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***Bambusa arundinacea* (Retz.) Willd. (Tabasheer): Effects on behaviour, memory, and immunity stressed rat model**

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Abstract

The present study investigated the psychoimmunomodulatory effects of Tabasheer (*Bambusa arundinacea* (Retz.) Willd.) on stress-induced behavioral, memory, and immune changes in rats. Sprague-dawleyrats were divided into five groups: normal control, stress control, and three groups receiving 200, 400, or 600 mg/kg *B. arundinacea* orally for 21 days. Stress was induced by forced swimming in cold water. Behavioral and memory parameters were evaluated using spontaneous alternation behavior and passive avoidance tests. Biochemical markers, including brain acetylcholinesterase activity and blood glucose levels, were measured. Immune function was evaluated by total leukocyte count, footpad reaction test, and agglutination method. The relative weights of the spleen, liver, and kidney were also determined. The results showed that stress significantly altered spontaneous alternation behavior, memory retention, and immune function, while increasing brain acetylcholinesterase activity and blood glucose levels compared to normal controls. Tabasheer treatment at 600 mg/kg significantly improved behavioral and memory parameters, reduced brain acetylcholinesterase activity, and regulated blood glucose levels in stressed rats. However, Tabasheer did not significantly affect immune parameters or organ weights. In conclusion, Tabasheer demonstrated protective effects against stress-induced behavioral and memory deficits, supporting its traditional use as a memory enhancer. Further clinical and pharmacological studies are warranted to explore its therapeutic potential and isolate active compounds.

1. Introduction

Medicinal plants have long been integral to traditional healthcare systems, especially in Ayurveda and Unani medicine, where they are widely used to treat chronic disorders. However, many such remedies lack sufficient scientific validation despite their widespread use (Irwin and Vedhara, 2005). One such remedy is Tabasheer, a siliceous secretion obtained from the internodes of female bamboo *B. arundinacea* (family: Bambusaceae), traditionally valued for its rejuvenating and neuroprotective properties. Tabasheer, also known as Vanshlochan, is traditionally used in India to treat various ailments including respiratory diseases, joint disorders, and cognitive decline. Its high silica content (up to 97%) makes it an effective remineralizing agent, contributing to bone, joint, and connective tissue health (Hartwell, 1971). Anecdotal evidence and folk medicine suggest its role in memory enhancement and immune support, particularly in the elderly, where silica levels decline.

Stress, a common physiological and psychological burden, has profound implications on both the central nervous and immune systems (Ramsey, 1982; Chrousos and Gold, 1992). Chronic stress is associated with the onset and progression of diseases such as Alzheimer's, depression, diabetes, and cardiovascular disorders. It activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS), leading to elevated levels of

glucocorticoid and catecholamine that impair neurotransmitter balance of neurotransmitters and immune function (Covelli *et al.*, 2005; McCormick *et al.*, 2008). Behavioral changes, impaired memory, and immuno suppression are common outcomes of such dysregulation (Maier and Watkins, 2003).

Psychoneuroimmunology (PI) investigates the interaction between the brain and the immune system, emphasizing how psychological stress can modulate immune responses (Irwin and Vedhara, 2005). Chronic stress can lead to thymic atrophy, lymphoid suppression, altered leukocyte distribution, and increased brain acetylcholinesterase (AChE) activity, an enzyme linked to cognitive decline (Neylan, 1998; Chrousos and Gold, 1992). Additionally, stress-induced hyperglycemia adversely affects brain glucose metabolism and affects memory functions (Gibson and Blass, 1976; Ragozzino *et al.*, 1996).

Glucose is vital for cognitive performance, as it fuels acetylcholine synthesis, a neurotransmitter essential for learning and memory. Studies suggest that improving blood glucose levels or cholinergic activity can improve memory retention in rodents and humans (Kaplan *et al.*, 2000; Craft *et al.*, 1996). Thus, modulation of glucose levels and AChE activity could serve as a mechanism for stress-related cognitive impairment and recovery.

