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Research Article

SKELETAL MUSCLE RELAXANT ACTIVITY OF CHLOROFORM EXTRACT OF *PHYLLOSTACHYS BAMBUSOIDES* ON WISTAR RATS

***SUNIL K TOMAR[1], GAURAV SHARMA[1], MATHEW GEORGE[1]**

[1]Department of Pharmacology, School of Pharmaceutical Sciences, Shoolini University, Solan (H.P.) 173229. Email: suny1435@gmail.com

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ABSTRACT

Skeletal muscle relaxant activity of the chloroform leaf extract of *Phyllostachys bambusoides* was investigated by testing the effects of the extract on wistar rat using rota-rod apparatus model, inclined screen test, climbing test. 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 2000 mg/kg body weight, did not show any toxic manifestations or death. The extract was administered orally at a dose of 200 mg/kg. Diazepam in a dose of 4 mg/kg (s.c.) was used as a standard. Chloroform extract at the dose level of 200 mg/kg body weight showed significant skeletal muscle relaxant activity. On the bases of these results we can conclude that the *Phyllostachys bambusoides* may be used to develop herbal medicines against the same.

Keywords: *Phyllostachys bambusoides,* Muscle relaxant, Rota-rod, Flavonoids, Diazepam

INTRODUCTION

In recent years, there has been an increasing interest worldwide in the use of herbal plants as health supplements or medicines. Systematic studies on the effect of specific medicinal herbs on the immune system are designed to obtain evidence-based scientific knowledge on the appropriate use of traditional medicinal herbs. *Phyllostachys bambusoides* (Gramineae) is commonly known as bamboo. It is one of the fastest growing plant in the world. *Phyllostachys* genera belonging to bamboos, which are perennial grasses distributed widely in Asian countries including Korea, India, China and Japan. The bamboo leaves originating from *Phyllostachys bambusoides* have been used in traditional medicine for their anti-inflammatory, antipyretic and diuretic. In addition, clinical use of bamboo leaves for treating hypertension[1], arteriosclerosis, antioxidant[2], antimicrobial[3], cardiovascular disease and anticancer[4] have been described. In the present work is to evaluate the skeletal muscle relaxant activity of the chloroform extract of leaves of *Phyllostachys bambusoides.*

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 apparatus[5-6]. The sample (powder of *Phyllostachys bambusoides* 40 gm.) was weighed and placed in the thimble made from thick filter paper, which was then loaded into the main chamber of the Soxhlet extractor[7]. The extractor was then placed onto a flask containing the extraction solvent (chloroform 500 ml).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[8]. 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[9-13].

Animals used

Adult Albino rats (Wistar strain) of either sex with weighing 100-150 g were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals were fed with commercially available rat pelleted diet. Water was allowed ad libitum under strict hygienic conditions.

The experimental protocols were duly approved by the Institutional Animal Ethical Committee, (IAEC, Approval No: 002/2010/IAEC/SU) of School of Pharmaceutical Sciences, Shoolini, University, Solan, Himachal Pradesh.

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 extract[14-16]. Male wistar rats150-200 g was 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[17]. 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[18].

Rota-rod apparatus test (motor coordination)

Rats were divided into three groups consisting of five animals each. Group I served as control which received 1% acacia. Animals of group II received standard drug Diazepam at a dose of (4 mg/kg, *i.p.*). Group III received the chloroform extract of *Phyllostachys bambusoides* orally at a dose of 200 mg/kg. Animals remaining on Rota-Rod (22 rpm) 2 min or more in low successive trials after the administration of test material or control vehicle the same test of 30 min for 2 hr. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and treated rats was taken as an index of muscle relaxation[19-20].

Inclined screen test

Each group of rats (n=5) were left for 1hr on a flat, slippery, rectangular glass (42cm × 37cm) inclined at 30º to the horizontal, 30 min after the administration of *Phyllostachys bambusoides* (200 mg/kg oral), Diazepam (4 mg/kg, i.p.), Acacia (1% oral) to observe for a paralyzing effect severe enough to cause the rats to slide off the screen[21].

Climbing test

Male rats were trained to climb a chain, 50 cm long by placing the fore paws of each one on the free end of the chain. The chain was suspended form a clamp standing on a laboratory bench. A normal rat grasped the chain with the fore paws and allowed to hang free, placed the two feet on chain and clambed till it got a marked point 2 cm to the top of the chain. Rats, which got to the mark within 30 sec, were selected for the further tests. Previously screened rats were used for the test, 30 min. after administration of *Phyllostachys bambusoides* (200 mg/kg, oral), Diazepam (4 mg/kg, i.p.), Acacia (1% oral) to different groups (n=5)[22].

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[23-26].

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 of 1000 mg/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 guidelines no.425 the therapeutic dose is 1/10th of toxic dose the therapeutic dose was calculated which was 200 mg.kg.

Table 1: Table showing result for acute toxicity studies

Dose	Observation	Inference
200	00000	
500	00000	
750	00000	$LD_{50} \leq 2000$ mg/kg
1000	00000	
2000	00000	

Skeletal muscle relaxant activity (motor coordination)

The skeletal muscle relaxant effect of methanolic extract of *Phyllostachys bambusoides* leaves has been shown in Fig 1, 2 and 3. Treatment with extract at a dose of 200 mg/kg and Diazepam at dose of 4 mg/kg decreased fall off and sliding time and increase climbing time (motor coordination). The result obtained from both standard and extract treated groups were compared with the control group. A highly significant *P<0.05 and **P<0.001 reduction in the motor coordination was observed in the test drug at 30 min of duration.

