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Evaluation of Analgesic activity of an ethanolic extract of Bambusa Vulgaris in Mice

Anish Kumar*, Nishpa Kumari, Priyanka Chaturvedi
Faculty of Pharmacy, VNS Group of InstitutionsNeelbud, Bhopal, MP, India
Ravishankar College of Pharmacy Bhopal, Madya Pradesh, India

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ABSTRACT
Bambusa vulgaris (Poaceae) is a perennial plant found all over the Earth except alkaline soils, desert and marsh. In recent year, focus on plant research has increased all over the world. The plant is a rich source of phytochemicals like tannin, flavanoid, alkaloid, carbohydrate, protein, monosaccharides, animo acid, and phytosterol through lacking substantial phytopharmacological studies. This project main aims at exploration of central nervous system (CNS) activity profile of enriched phytosterol fraction. The Analgesic activity was evaluated by using protocol i.e. locomotor activity and rotarod test, used to study any defects in motor coordination in mice. Analgesic experiments were conducted following tail suspension test in mice. Bambusa Vulgaris total steroid (BVTS) at dose 750 mg/kg showed a significant increase ($p < 0.001$) in sleeping time, depression of locomotor activity ($p < 0.001$) and muscle grip performance, and increased duration of tail suspension induced immobility ($p < 0.01$). Bambusa Vulgaris steroid showed marked Analgesic activity and muscle relaxant activity with sub-maximal anti-stress effect.

Keywords-Bambusa Vulgaris, Poaceae, Phytosterol, Analgesic activity, Muscle relaxant.

I. INTRODUCTION

Bambusa vulgaris is a large woody grass that belonging to the family Poaceae. This ancient woody grass widely found in tropical, subtropical and mild temperate zones of the world. Phytochemicals consist of fresh leaves & dried fruits in Bambusa vulgaris.

According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times. Herbal drugs consstitute a major share of all the officially recorgnised syatems of helth in india viz.Ayurveda, Yoga, Unani, Shidha, Homeopathy and Naturopathy.The medicinal plants also contain other beneficial compound like ingredients for functional foods. Hence, the global knowledge about Ayurveda and Indian herbals will hopefully be inhenced by information on the evidence- base of thes plants.This will yield rich dividends in the coming years. Herbal medicine also called botanical medicine or phytomedicine referes to using a plant's seeds, berries, roots, leaves,bark or flowers for medicinal purposes. Herbalism has a long tradition of use out side of conventional medicine. It is becoming more mainstream with improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treatment and prevention of disease(Vaidya and Devasagayam,2007).

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses of plants. African and Native American indigenous cultures used herbs as healing rituals, while others developed traditional medicinal systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purpose. In the early 19th century, when the chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later,chemists began making their own version of plant compounds, and over time, the use of herbal medicines declined infavor of eco-conservation. The World Health Oraganization(WHO) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. Herbal medicines have a good potential in the emerging nutraceutical industry as these materials are often considered food, as well as medicines. They are used in preventive and curative treatments throughout the world.

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population globally is estimated to be around five percent.

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II. MATERIALS AND METHODS

2.1 Collection of plant material

Herb was collected from, Ratibad Bhopal (M.P.). The plant was collected in the month of February 2019. It was made completely clean, dust free and allowed to get dried under the shade.

2.2 Drying and size reduction of plant material

The plant material was dried under shade. It was pulverised to coarse powder with the help of hand grinder. The coarse powder was packed into airtight container and stored in cool and dry place. This material was used for the further study.

2.3 Preparation of crude extract

170 gram of plant material was extracted with petroleum ether by hot maceration process for 24 hrs. The marc was pressed and filtered. The solvent was concentration under reduced pressure using rotator evaporator and dried below 40C. The extract was dark green in colour and having characteristic odour.