Despite the extensive ethnopharmacological use of *B. arundinacea* to promote vitality, memory, and immunity, its psychoimmunomodulatory effects under experimentally induced stress have not been adequately explored. Traditional texts mention its use in cognitive and inflammatory disorders, but scientific studies that validate its efficacy, especially in an integrated stress model, are lacking (Muniappan and Sundararaj, 2003).

To address this gap, the present study evaluated the effects of Tabasheer on behavior, memory, immune function, AChE activity,

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and blood glucose levels in a forced cold-water swim stress model in rats. Given the traditional use and its biochemical profile, it was hypothesized that Tabasheer could mitigate stress induced impairments in cognition and immune response, potentially validating its role as a natural adaptogen and cognitive enhancer.

2. Materials and Methods

2.1 Chemicals and reagents

Bovine serum albumin, Folin Ciocalteu phenol reagent was obtained from Merck Specialties Pvt. Limited, India, Acetylthiocholine iodide from across Organic, USA, and DTNB (5',5'-bisdithionitrobenzoic acid) from Himedia Laboratories Pvt. Ltd. India. Glucose estimation kits were obtained from Span Diagnosis, India.

2.2 Animals

Sprague dawleyrats (body weight: 150-250 g; Age: 8-10 weeks old) either sex were procured from the Central Animal House Facility, Central Drug Research Institute, Lucknow, India. Animal experiments were performed according to the CCSEA rules and regulations and the proposed work was approved by the Integral University's Institutional Animal Ethics Committee (IAEC), Lucknow, India (Approval No: IU/Pharm/PhD/CPCSEA/02/2009).

2.3 Drugs

The crude herbal drug Tabasheer (*Bombusa arundinacea* (Retz.) Willd.) was purchased from Hamdard Dawakhana/Aminabad local market and was authenticated by chemotaxonomist.

2.4 Acute toxicity test

This study was carried out only for the evaluation of the safety of the selected doses of drugs according to the OECD guideline.

2.5 Preparation and administration

Tabasheer was prepared by grinding the crudeherbal drugs to a fine powder, and then freshly prepared in vehicle (d.w) immediately before administration to the rats. The prepared drug sample were administered intragastrically or by oral rout using animal feeding intubations needles at the same time of day.

2.6 Experimental framework

The rats were divided into five groups of 6 rats (Table 1).

- A. Normal control (NC)/non-stress (Gr-I) group was housed and fed in the normal condition.
- B. Stress groups were further divided into two groups:
 - (i) Non-drugtreated group or stress control (SC or Gr-II)
 - (ii) Drug-treated groups (Gr-III, Gr-IV and Gr-V).

Stress was induced in experimental SD rats by making them swim in cold water (temperature $10 \pm 5^\circ\text{C}$) maximum for 120 min/day at least for three consecutive weeks 30 min after treatment (modified method used by Ferry *et al.*, 1991). Rats were made to swim in a tub of 50 cm diameter and filled to a height of 20 cm with 30 cm of space above the head of the rat.

The number of groups with their treatment schedule is presented below;

Table 1: Treatment schedule of Tabasheer (*B. arundinacea*)

Groups (n= 6)	Treatment	Dosage, route of administration and duration
I (NC)	Vehicle	10 ml/kg p.o., once a day for 21 days
II (SC)	Vehicle + Stress	10 ml/kg p.o., once a day for 21 days
III	Tabasheer + Stress	200 mg/kg p.o., once a day for 21 days
IV	Tabasheer + Stress	400 mg/kg p.o., once a day for 21 days
V	Tabasheer + Stress	600 mg/kg p.o., once a day for 21 days

n = Number of animals used in each group

All behavioral and biochemical tests were evaluated 2.5 h after treatment (drugs or vehicle).

