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Evaluation of Memory Enhancing Potential of *Dendrocalamus Strictus* Leaf Extracts on Suitable Animal Model

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Abstract

Medicinal plants have always been the principle sources of medicine worldwide. India sustains a very rich traditional medicinal plant wealth and inherits unique plant and animal communities. Present study enumerates the phytochemical screening followed by antioxidant and memory enhancing evaluation of aqueous and methanolic extract of *Dendrocalamus strictus (DS)* leaves. Freshly collected and authenticated leaves were studied for its morphological and pharmacognostic character followed by physical and phytochemical evaluation. Phytochemical screening showed the presence of alkaloids, glycosides, carbohydrates, steroids and flavonoids in both the extracts. Physical parameters like solubility, ash values, LOD, extractive value etc. has been studied. The antioxidant activity of the extracts was done by using DPPH method. The results showed that aqueous extract at 100µg/ml concentration and methanolic extract at 150µg/ml concentration showed the significant antioxidant effect as compared with ascorbic acid as standard. The *In-Vivo* memory enhancing activity of *DS* leaf extracts was evaluated by radial arm maze model in rats using Piracetam as a standard. Both the extracts at 200mg/kg conc^n showed significant to highly significant increase in number of entries & time spent in P zone (from $P < 0.05$ to $P < 0.001$). The result suggested that *DS* leaf extracts possess memory enhancing activity and this might be due to flavonoids, Phenolic compounds, Steroids present in extracts.

Keywords: *Dendrocalamus strictus* (DS), Phytochemical, Antioxidant, Memory enhancing activity.

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Introduction

Memory is the ability of an individual to record sensory stimuli, events, information etc., retain them over a short or long period of time and recall the same at a later date when needed. Learning is the process of acquisition of information and skills, while subsequent retention of that information is called memory. (S. Shajahan *et al*., 2014). Learning is the process of acquiring knowledge about the world and memory could be considered as the retention of the acquired knowledge, which can be retrieved as and when, required. Poor learning abilities, impaired memory, lower retention and slow recall are the common problems in stressful situations. Moreover, age, stress and emotions are conditions that may lead to impaired learning, memory loss, amnesia, and dementia or to more ominous threats like Schizophrenia and Alzheimer's disease. Memory is understood as an informational processing system with explicit and implicit functioning that is made up of a sensory processor, short-term (or working) memory, and long-term memory. (Baddely, 2007).

Dendrocalamus strictus (DS) commonly known as Bamboo plant constitutes one of the most important renewable natural resources of India. The use of bamboo is not only restricted as structural and building materials but with the advent of time has become an important component of the medicinal field. From ancient time bamboo has been an important ingredient of traditional Asian Medicines in general and Chinese medicine in particular. Modern scientific approaches are now used to validate the traditional uses and researchers from round the globe have been successful in isolating active chemical constituents from different parts of this *green gold*. They also provide an insight into the potential health benefits of bamboo parts.

Dendrocalamus strictus is found to be an excellent source of many bioactive compounds such as crude protein, crude fiber, ash and other minerals. Leaves were found to be rich in calcium, magnesium, copper, and manganese; moderate in zinc. Different parts of this plant have been investigated and confirmed to possess various pharmacological activities such as anti-diabetic, antimicrobial, antidepressant, anticancer, anti-obesity, anti-inflammatory (Arvind Kumar Goyal et al 2014). Therefore; it was thought worthwhile to target the study to reveal the evaluation of Memory enhancing potential of *Dendrocalamus strictus* leaf extracts on suitable animal model.

Material and Method

Plant material

The leaves of *Dendrocalamus strictus* were collected from local region of Nanded District,

Maharashtra, India. The plant was identified and authenticated by Dr. S. S. Bodke, Head Department of botany & Horticulture, Yeshwant Mahavidyalaya, Nanded. The voucher specimen of the plant was deposited at the college for further reference. The leaves were dried under shade, powdered and stored in an air tight container.

Preparation of extract

The collected leaves were dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 150g of powdered materials were extracted with methanol using Soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts were then concentrated and dried under reduced pressure. (Kokate CK., 1994)

Phytochemical analysis

The aqueous and methanolic extract of *Dendrocalamus strictus* was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used for preliminary qualitative phytochemical analysis of extracts. (Khandelwal KR., 2005)

Animals

Wistar rats of either sex weighing (150-200 gm) were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol (Registration No. 1613/PO/Re/S/12/CPCSEA). Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*.

Toxicity study

Safe dose calculation

Acute toxicity was involved in estimation of LD_{50}. As per reported reference, Safe dose of *Dendrocalamus strictus leaf* extracts was 2000 mg/kg carried out as per OECD Guidelines for Acute Toxicity study: Class Method (423).As per reported reference, studies conducted revealed that the administration of graded doses extracts (up to a dose of 2000 mg/kg) of extract did not produced significant changes in behaviours such as alertness, motor activity, breathing, restlessness, diarrhoea, convulsions, coma and appearance of the animals. Accordingly, maximum therapeutic experimental dose was calculated as 200 mg/kg. (OECD Guidelines 423)

Radial arm maze model

Rats were divided into six groups of 6 animals each as follows: Group-I animals served as control group and received (vehicle p.o.) Group-II standard group received Piracetam (200mg/kg p.o.) Group-III received Methanolic extract (100 mg/kg p.o). Group-IV received

Methanolic extract (200 mg/kg p.o.) Group-V received Aqueous extract (100 mg/kg p.o.). Group-VI received Aqueous extract (200 mg/kg p.o.)