2.4 Extraction of plant by different solvents.

170grams of crude Bambusa vulgaris was extracted with different – solvent e.g. 70% ethanol, 70% hydro-alcohol. by hot maceration. Filtered and dried, then salkowski and Lieberman burchard test was carried out on the extract- before and after hydrolysis with filter to explore the nature of non-polar component on steroid or triterpenoid.

2.5. Separation of Phytosterol

The extract was dark green in colour, having characteristic odour. The hydro-alcoholic extract was refluxed with 5% alcoholic KOH for 4 hr and filtered. The filtrate was extracted thrice with diethyl ether. The
diethyl layer was separated, dried and the percentage yield was calculated. The extract showed positive test for steroid.

Dried aerial part of plant
↓hot maceration
Extracted with hydro-alcohol
↓filter
Extracted lipid (filtrate)
↓volume reduction
Extracted lipid refluxed with 5% alcoholic KOH for 4 hr.
↓filter
Filtrate extracted with Di-ethyl ether
↓Di-ethyl ether layer
Dried and yield calculated
↓Positive test for steroid
Separated total phytosterol

2.6 Preparation of Extract Dosage Form

All the dosage form of extracts was made in water for injection. Suspension was stored in air tight bottles in a cool place. Suspension of sterol was administered by oral route. All the standard drug solution was made in water for injection.

2.7 Animals

Male and female breed Swiss Albino mice weighing between 25-32 gm were used in the experiments. All the experiments were performed between 9:30 to 16:30 hr to overcome diurnal and circadian variations. All the animals were housed at a temperature of 24 ± 2°C and in a relative humidity of 65 ± 5%. A 12:12 hr L: D cycle was followed. All the animals were housed in polypropylene cages with paddy husk as bedding with free access to water and fed with standard commercial pelleted chow (Hindustan Lever). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee of Faculty of Pharmacy, VNS Group of Institutions, Bhopal, MP, Indiaand were in accordance with the guidelines of the IAEC.

2.8 Acute toxicity studies (LD50)

The acute toxicity study was performed for Bambusa Vulgaris total sterol (BVTS) using Swiss Albino mice. The animals were fasted for 12 hr prior to the experiment and were administered orally with different dose of BVTS and observed for mortality up to 48 hr (short term toxicity). All the animals dosed at 2000 mg/kg body weight did not show evident toxicity throughout the experimental period A dose range of 250, 500 and 750 mg/kg was selected for evaluation of pharmacological activities. All the animals were also observed for long term toxicity up to 14 days.

2.9 Effect of BVTS on locomotor activity of mice on Actophotometer

Locomotor activity was recorded with a digital activity cage (Dolphin, India). In a pre-test session mouse was individually placed in the actophotometer for 10 min to score the basal reading. All the animals were treated with vehicle, different doses of BVTS and standard drug diazepam (2 mg/kg) then placed individually in the actophotometer to score locomotor activity after 30 min. Mean change in the locomotor activity was calculated for each group.

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2.10 Effect of BVTS on muscle grip performance of mice on rotarod apparatus

Digital rotarod apparatus (Jyoti Scientific, India) was used to evaluate the muscle relaxing and sedative effects of BVTS. The animals were placed individually on the rotarod, rotating at a speed of 25 rpm to score the fall off time. Respective groups of animals were treated with vehicle, different doses of BVTS and reference standard diazepam (2 mg/kg), and were subsequently assessed for their performance on the rotarod after 30 min of drug treatment. Percentage changes in fall off time were calculated for each group.

2.11 Effect of BVTS on tail suspension induced immobility on mice

The animals were suspended by a plastic string 75 cm long, about 20 cm above a table top. The duration of immobility was recorded for a period of 6 min (after discarding activity in the first 2 min because animals try to escape during this period). Mice were considered immobile only when they hung passively and remain motionless. The same procedure was followed animals treated with vehicle, standard drug (diazepam in doses of 2 mg/kg, orally) and BVTS 30 min before the test, and percentage change in immobility was calculated

III. RESULT:-

3.1 Phytochemical Sreening of separated BVTS.

Tabale:-1 Phytochemical Sreening of separated BVTS.