2.7 Behavior in maze (Spontaneous alteration behavior)

The assessment was made on a plus maze made of polywood painted blue/grey. It consisted of a symmetric arm (50 cm long \times 10 cm wide) with 15 cm high side walls. The arms extend from a central platform (10 \times 10 cm) at a height of 50 cm above the floor and were labeled A, B, C and D. The rat was placed in the center and allowed to travel freely through the maze for 6 min. The sequence of arm entries into different arms was recorded. A 4/5 alternation is defined as the entry into four different arms overlapping quintuplet sets of five consecutive arm entries/choices within the total set of arm entries. A B C A C was not considered as alternation. Using this procedure, possible alternation sequences are equal to the number of arm entries minus four (Ragozzino *et al.*, 1998). Therefore, the percent alternation was scored as follows:

$$\frac{\text{Actual number of alternations}}{\text{Number of arm entries} - 4} \times 100$$

2.8 Passive avoidance test

The step-by-step passive avoidance apparatus (shuttle box) was used for the evaluation of the memory retention deficit in rodents. The apparatus consists of light and dark chambers of equal size (30 cm long \times 30 cm wide \times 40 cm high) by a central wall. This wall has an opening through which the animal can pass through to chambers. The floor consists of a metal grid connected to a shock scrambler. The test consists of an acquisition and retention trial. On day 20 after treatment, the rat was placed in the light chamber. A trap-door separating the chamber was open, and latency to enter the dark chamber is measured in seconds. Immediately after the rat entered the dark chamber, the trap door was closed and an electric shock (1 mA) was delivered for 3 s. Five seconds later, the rat was removed from the dark chamber and returned to its home cage. The retention test was performed 24 h later in the same way as in acquisition trial and termed retention latency, without applying foot shock the latency time was recorded to a maximum of 3 min (Kumar and Gupta, 2002).

2.9 Brain acetylcholinesterase activity

The rate of thiocholine formation from acetylthiocholine iodide in the presence of tissue cholinesterase is measured by first treating with DTNB and then measuring the optical density (OD) of the yellow-colored compound formed, during the reaction at 412 nm using UV visible spectrophotometer (UV Pharmaspec-1700, SHIMADZU), every min for a period of 3 min. Rats were sacrificed by using ether anesthesia. The brain was quickly removed and kept in an ice bath. The entire brain was used to measure acetylcholinesterase activity. A known weight of brain tissue is homogenized in 0.32 M sucrose solution to obtain a 10% homogenate. The homogenate was centrifuged at 3,000 rpm for 15 min, followed by centrifugation at 10,000 rpm for 10 min at a constant temperature of 4°C. After centrifugation, 1 ml of the supernatant is mixed with 9 ml of sucrose solution to obtain 1% post mitochondrial supernatant (PMS). Acetylcholinesterase estimation was performed in the above 1% PMS using the Ellman method (Lowry *et al.*, 1951; Ellman *et al.*, 1961).

2.10 Blood glucose

Blood was collected from tail vein and blood glucose levels were estimated using glucose estimating kit (Span Diagnosis, India). The procedure and time indicated for the kit were carefully followed. Dual measurements were made and the average value was used as the measured value. The absorbance was measured with a UV visible spectrophotometer (UV Pharmaspec-1700, SHIMADZU) at a wavelength of 505 nm.

2.11 TLC (Total leukocyte count)

TLC was evaluated by a routine hematological method using Neubauer's chamber with haemocytometer (Johrapurkar *et al.*, 2003).

2.12 Footpad reaction test

The cell-mediated immune response was assessed by the footpad reaction test in rat. Increase in paw volume induced by an injection of sheep RBC (5×10^8 cells/ml in normal saline), in the subplantar region of the right hind paw. The mean percent increase in paw volume was considered as an index of cell-mediated immunity (Bin Hafeez *et al.*, 2001).

2.13 Agglutination method

Blood samples were collected from the tail vein and the measurement of antibody titer by hemagglutinations was carried out using method Micro technique, employing 96 wells micro test plate flat bottom (TARSONS, CAT No. 941196). Briefly, each well in the plate received 25 micro liter of serial two-fold dilutions in sera in normal saline and an additional 25 microliter volume of 1 % (v/v) sheep erythrocyte suspension (SES) in normal saline (5×10^8 cell/ml). One hour after incubation of the mixtures at room temperature, the hemagglutination capacity was read. The positive hemagglutination reaction was visualized as a mat formation at the bottom, whereas the button formation indicated a negative hemagglutination reaction. The sera were determined as the reciprocal of the maximum dilution that presents positive hemagglutinations (Bin Hafeez *et al.*, 2001).

