic neurotransmission through inhibition of AChE activity.

Keywords: Butea monosperma, Streptozotocin, Plus Maze, Y-Maze, Acetylcholinesterase

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INTRODUCTION

Alzheimer's disease is one of the most common neurodegenerative disease that has been characterized by the progressive impairment of cognitive function, changes in behavior [1]. Cholinergic hypo-function plays a basic role in cognitive dysfunction and memory loss. [2]. Pathology of AD constitutes neuronal loss in the basal forebrain particularly within the septo-hippocampal cholinergic systems involved in the learning and memory processes. Various cholinomimetics drugs have been approved for the management of AD.

These drugs show therapeutic effects by acting on acetylcholine deficits and, consequently, increase acetylcholine levels in the brain [3]. Dementia is a term used for memory loss and other intellectual abilities that are severe enough to disrupt daily activities later in life [4]. The most common types of dementia include Alzheimer's disease (AD), cerebrovascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinson's disease [5]. AD is the most common type of dementia and is responsible for 50-60% of all cases [6]

AD is a progressive neurodegenerative brain disease. AD is characterized by memory loss and behavioral changes [7]. Memory loss is usually the main symptom of the AD. The therapeutic approach in the treatment of AD is the use of AChE inhibitors (acetylcholinesterase inhibitors). AChEI blocks the activity of acetylcholinesterase that normally breaks down acetylcholine (ACh).

The inhibition of this acetylcholinesterase prevents the decomposition of acetylcholine, which is known to be deficient in AD. [8]

Tacrine (AchEI) is the first drug approved for treatment of AD [9]. Though, tacrine has many disadvantages, such as low bioavailability, narrow therapeutic window and hepatotoxicity in the treatment of AD [10].

Other acetylcholine esterase inhibitor such as donepezil, rivastigmine and galantamine have limited therapeutic success as they only improve memory in mild dementia and cannot stop the process of neurodegeneration [11]. Butea monosperma (Lam.) belonging to the family Leguminoceae.It is reported to have numerous uses in the indigenous system of medicine in India.

Various medicinal properties are reported are hepatoprotective activity, astringent, aphrodisiac, and tonic properties. In the ancient time various parts are to treat disease like night blindness, elephantiasis, anorexia, constipation, inflammation, gonorrhoea, burning urine etc.

Streptozotocin (STZ), a glucosamine-nitrosourea compound derived from soil bacteria and originally developed as an antitumor agent, was found in 1963 to induce diabetes in experimental animals. Since then, the systemic application of STZ has become the most studied experimental model of insulin-dependent diabetes (type 1). The compound is selectively toxic to pancreatic beta-producing insulin cells, which is explained as a result of its cellular uptake by the low affinity glucose transporter protein 2 (GLUT2) located in its cell membranes. The cytotoxicity of STZ is mainly due to the alkylation of DNA that causes cell necrosis. In addition to pancreatic beta cells, the applied STZ also systematically damages other organs that express GLUT2, such as the kidney and the liver, while the brain is not directly affected because the blood-brain barrier lacks this transporter protein (Fig. 1). However, single or double injection of intracerebroventricular STZ (icv) chronically reduces glucose uptake in the brain and produces many other effects that resemble the molecular, pathological and behavioral characteristics of Alzheimer's disease (AD)

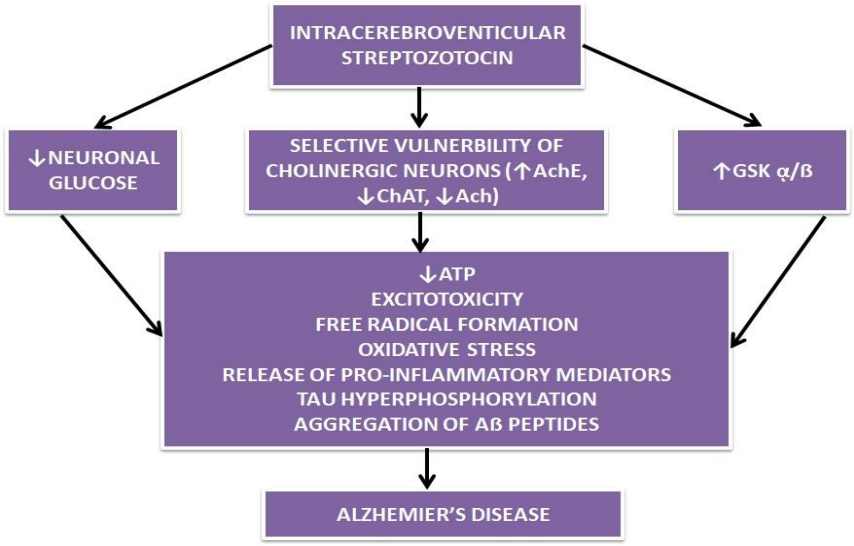

Figure 1: Streptozotocin Meachanism of Action for Induction of Alzhemier's disease

MATERIAL AND METHODS

Preparation of the extract.