The apparatus was fabric elevated eight arm radial arm maze with the arms extending from a central platform. 26cm in diameter. Each arm is 56cm long, 5cm wide with 2cm high rails along the of the arm. The maze was well illuminated and numerous cues were present. Food pellets were placed at the end of the arm. To motivate the rat to run the maze. Animals were trained on a daily basis in the maze. (Vogel G, Sandow J 2002)

Each rat maintains at 85% of its total diet weight, was exposed to the maze daily with the food pellet in a fix arm. The apparatus was cleaned with damp cloth after each trial. The evaluation was carried out on 8^{th} day, 30 minutes after the respective drug treatment. Food pellet was placed in a variable arm for evaluation of working memory (Kadiri S, *et al*., 2016).

Evaluation

Latency to find food was recorded as a measure of working memory evaluation.

Statistical Analysis

The data were expressed as mean ± Standard error of mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) test.

Results and Discussion

The table 1 shows the consolidated data of number of entries as well as time spent in C and P zone respectively. When the drug treated groups where compared with control and standard, groups it showed that all four drug treated groups (MDS-100, MDS-200, ADS-100, & ADS-200) showed increased in no. of entries of the animals at P zone. The maximum was showed by ADS-200 group as the number of entries of this group showed significant increase in number of entries i.e. 40.6±0.61****#** as compared to standard group. Similarly, when the drug treated group screened for Time spent in P zone with Standard and control, here also all the groups showed moderate to significant increase in time spent at P zone by drug treated groups. Here also group no.6 i.e. ADS-200 showed highly significant memory enhancing effect when compared with control group. The result confirms memory enhancing potential of all four tested groups when compared with control and standard groups. Although the effect showed by ADS-200 was found to be significant as compare to other groups.

Table 1: Comparative study of Number of entries and Time spent in C and P zone of rats for Memory enhancing activity of *Dendrocalamus strictus* (Aqueous and Methanolic) leaf extracts.

Group	Number of entries in				Time spent in (Sec)			
	C zone		P zone		C zone		P zone	
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
Ctrl	25.6±1.11	24.2± 1.70	15.2± 0.30	23.2± 0.47	74.6± 2.16	73.4±1.51	161.2±7.85	161.0± 0.61
Std	24.0±4.41	12.4± 1.72**	23.4± 0.55*	38.0± 0.57**	62.2± 5.25*	31.0±1.39**	145.8±16.4	220.2± 6.02**
MDS-100	23.2±3.71	18.0± 0.8 #	27.8± 2.52**#	38.8± 0.94**#	64.0± 2.04 #	50.0±1.09**	149.4±2.45	179.0± 1.46*Δ
MDS-200	26.8±1.93	20.0± 0.44#	28.4± 1.89**#	40.2± 0.54**#	60.4± 1.76*#	49.8±1.07**	157.2±2.28	188.2± 2.84**Δ
ADS-100	25.0±0.77	18.8± 4.10#	26.6± 1.08**#	39.0± 0.51**#	65.0± 1.23 #	48.4±1.56**	150.2±2.24	181.0± 2.72*Δ
ADS-200	26.2±1.62	19.4± 0.84#	27.2± 1.07**#	40.6± 0.61**#	62.0± 1.03*#	47.4±2.58**	159.4±1.25	190.2± 1.98**Δ