2.14 Relative organ weight

Rats of all groups were weighed and sacrificed on the last day of treatment and relative organ weight (organ weight/100 g body weight) of kidney, liver and spleen was determined for each animal.

2.15 Statistical analysis

Data were expressed as mean \pm SEM and the results were analyzed by ANOVA followed by Dunnett's t-multicomparison test. p values <0.05 were considered significant.

3. Results

3.1 Effect of Tabasheer (*B. arundinacea*) on acquisition and retention latency (Passive avoidance test)

In Table 2, SC acquisition transfer latency of SC was increased ($p < 0.01$) and retention transfer latency decreased ($p < 0.01$) significantly, compared with NC. However, the acquisition transfer latency decreased significantly ($p < 0.05$) with HD, was not significant with LD and the MD and retention transfer latency increased ($p < 0.01$) with HD, significantly, was increased ($p < 0.01$) with HD significantly, non-significant with both the LD and MD of *B. arundinacea* treated, compared with SC.

Table 2: Effect of Tabasheer (*B. arundinacea*) on acquisition and transfer latency of passive avoidance paradigm in rats

Groups/Treatment	Dose/kg (p.o.)	Acquisition TL (s)	Retention TL (s)
Vehicle (d.w.)	10 ml	9.50 \pm 1.44	177.50 \pm 2.50
Vehicle + Stress	10 ml	17.00 \pm 0.91**	90.25 \pm 14.59**
<i>B. arundinacea</i> + Stress	200 mg	13.00 \pm 1.63 ^{ns}	115.00 \pm 10.41 ^{ns}
<i>B. arundinacea</i> + Stress	400 mg	12.50 \pm 1.17 ^{ns}	126.25 \pm 10.08 ^{ns}
<i>B. arundinacea</i> + Stress	600 mg	12.00 \pm 1.08 [#]	150.75 \pm 3.95 ^{##}

All values were expressed as Mean \pm SEM, Significant difference between various groups (ANOVA) and individual comparison was performed using the Dunnett t test. ** $p < 0.01$ = significant, compared with normal group (Gr-I), ns = non-significant, compared with stress control (Gr-II), ^{##} $p < 0.01$ and [#] $p < 0.05$ = significant, compared with stress control (Gr-II)

3.2 Effect of Tabasheer (*B. arundinacea*) on spontaneous alteration behavior in maze

In Table 3, the % alternation on the plus maze (SC) was significantly decreased ($p < 0.05$), when compared to NC. The % of alternation increased significantly ($p < 0.05$) with a higher dose (HD =

600 mg / kg, *i.e.* Gr-V) on day 10 and 20th and median dose (MD = 400 mg, *i.e.*, Gr-IV) on day 10th, respectively, and was non-significant ($p > 0.05$) with lower dose (LD = 200 mg / kg, *i.e.* Gr-III) on day 10th and 20 and MD on day 20th, respectively, of treated *B. arundinacea* treated, when compared with SC.

Table 3: Effect of Tabasheer (*B. arundinacea*) on spontaneous alteration behavior in rats

Groups/Treatment	Dose/kg (p.o.)	% Alternations on	
		10 th day	20 th day
Vehicle (d.w.)	10 ml	65.83 ± 13.72	67.08 ± 12.10
Vehicle + Stress	10 ml	30.83 ± 3.63*	23.33 ± 4.86*
<i>B. arundinacea</i> + Stress	200 mg	47.92 ± 13.34 ^{n.s.}	49.24 ± 11.94 ^{n.s.}
<i>B. arundinacea</i> + Stress	400 mg	60.35 ± 2.84 [#]	59.27 ± 12.95 ^{n.s.}
<i>B. arundinacea</i> + Stress	600 mg	63.40 ± 5.77 [#]	64.29 ± 9.72 [#]

All values were expressed as Mean ± SEM, significant difference between various groups (ANOVA) and individual comparison was performed using the Dunnett t test. * $p < 0.05$ = significant, compared with normal group (Gr-I), ns = not significant, compared with stress control (Gr-II), [#] $p < 0.05$ = significant, compared with stress control (Gr-II).