S.no.	TEST	OBSERVATION	INFERENCES
1	Liebermann burchard	Lower layer dark red colour	++++(steroid)
2	Salkowski	Lower layer dark red colour	++++(steroid)
3	Saponin	No dark red colour in lower layer	-ve
4	Alkaloid	Dark red colour in lower layer	+ve

3.2 Acute toxity

The estimated LD_{50} of ETTS wasfound to be 2000.00 mg/kg, i.p.

Tabale:-2 Ld50 determination ofBambusa Vulgaris steroidal fraction (log dose-probit) on mice.

Dose (mg/kg)	Log dose	% mortality	Correct% mortality	Probit value
1000	3	100	95	6.64
750	2.87	60	60	5.25
500	2.69	40	40	4.75
150	2.17	20	20	4.16
50	1.69	0	0	5.00

3.3 Effect of Bambusa vulgaris total sterol onanalgesic activity on hot plate method.

The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments.

Table 3: Analgesic effect of methanol extract from Bambusa vulgaris by hot plate method in rats.

TGroups	Reaction time in seconds (mean ± SEM)				
	0 min	15 min	30 min	45 min	60 min
Control Normal saline	30.67 ± 5.15	28.25 ± 4.87	31.50 ± 4.57	24.08 ± 6.37	24.83 ± 6.02

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Morphine sulfate	30.75 ± 5.64	37.54 ± 5.55	41.58 ± 3.22[b]	41.79 ± 2.87[ab]	41.46 ± 2.55[ab]
BVTS 500	32.04 ± 4.59	27.92 ± 4.04	31.42 ± 2.61	34.38 ± 2.08	31.73 ± 1.46
BVTS 750	30.88 ± 4.08	27.67 ± 2.17	24.50 ± 3.08	27.17 ± 3.92	26.71 ± 4.06

3.4Effect of BVTS on locomootor activity of mice

The BVTS significantly decreased the locomotor activity in a dose dependent manner. The depression of locomotor activity was found maximum at a dose of 750 mg/kg (show in table no.3)

Table.4.Effect of Bambusa Vulgaris total sterol on locomotor activity of mice on actophotometer apparatus

Groups	Dose (mg/kg, i.p)	Locomotion score (M ± SEM)		% Change in locomotion
		Basal	After drug administration	
Vehicle control	0.5 ml/ 100 gm	492.52 ± 12.19	-	-
Diazepam	2	361.52 ± 12.19	140.26 ± 10.50	□ 82.85
BVTS	250	354 ± 12.29	144.09 ± 10.57***	□ 51.96
BVTS	500	415.41 ± 27.31	139.03 ± 30.23***	□ 55.37
BVTS	750	475.11 ± 45.77	115.65 ± 10.91***	□ 58.56
		450.21 ± 11.69		

Values are expressed as mean±S.E.M with (n = 6) per group. BVTS: Bambusa Vulgaris total sterol. ***p < 0.001 compared to standard drug diazepam treated group.

3.5 Effect of BVTS on muscle grip performance ofmice

BVTS at 750 mg/kg dose caused significant (p < 0.05) decrease in fall off time with 80.04% decrement while the standard drug diazepam had fall off time 73.92 ± 12.01 sec with 93.62% decrease (Table4).

Table.5. Effect of Bambusa vulgars total sterol on muscle grip performance of mice on rotarod apparatus

Groups	Dose (mg/kg, i.p)	Fall off time in sec (M ± SEM)		% Change in fall off time
		Basal	After drug administration	
Vehicle control	0.5 ml/100 gm	199.50 ± 10.00	-	-
Diazepam	2	143.12 ± 13.13	73.92 ± 12.01	□ 93.62
BVTS	250	98.39 ± 14.50	74.65 ± 12.43***	+ 53.35
BVTS	500	146.44 ± 10.20	78.86 ± 4.53**	□ 39.07
BVTS	750	136.14 ± 13.51	25.36 ± 2.05*	□ 22.04

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Values are expressed as mean ± SEM with (n = 6) per group. BVTS: Bambusa Vulgaris total sterol. $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$ and ns = not significant compared to standard drug diazepam treated group.