The leaves of *butea monosperma* were collected in the local area of the city of Indore and authenticated. The leaves were washed, dried in the air under shade and powdered. 50 g of powdered drug was subjected to soxhletion with hydroalcoholic solution in the ratio of 70:30.

Extraction was done at temperature of 50 ° C until the clear solvent was observed in the siphon tube. The extract was concentrated in a water bath at 40 ° C. The concentrated extract was then dried at 40 ° C in a hot air oven. The dried extract has been packed in an airtight container.

Phytochemical screening

The extract obtained was subjected to preliminary phytochemicals screening. The extracts were examined for alkaloids, flavonoids, phenols, tannins, saponins, fixed oil, vitamin C, proteins and amino acids, glycosides, flavonoids, terpenoids, tannins [16-17].

Acute toxicity studies

In this study, male Wistar rats weighing 250-300 g were used. They had free access to food and water ad libitum. Acute toxicity studies have been conducted in accordance with OECD (Organization for Economic Cooperation and Development) guidelines n. 425 (Up and Down procedure).

It was found that the test samples were safe up to 2000 mg / kg. The protocol were approved by the Institutional Animal Ethics Committee under the CPSCEA. (IAEC / MIPS / 01/2017/04)

Group I: Normal control

Group II: Streptozotocin (3 mg / kg) (Negative control)

Group III: Donepezil (5 mg / kg) + Streptozotocin (3 mg / kg)) (Positive control)

Group IV: BMLE (100 mg / kg + streptozotocin (3 mg / kg)

Group V: BMLE (200 mg / kg) + streptozotocin (3 mg / kg)

Induction of Alzheimer:

MaleWistar rats (250-300 g) were anesthetized by intraperitoneal injection of a combination of ketamine and xylazine (100 and 5 mg / kg, respectively) and then all rats were operated with a stereotaxic apparatus. .

For the surgery, the stereotactic apparatus (Pxinos and Watson) were used, the scalp was washed with iodine

solution, it was recorded in the midline and drilled through the post bregma skull of 0.8 mm, 1.4 mm laterally from the sagittal line and 3.4 mm below the dura mater. The STZ groups received a bilateral ICV injection of streptozotocin (3 mg / kg, body weight) which was dissolved in the citrate buffer (pH 4.4).

The concentration of STZ was prepared to provide 5 µl / site of injection of the solution. The rats in the control group received an ICV injection of the same volume of citrate buffer compared to the treated STZ and the injection was repeated on the third day [18]. Elevated Plus Maze: The EPM functioned as a model of exteroceptive behavior (in which the stimulus existed outside the body) to evaluate learning and memory in rats.