3.3 Effect of Tabasheer (*B. arundinacea*) on brain acetylcholinesterase (AChE) activity

In Table 4, AChE activity in SC was significantly increased ($p < 0.01$), when compared to NC. AChE activity was significantly decreased ($p < 0.05$) with HD and was not significant with LD and MD of *B. arundinacea* treated, compared with SC.

3.4 Effect of Tabasheer (*B. arundinacea*) on blood glucose level

In Table 4, the blood glucose level of SC was significantly increased ($p < 0.01$), when compared to NC. Blood glucose level was significantly decreased ($p < 0.01$) with MD and HD doses, and was not significant with LD of *B. arundinacea* treated, compared with SC.

3.5 Effect of Tabasheer (*B. arundinacea*) on cellular and humoral immunity

In SC (Table 5), the total leukocytes counts (TLC) decreased ($p < 0.01$) and total paw edema increased ($p < 0.01$) significantly, and the agglutination (mean net formation in cup number) disappeared significantly disappeared ($p < 0.01$) with dilution (Table 5), compared with NC. While *B. arundinacea* treated did not show any significant effect on TLC, total paw edema, and agglutination, when compared to SC.

3.6 Effect on organ weight/100 g body weight

In Table 6, the size of the SC spleen and liver decreased ($p < 0.01$) and size of kidney increased ($p < 0.05$) significantly, when compared to NC. While *B. arundinacea* treated did not show any significant effect on spleen, liver and kidney, when compared with SC.

Table 4: Effect of Tabasheer (*B. arundinacea*) on brain acetylcholinesterase activity and blood glucose level in rats

Groups/Treatment	Dose/kg(p.o.)	Acetylcholinesterase activity (µmoles/mg of protein)	Blood glucose level (mg/dl)
Vehicle (d.w.)	10 ml	127.28 ± 33.15	84.71 ± 9.66
Vehicle + Stress	10 ml	328.50 ± 54.98**	192.46 ± 8.82**
<i>B. arundinacea</i> + Stress	200 mg	197.36 ± 69.18 ^{n.s.}	170.75 ± 15.03 ^{n.s.}
<i>B. arundinacea</i> + Stress	400 mg	182.66 ± 37.97 ^{n.s.}	133.83 ± 3.15 [#]
<i>B. arundinacea</i> + Stress	600 mg	160.58 ± 22.12 [#]	124.25 ± 8.94 [#]

All values were expressed as Mean ± SEM, significant difference between various groups (ANOVA) and individual comparison was performed using the Dunnett t test. ** $p < 0.01$ = significant, compared with normal group (Gr-I), n.s. = not significant, when compared to stress control (Gr-II), [#] $p < 0.01$, [#] $p < 0.05$ = significant, when compared with stress control (Gr-II).

Table 5: Effect of Tabasheer (*B. arundinacea*) on cellular and humoral immunity in rats

Groups/Treatment	Dose/kg (p.o.)	Cellular immunity		Humoral immunity Paw edema (% V/V)
		TLC (Cell/mm ³)	Agglutination (in cup number)	
Vehicle (d.w.)	10 ml	6224 ± 497.19	11.00 ± 0.41	31.12 ± 3.82
Vehicle + Stress	10 ml	2627 ± 177.78**	3.50 ± 0.65**	67.02 ± 3.84**
<i>B. arundinacea</i> + Stress	200 mg	2810 ± 527.86 ^{n.s.}	4.50 ± 0.71 ^{n.s.}	47.57 ± 6.52 ^{n.s.}
<i>B. arundinacea</i> + Stress	400 mg	3068 ± 302.01 ^{n.s.}	3.75 ± 0.86 ^{n.s.}	61.24 ± 8.24 ^{n.s.}
<i>B. arundinacea</i> + Stress	600 mg	3239 ± 402.13 ^{n.s.}	4.00 ± 0.91 ^{n.s.}	50.42 ± 7.77 ^{n.s.}

All values were expressed as Mean ± SEM, significant difference between various groups (ANOVA) and individual comparison was performed using the Dunnett t test. ** $p < 0.01$ = significant, compared with normal group (Gr-I), n.s. = not significant, when compared to the stress control (Gr-II).