3.6 Effect of BVTS on tail suspension induced immobility onmice

Treatment of diazepam showed extremely significant increase (96.32%) in duration of immobility while Imipramine decreased the duration of immobility (89.32%) as tabulated in Table 5. BVTS at dose of 750 mg/kg showed highly significant increase ($p < 0.01$) in duration of immobility(46.83%).

Groups	Dose (mg/kg, i.p)	Duration of immobility in sec (M ± SEM)	% Change in immobility duration
Vehicle control	0.5 ml/100 gm	125.55 ± 7.23	-
Diazepam	2	246.48 ± 15.30**	+ 96.32
Imipramine	20	13.41 ± 4.95***	- 89.32
BVTS	250	70.22 ± 7.82^{ns}	- 30.52
BVTS	500	122.12 ± 10.07^{ns}	+ 14.57
BVTS	750	144.34 ± 12.33**	+ 12.83

Values are expressed as mean ± SEM with (n = 6) per group. BVTS: Bambusa Vulgaris total sterol. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ and ns = not significant compared to vehicle controlgroup.

IV. DISCUSSION

In the present study Bambusa vulgaris extract prepared in different solvents depending on polarity Bambusa vulgaris was tested for the presence of different phytoconstituents. The plant was found to be rich in steroidal content so the extraction of total sterol was carried out.

The steroids are the centrally active class of phytoconstituents which are responsible for many pharmacological activity.

This study was conducted on several central nervous system related experimental models that induces hypnosis, locomotor activity, rotarod, elevated plus maze, tail immersion test, forced swin test and MES induced convulsion to investigate the possible central effect of Bambusa vulgaris. These tests are classical models for screening CNS action providing information on depressant or stimulant property, psychomotor performance, anxiolytic, myorelaxant activity.

Analgesic activity is considered as an index of hot plate method in which the activity of mice is decreases in its activating of sedative activity.

Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity. BVTS significantly decreased locomotor activity in all the tested doses diazepam act as a centrally active muscle relaxant interacting with specific receptors enhancing GABAergic transmission (Tripathi,2002). Decrease in locomotion reveals depressant effect on CNS may be due to increase in the concentration GABA in brain.

Controlled grip of mice on rotarod ensures its staying over the rod for longer duration. Enhanced stay on rotarod delays the fall off time signifying better muscle grip strength. CNS depressant drug decrease the grip strength and mice may fall from the rotarod due loss of muscle coordination. Loss of grip strength is measured as motor in coordination or muscle relaxant effect of drug. BVTS decrease the fall off time of mice from the rotating rod suggesting the muscle relaxant effect.

The elevated plus maze test is principally based on the exposure of animal to an elevated maze array evoking an approach avoidance conflict. The fear due to height induces anxiety in

the animals when placed on the elevated plus maze(EPM). The animals being exposed to the new environment tends to avoid open entries and prefer to stay in closed arn due to fear . the ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolotic agents are expected to increase the motor activity, which is measured as increase in the time spent by the animal in the open arms. Diazepam produced significant increase in open arm duration and also number of entries into the open arms.

GABA is the major inhibitory neurotransmitter in the CNS. Different anxiolytic muscle relaxant sedative drugs implicate their through GABAa receptor intraction.

Bambusa vulgaris total sterol may act on GABAergic transmission by direct activation of GABAa receptor indirectly by potentiation of GABAa receptor mediated hyper-polarization.

The test substance, steroid also showed same activity profil like diazepam with increased immobility in tail suspension test and effect on forced swim test. In the etiology of depression catecholamine and 5-HT have been implicated as very important central neuro-transmitters.

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