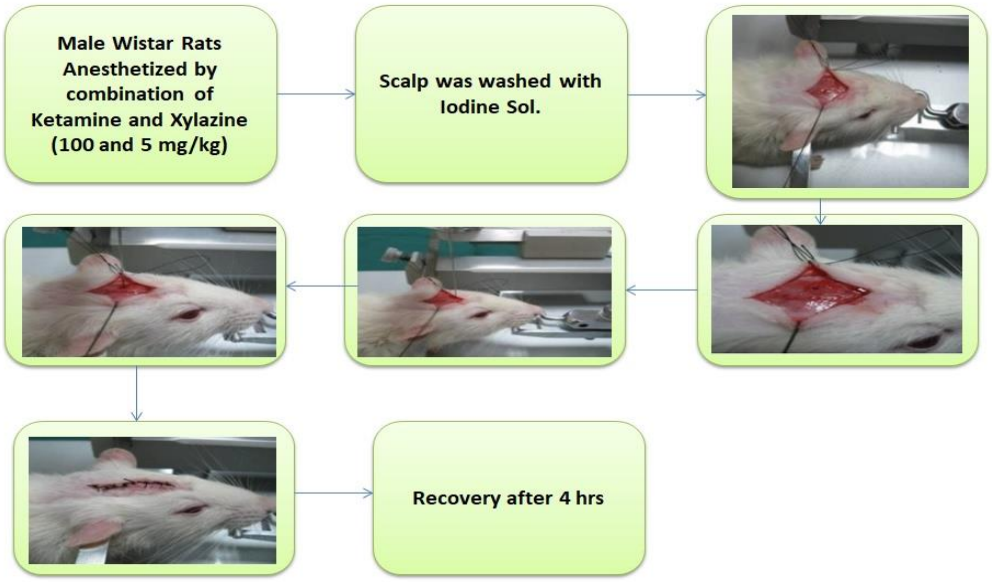

Figure 2: Induction of Alzheimer's Disease

Elevated Plus Maze

The elevated plus maze consisted of two open arms (50 cm x 10 cm x 40 cm) and two closed arms (50 cm x 10 cm x 40 cm), with an open roof, arranged so that the two arms open one in front of the another The maze was raised to a height of 50 cm from the ground to measure the anxiety index in rats. [19-21] It is a simple test of recognition of two tests to measure the memory of spatial recognition, it does not require the learning of a rule and, therefore, it is useful for the study of memory in rodents.

Y Maze

The Y Maze consists of three arms with an angle of 120 ° between each of the two arms. The dimensions of the arm are 8 cm x 30 cm x 15 cm (width x length x height). The three identical arms were designated randomly: the initial arm, in which the rats began to explore (A), the new arm (B, with food stimuli) and the other arm (C). [22] Mice tend to systematically explore the maze, inserting each arm in turn.

On the first day, all rats were allowed to explore Y Maze for a period of ten minutes each. From the 2nd to the 5th day, the rats received four consecutive training sessions per day in the maze that lasted 8 minutes. In each trial, the rats were placed in the entrance chamber (A) and the range of arm entry in all three arms was visually recorded, including possible return in the same arm. The modification is defined as the number of successive entries in the three arms in sets of overlapping triplets.

The alteration rate is calculated as the total number of entries in the arm minus two and multiplied by 100. The amnestic pretreatment 30 minutes before the tests induces a marked decrease in the performance of the spontaneous alteration with a concomitant increase in the total number of the arm. It is expected that the administration of agents that have memory improvement effects will reverse the changes.

During the evaluation of the learning, the animals were exposed to water and food ad libitum for only 1 hour after the exposure of the maze of the day was completed to ensure motivation towards the reward area (B). [23]

Estimation of ACh levels in the brain by quantifying AChE inhibition:

Brain cholinesterase activity was measured using the Ellman method [24]. An aliquot of 0.4 ml of the prepared homogenate was added to a cuvette containing 2.6 ml of sodium phosphate buffer (pH 7.2, 0.1 M).

To this, 100µl of Ellman's reagent (DTNB) was added and taken into a photocell. The absorbance was set to zero at 412 nm when the fluctuations stopped. of the substrate (Acetyl thiocholine iodide) 20µl was added. A change in the absorbance per minute was noted Calculations AChE activity is calculated using the following formula

Calculations

AChE activity is calculated using the following formula

$$R = \frac{\Delta A}{1.36 \times 10^4} \times \frac{1}{\left(\frac{400}{3120}\right) C_0} = 5.74(10^{-4}) \frac{\Delta A}{C_0}$$

Where,

ΔA = Change in absorbance per minute

C0 = Original concentration of the tissue (mg/ml)

R = Rate in moles substrate hydrolyzed per minute per gram of tissue

RESULT AND DISCUSSION

Phytochemical Screening

The preliminary phytochemical analysis of ethanol extract of *Butea monosperma* leaves showed the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, saponins,fixed oil, vitamin C, proteins and amino acids. Acute oral toxicity study of *Butea monosperma* was carried out as per OECD guideline 425. From the limit test results, it was ob- served that the *Butea monosperma* leaves were safe up to a dose level of 2000 mg/kg. There was no mortality and the experimental animals did not show any toxic effect throughout the observation period of 14 days.

