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Research Article

# ANTICONVULSANT ACTIVITY OF CHLOROFORM EXTRACT OF *PHYLLOSTACHYS BAMBUSOIDES*

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**ABSTRACT**

The anticonvulsant activity of the chloroform leaf extract of *Phyllostachys bambusoides* was investigated by testing the effects of the extract on electroshock induced seizures in rat. Experiments were carried out on male rat and the animals were randomly allotted to the different control and test groups. The extracts (chloroform) contain glycosides, carbohydrates, tannins, proteins and flavonoids. It was found that chloroform extracts up to a dose of 1000mg/kg body weight, did not show any toxic manifestations or death. In electroshock induced seizures, the administration of *Phyllostachys bambusoides* chloroform extract at a dose of 200 mg/kg 1 h prior to the electroshock. Chloroform extracts at the dose of 100 mg/kg body weight could not exert any significant protective effect on electroshock induced convulsions. Diazepam in a dose of 4mg/ kg, totally abolished the episodes of convulsions. Chloroform extract at the dose level of 200 mg/kg body weight showed significant antiepileptic activity.

**Keywords:** *Phyllostachys bambusoides,* Anticonvulsant, Flavonoids, Electroshock induced seizures.

## INTRODUCTION

Seizures are the most common neurological disorders with an incidence of 3% in the general population (Annegers, 2001). Anti-epileptic drugs (AEDs) available currently do not provide cure nor prevent relapse and they are often associated with serious side effects, including chronic toxicity, teratogenicity and adverse effects on cognition and behavior[1]. Remedies from plant play an important role in developing countries in the health care of millions of people. Many people in developing countries despite immense technological advancement in modern medicine still depend on traditional healing practices and medicinal plants for their daily health care needs (Ojewole, 2004). The unregulated destruction of the flora of tropical rain forest poses a threat to the medicinal plants. There is every virtue in intensifying research into medicinal flora, especially those claimed to have beneficial effects in serious disorders such as epilepsy[2]. However, only limited efforts have been made to evaluate the potentials of such plants for their use in modern medicine or to scientifically justify their traditional use in the treatment of CNS disorders including epilepsy[3].

*Phyllostachys bambusoides* is a group of perennial evergreens in the true grass family Phocaea, subfamily Bambusoideae, tribe Bambuseae. Commonly it is known as madake. *Phyllostachys* genera belonging to bamboos, which are perennial grasses distributed widely in Asian countries including Korea, China and Japan4. In Chinese traditional medicine, the plant is used for fever, in the treatment of cancer, convulsion, as an analgesic, anti-inflammatory agent, in the treatment of diabetes and as an antimicrobial agent. This study was therefore designed to evaluate the anticonvulsant activities of *Phyllostachys bambusoids* in order to scientifically justify its use in traditional medicine to treat epilepsy[5].

## MATERIALS AND METHODS

### Collection and authentication of plant material

The leaves of plant *Phyllostachys bambusoides* was collected from the field of Department of Silviculture, Nauni University, Solan. The botanical identity was confirmed by Dr. R. Raina, qualified taxonomist from the Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). Voucher specimens were deposited with the Herbarium at Nauni and are entered in the UHF-Herbarium Field book no. 12530.

### Extraction

The extraction is done through soxhlet apparatus6. The sample (powder of *Phyllostachys bambusoides*40gm.) was weighed and placed in the thimble made from thick filter paper, which was then loaded into the main chamber of the Soxhlet extractor. The extractor was then placed onto a flask containing the extraction solvent (chloroform 500ml).The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. The chamber containing the solid material was slowly filled with warm solvent to dissolve some of the desired compound[7]. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times, over 36 hrs. During each cycle, a portion of the non-volatile compound dissolved in the solvent. The extract was passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 7.93%, which was stored in universal bottles and refrigerated at 4°C prior to use.

### Phytochemical screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on the Leaf powdered using standard procedures[8].

### Animals

Male wistar rats weighing 150-200 gm of either sex were procured from animal house. All the animals are kept in standard polypropylene cages and maintained under standard conditions: temperature (24 ± 10 C), relative humidity (45-55 %) and 12:12 light: dark cycle. The animals were fed with standard rat pallet and water. The animals were allowed to acclimatize to laboratory conditions 48 hrs before the start of the experiment. Groups of 5 rats (150-200gm.) were used in all sets of experiments.

### Acute toxicity studies

Acute toxicity studies were carried out following OECD guideline no. 425 to study the acute Toxic effects and to determine the minimum lethal doses of the drug extracts[9]. Male wistar rats150-200 g were used for the study. The chloroform extract was administered orally to overnight fasted animals at doses of 200 mg/kg,500 mg/kg, 750 mg/kg, 1000 mg/kg and 2000 mg/kg of body weight[10]. After administration of the extracts, the animals were observed continuously for the first two hours, for any toxic manifestation. Thereafter, observations were made at regular intervals for 48 hours. Further the animals were under investigation up to a period of 2 week[11].