Table 6: Effect of Tabasheer (*B. arundinacea*) on relative organ weight in rats

Groups/Treatment/dose	Organ wt. g/100 g body wt		
	Spleen	Liver	Kidney
Vehicle (10 ml/kg)	0.443 ± 0.07	3.29 ± 0.14	0.67 ± 0.04
Vehicle (10 ml/kg) + Stress	0.212 ± 0.01**	2.05 ± 0.02**	0.95 ± 0.03*
<i>B. arundinacea</i> (200 mg/kg) + Stress	0.351 ± 0.04 ^{n.s.}	2.15 ± 0.21 ^{n.s.}	0.83 ± 0.06 ^{n.s.}
<i>B. arundinacea</i> (400 mg/kg) + Stress	0.303 ± 0.06 ^{n.s.}	2.21 ± 0.15 ^{n.s.}	0.91 ± 0.07 ^{n.s.}
<i>B. arundinacea</i> (600 mg/kg) + Stress	0.326 ± 0.04 ^{n.s.}	2.19 ± 0.08 ^{n.s.}	0.89 ± 0.04 ^{n.s.}

All values were expressed as Mean ± SEM, significant difference between various groups (ANOVA) and individual comparison was performed using the Dunnett t test. ** $p < 0.01$ and * $p < 0.05$ = significant, compared with normal group (Gr-I), n.s. = not significant, when compared to the stress control (Gr-II).

4. Discussion and Conclusion

Stress is thought to impaired immune function (Herbert *et al.*, 1993) through emotional or behavioral manifestations such as anxiety, fear, tension, cognition, anger and sadness and physiological changes like heart rate, blood pressure, glucose metabolism and sweating. Naturalistic stressors are associated with increase in number of circulating neutrophils, decrease in number and percentages of total T cells, decreased antibody production which indicates weak immune functions (Chrousos and Gold, 1992), decrease the size of spleen and liver, increase size of the kidney, altered cognitive function (*e.g.*, loss of memory) and increased circulating glucose level. In the present study, *B. arundinacea* did not show any significant effect on immune parameters, that is, ineffective in the loss of immunity under stress condition.

Circulating glucose concentration regulates many brain functions, including learning and memory (Kaplan *et al.*, 2000). It has been suggested that glucose improves cognitive performance (Tucek and Cheng, 1974). Also, glucose is critical for the production of acetyl-CoA, a precursor of acetylcholine (Gibson and Blass, 1976). Thus, it is that glucose enhances memory processes by increasing acetylcholine synthesis and release (Ragozzino, 1996). Brain acetylcholine is associated with memory consolidation and is hydrolyzed by brain cholinesterase. In the pathogenesis of Alzheimer's disease (the main memory symptom), acetylcholine deficiency is indicated as one of the possible reasons. Therefore, cholinesterase inhibitors are beneficial and protect memory loss.

In this study, *B. arundinacea* (600 mg/kg) showed an inhibitory effect on brain cholinesterase activity and improved behavior in stress condition. Consistent increase in blood glucose level in condition of chronic stress or diabetes depresses cognitive functions and immune function. In this study, *B. arundinacea* (400 and 600 mg/kg) showed regulatory effects on circulating glucose under stress condition. The study concluded that Tabasheer (*B. arundinacea*) had only the protective effect against stress-associated abnormal behaviors or memory deficit. The present study scientifically supports the traditional uses, religious beliefs and uses of these drugs in Unani/Ayurveda formulations as memory enhancers. Therefore, it is suggested that more clinical and pharmacological studies be conducted to investigate other unexploited potentials of these drugs and further investigations are also required to isolate and purify new compounds of industrial importance.

Conflicts of interest

The authors declare no conflict of interest relevant to this article.

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