Elevated Plus maze

The effect of the vehicle, streptozotocin control, *Butea monosperma* (100 and 200 mg/kg) and Donepezil (5mg/kg) were evaluated at the end of days 7 and 14. The streptozotocin (1 mg/kg) control group showed a significant increase in TL values on the acquisition as well as on the retention days as compared with vehicle control rats, indicating impairment in learning and memory. In the AT on day 7 and day 14, the *Butea monosperma* at dose levels 100 and 200 mg/kg demonstrated decrease in the TL as compared to the streptozotocin control group. The results obtained were found to be statistically significant . Donepezil (5mg/kg) exhibited marked decrease in TL in comparison with the streptozotocin control group. However, *Butea monosperma* at the dose levels 100 and 200 mg/kg showed a decrease in the TL, which is comparable to that shown by Donepezil [Table 1].

Table 1: Effect of the extract of *Butea monosperma* on transfer latency (elevated plus maze paradigm) in streptozotocin-induced amnesia in rats

Groups	Treatment	TL on acquisition day (sec)	
		7 day	14 day
Group I	Normal Control	94.17±13.92	35.83±5.89
Group II	Streptozotocin (3mg/kg) (Negative Control)	140.67±17.2^{ns}	117.8±11.03^{ns}
Group III	Donepezil (5mg/kg) + Streptozotocin (3mg/kg) (Positive Control)	48.17±5.21***	7.33±2.13***
Group IV	*Butea monosperma(100mg/kg+* Streptozotocin (3mg/kg)	52.5±4.75**	17.33±4.73**
Group V	*Butea monosperma(200mg/kg)+* Streptozotocin (3mg/kg)	26.5±6.67**	15.33±3.05**

All values are expressed as mean ± S.E.M, *n* = 6 in each group. Values are significantly different from group I control; ns - non-significant; *P* values: * < 0.05, ** < 0.01, *** < 0.001 (Student t. test analysis). [b] Values are significantly different from group II negative control, ns- non significant, *P* values: * < 0.05, ** < 0.01, *** < 0.001 (one way ANOVA followed by Dunnett's test)

Y maze Model

Y maze model used in the present study proved to be a sensitive measure of spatial recognition memory. The effect on alteration behavior was studied on two parameters, % alteration (Table 2) and No. of arm entries (Table 3).

Effect on % alteration: Normally rats exhibit an alteration of around 60-70% as exhibited by the normal group (Group 1). Group 3 achieving a slightly higher alteration than the vehicle group (Group 1). The alteration showcased by Groups 1, 3 is indicative of the natural tendency of rats to exhibit an alteration of around 60-70% in a 5 min session, however the alteration achieved on the second trial (Day 14) was higher elaborating higher % alteration (ability to alternate) on account of acquisition of memory. Group 2 exhibited a marked decrease in spontaneous alteration (a characteristic feature of amnestic agents), elaborating the amnestic effects of streptozotocin, however in Groups 4-5; there was a significant increase in % alteration thus supporting their memory enhancing effects to reverse the effects of streptozotocin. The greatest alteration was achieved by the standard Donepezil; in presence of amnesia (Group 4) followed by the higher dose of *Butea monosperma* extract in presence of amnesia (Group 5). It succeeded in closely approximating the alteration response of the standard Donepezil. Assuming generalization % alteration on Day 14 was found to be greater than that observed on Day 13, thus elucidating the retention characteristics of the purported nootropics

Table 2: Effect of the extract of *Butea monosperma* on Assessment of learning and memory using Y Maze Apparatus % Alteration

Groups	Treatment	% Alteration	
		% Alteration on Day 13	% Alteration on Day 14
Group I	Normal Control	65.50±0.072	71.25±0.087
Group II	Streptozotocin (3mg/kg) (Negative Control)	42.38± 0.594^{ns}	53.33± 0.259^{ns}
Group III	Donepezil (5mg/kg)+Streptozotocin (3mg/kg) (Positive Control)	69.33±0.594***	71.49±0.389***
Group IV	*Butea monosperma(100mg/kg+* Streptozotocin (3mg/kg)	65.42±0.277**	69.14±0.260**
Group V	*Butea monosperma(200mg/kg)+* Streptozotocin (3mg/kg)	62.56±0.371**	65.43±0.313**

All values are expressed as mean ± S.E.M, *n* = 6 in each group. Values are significantly different from group I control; ns - non-significant; *P* values: * < 0.05, ** < 0.01, *** < 0.001 (Student t. test analysis). [b] Values are significantly different from group II negative control, ns- non significant, *P* values: * < 0.05, ** < 0.01, *** < 0.001 (one way ANOVA followed by Dunnett's test)