### MES induced convulsions

The effect of the aqueous stem bark extract of *P .bambusoides* on generalized seizures was evaluated by the maximal electroshock (MES) method as described by Swinyard and Wood head, (1982). Wistar rats (150 – 200g)fasted overnight but had access to water which was only withdrawn during the experiment were randomly allotted to groups of at least five animals per group. The animals

were administered orally, distilled water (5ml/kg), extract (200 mg/kg), or diazepam (4 mg/kg, i.p.). Generalized seizures were induced one hour later with electroshock (Electroshock unit) through a pair of ear electrodes which delivered an alternating current of constant frequency (60Hz) and 150mA for 0.2sec to elicit tonic hind-limb extension in the animals[12,13]. Distilled water treated animals receiving this electrical stimulation undergo convulsive seizures pattern having a tonic flexor phase, a tonic extensor phase and clonic phase. An animal was considered to be protected if the characteristic electroshock convulsive seizure pattern was absent[14,15]. The ratio of animals protected in each group as well as the percentage protected was determined.

**Statistical analysis**

All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test. Data from distilled water treated animals were used as the control and data from diazepam treated animals were used as standard values. All values are expressed as Mean ± S.E.M. Results were regarded as significant at $P< 0.05$[16,17].

**RESULTS**

**Preliminary phytochemical studies**

The preliminary phytochemical screening of chloroform extract shows the presence of glycosides, carbohydrates, flavonoids, tannins and proteins.

**Acute toxicity studies**

Acute toxicity studies were carried out to evaluate the drug's toxicity and to determine the minimum lethal dose of the drug extracts, using wistar rats. It was found that chloroform extracts up to a dose of1000mg/kg body weight, did not show any toxic manifestations or death. It shows toxicity at a dose of 2000 mg/kg. So according to OECD guidlines no.425 the therapeutic dose is $1/10^{th}$ of toxic dose the therapeutic dose was calculated which was 200 mg.kg.

**Table 1: Table showing result for acute toxicity studies**

| S.no. | Dose of plant extract (mg/ kg) | Observation | Infrence |
|---|---|---|---|
| I. | 200 | 00000 | $LD_{50} \leq 2000$ |
| II. | 500 | 00000 | mg/kg |
| III. | 750 | 00000 | |
| IV. | 1000 | 00000 | |
| V. | 2000 | toxicity | |

**Effect on MES induced convulsion**

The extract (200.0 mg/kg) protected the rats against maximal electroshock-induced convulsions. The extract also significantly reduced the mean recovery time of convulsed animals. Diazepam (4 mg/kg), the standard anticonvulsant used produced 100% inhibition of hind limb tonic extension (HLTE) of maximal electroshock test (MEST).

**Table 2: Antiepileptic activity of *phyllostachys bambusoides***

| **MES Induced Epilepsy**** | | |
|---|---|---|
| GROUP* | DOSE | EXTENSION + STUPOR TIME |
| CONTROL | - | 189.53± 0.677 |
| STANDARD(Diazepam) | 4mg/kg | 28.62 ± 0.373 |
| *AEPB* | 500mg/kg | 174.23± 0.069[c] |
| *AEPB* | 500mg/kg | 32.53±1.032[a,b] |

*- Each group consists of 5 animals

**- Data is in Mean ± SEM.

[a]- Significant decrease in time of fall as compared to vehicle treated group

[b]- Non significant difference in time of fall as compared to Diazepam treated group

[c]- Negligible effect on time of fall as compared to control group.

**DISCUSSION**

*Phyllostachys bambusoides* exhibits anticonvulsant activity in electroshock induced seizure model. MES induced human generalized seizures (Loscher and Schmid, 1998). Compounds effective against this experimentally induced seizure models, are effective against generalized type of epilepsy.

The oral administration of *Phyllostachys bambusoides* at the dose of 200 mg/kg1 hr before the electric shock, delayed the onset of seizures, and decreased the duration of seizures (Table2).

Studies have shown that benzodiazepine binds to a specific subunit on the $GABA_A$ receptor at a site that is distinct from the binding site of the endogenous GABA molecule. The $GABA_A$ receptor is an inhibitory channel which, when activated, decreases neuronal activity. Benzodiazepines do not supplement for the neurotransmitter GABA, rather benzodiazepines such as diazepam bind to a different location on the $GABA_A$ receptor with the result that the effects of GABA are enhanced. Benzodiazepines cause an increased opening of the chloride ion channel when GABA binds to its site on the $GABA_A$ receptor leading to more chloride ions entering the neuron which in turn leads to enhanced central nervous system depressant effects. Diazepam binds non-selectively to alpha1, alpha2, and alpha3 and alpha5 subunit containing $GABA_A$ receptors. Anticonvulsant activity of *Phyllostachys bambusoides* be attributed to the above mechanism.

**CONCLUSION**

The alcoholic extract of *Phyllostachys bambusoides* exhibited anticonvulsant activity in experimental animal models. The results of this study provide support for the traditional use of *Phyllostachys bambusoides* an anticonvulsant drug.

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