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

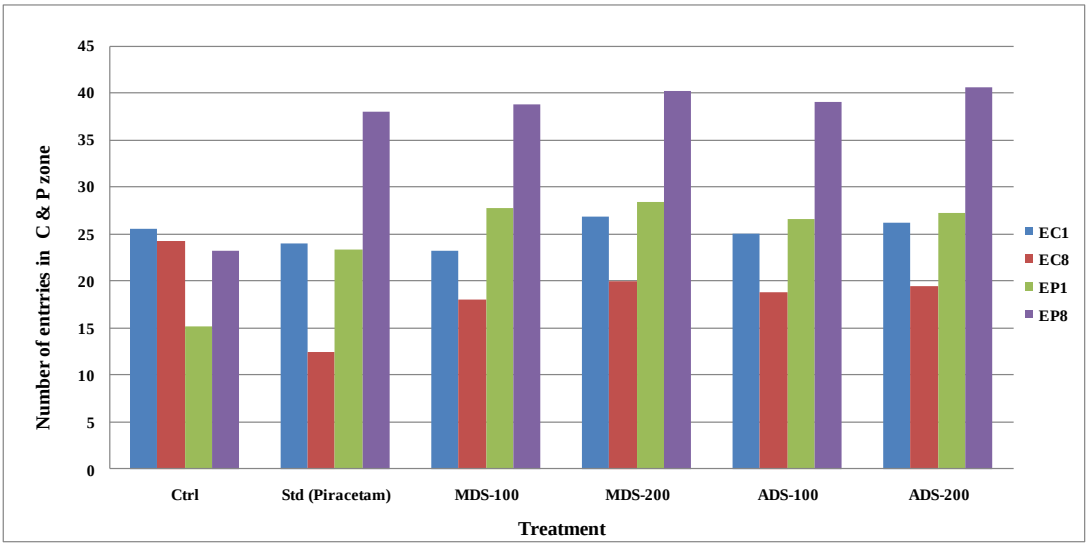

Fig. 1: Number of Entries in C and P zone of Rats during Experimental Period

EC1- Number of entries in C zone on day 1, EC8- Number of entries in C zone on day 8, EP1- Number of entries in P zone on day 1, EP8- Number of entries in P zone on day 8.

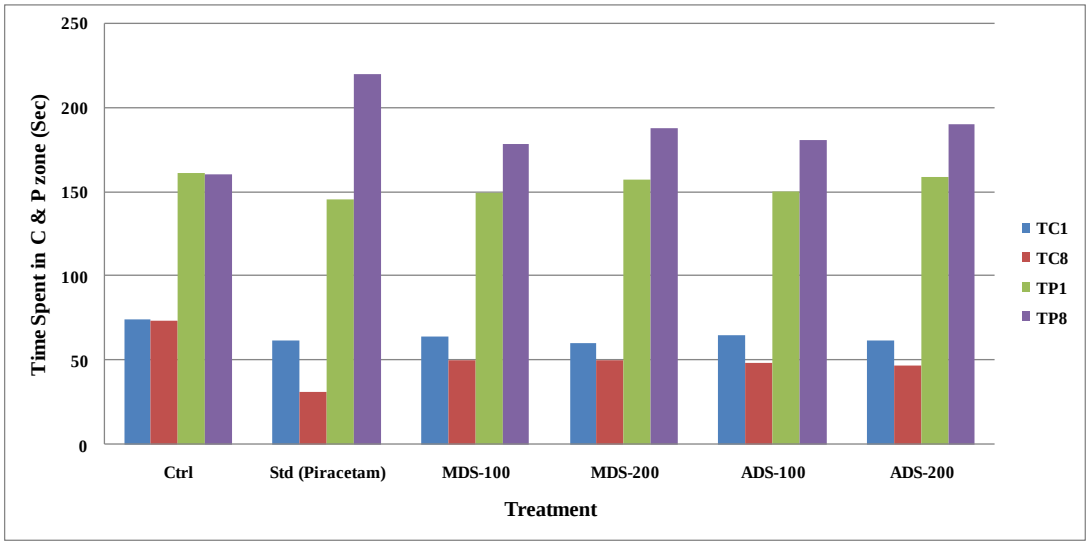

Fig. 2: Time Spent in C and P zone of Rats during Experimental Period

TC1- Time spent in C zone on day 1, TC8- Time spent in C zone on day 8, TP1- Time spent in P zone on day 1, TP8- Time spent in P zone on day 8.

Conclusion

Dendrocalamus strictus leaf contain several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like cancer, inflammation, infectious, cardiopathy, diabetes, hepatotoxicity and many microbial attacks where its memory enhancing potential was claimed to be useful. The extracts of *Dendrocalamus strictus* leaf tested for memory enhancing activity by researchers. No methodical report on memory enhancing activity of *Dendrocalamus strictus* leaf was available.

The present study aimed at evaluating the *In-Vivo* memory enhancing activity of *Dendrocalamus strictus* leaf extract in rats. Aqueous and methanolic extracts were prepared by the hot extraction process, i.e. by using Soxhlet apparatus. Preliminary phytochemical evaluation of aqueous and methanolic extract was carried out for the determination of presence of phytoconstituents. Antioxidant property of *Dendrocalamus strictus* leaf was carried out by using DPPH radical scavenging assay technique respectively. In DPPH assay all the extracts showed promising antioxidant activity, however aqueous extract of *Dendrocalamus strictus* leaf revealed significant antioxidant activity.

The result of acute oral toxicity studies of plant extracts as per standard references revealed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 2000 mg/kg body

weight in mice. Accordingly, safe experimental dose was calculated as ≤ 200mg/kg & was used accordingly for further screening of extracts.

In-Vivo study has showed that aqueous and methanolic extracts of *Dendrocalamus strictus* does possess significant memory enhancing activity with 100 mg/kg and 200 mg/kg. High doses of the aqueous extract 200 mg/kg being more superior and showed significant to highly significant percentage inhibition (from $P < 0.05$ to $P < 0.001$) when compared with standard Piracetam.

The finding of the present study reveals that *Dendrocalamus strictus* leaf has potent memory enhancing activity.

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