Table 3: Effect of the extract of *Butea monosperma* on Assessment of learning and memory using Y Maze Apparatus by Number of Arm Enteries

Groups	Treatments	Number of Arm entries	
		Number of Arm entries on Day 13	Number of Arm entries on Day 14
Group I	Normal Control	25.0±0.035	20.0±0.069
Group II	Streptozotocin (3mg/kg) (Negative Control)	33.50±0.119^{ns}	30.5±0.257^{ns}
Group III	Donepezil (5mg/kg)+Streptozotocin (3mg/kg) (Positive Control)	13.50±0.218***	8.0±0.239***
Group IV	*Butea monosperma(100mg/kg+* Streptozotocin (3mg/kg)	24.50±0.757**	18.0±0.719**
Group V	*Butea monosperma(200mg/kg)+* Streptozotocin (3mg/kg)	25.5±0.457**	19.5±0.485**

All values are expressed as mean ± S.E.M, *n* = 6 in each group. Values are significantly different from group I control; ns - non-significant; *P* values: * < 0.05, ** < 0.01, *** < 0.001 (Student t. test analysis). [b] Values are significantly different from group II negative control, ns- non significant, *P* values: * < 0.05, ** < 0.01, *** < 0.001 (one way ANOVA followed by Dunnett's test)

Estimation of AChE activity in the brain:

In the standard group, the animals treated with Donepezil (5mg/kg) produced a significant reduction of AChE enzyme activity. In the treatment group, the animals treated with *Butea monosperma* at 100 and 200 mg/kg produced a significant reduction of AChE enzyme activity and as compared to positive control. Percentage inhibition of AChE activity of treatment group has shown a significant increase when compared to positive control. [Table 4]

Table 4: Effect of the extract of *Butea monosperma* on AChE Inhibition Activity

Groups	Treatment	AChE concentrated (μMol /minute/g of tissue)	Inhibition of AChE activity (%)
Group I	Normal Control	6.742±0.18	
Group II	Streptozotocin (3mg/kg) (Negative Control)	10.39±0.35^{ns}	35.11^{ns}
Group III	Donepezil (5mg/kg) + Streptozotocin (3mg/kg) (Positive Control)	3.968±0.19***	61.8***
Group IV	*Butea monosperma(100mg/kg+* Streptozotocin (3mg/kg)	4.907±0.31**	50.12**
Group V	*Butea monosperma(200mg/kg)+* Streptozotocin (3mg/kg)	4.967±0.31**	52.19**

All values are expressed as mean ± S.E.M, *n* = 6 in each group. Values are significantly different from group I control; ns - non-significant; *P* values: * < 0.05, ** < 0.01, *** < 0.001 (Student t. test analysis). [b] Values are significantly different from group II negative control, ns- non significant, *P* values: * < 0.05, ** < 0.01, *** < 0.001 (one way ANOVA followed by Dunnett's test)

CONCLUSION

The present study investigated the Anti-Alzheimer activity of hydroalcholic extract of *Butea monnosperma* leaves in rats models. Progressive neurodegeneration in the brain, leading to the depletion of acetylcholine (ACh) stores is the primary cause of memory loss in the ageing population. Drugs or plant swith acetylcholinesterase (AChE) activity can thus increase levels of ACh that are known to be depleted in neurodegenerative disorders such as Alzheimer's disease

(AD) by inhibiting AChE, an enzyme that degrades Acetylcholine [25]. In humans, memory is generally accessed through spoken or written languages, whereas, in laboratory animals, cognitive functions must be accessed through behavioral models [26]. Two such models are the transfer latency by elevated plus maze and alternation performance (SAP) in the Y-maze and object recognition performance. Moreover, the streptozotocin used in this study to induce amnesia has a high selectivity for the muscarinic receptors, especially the M1 receptor subtypes found in the hippocampus [27].Cholinergic transmission generally ends by the acetylcholine hydrolysis through the enzyme AChE which is responsible for degradation of acetylcholine to acetate and choline at the level of the synaptic cleft [28]. Thus, estimating the acetylcholine esterase activity can provide valuable information on cholinergic function. Our results in- dicated a decrease in AChE activity in the brain of animals pre-treated with *Butea monosperma* leaf extract against streptozotocin induced memory impairment in both elevated plus model and Y-maze model. The effect might be due the activity of *Butea monosperma* leaves that produced enhancement in cholinergic transmission by potential AChE inhibitory activity.

Conflict of interest: None

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