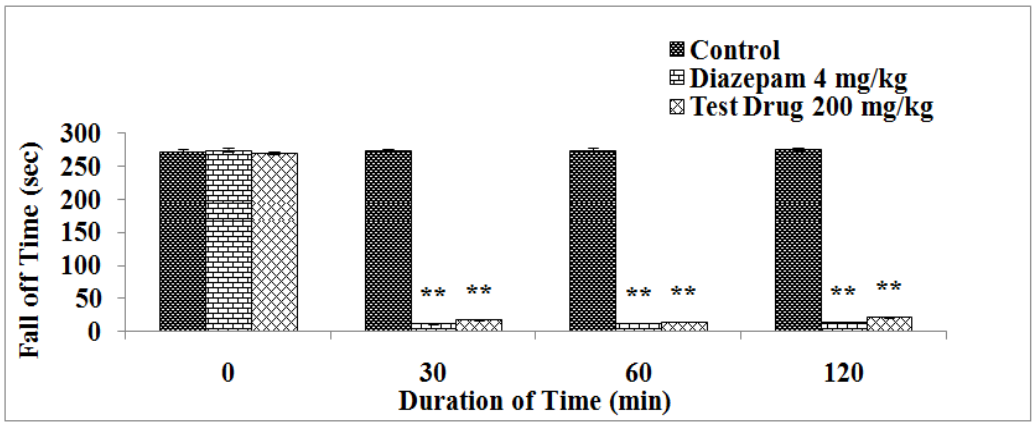

Fig. 1: Effect of *Phyllostachys bambusoides* on muscle relaxant activity (rota-rod apparatus model). Data are means ± S.E.M. of six animals.

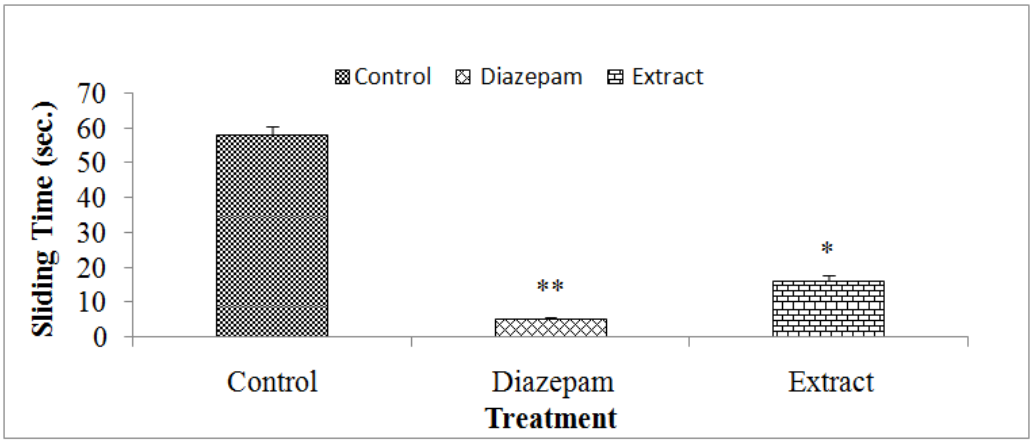

Fig. 2: Effect of *Phyllostachys bambusoides* on muscle relaxant activity (Inclined screen test). Data are means ± S.E.M. of six animals.

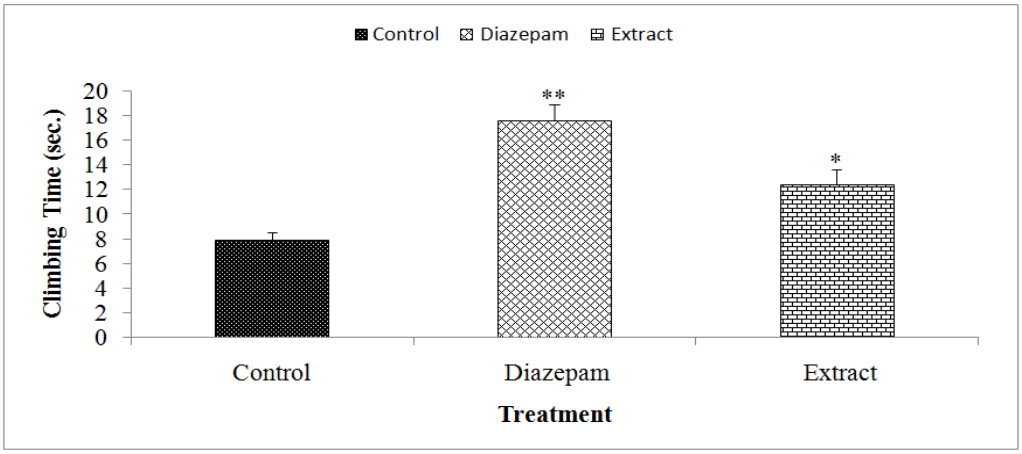

Fig. 3: Effect of *Phyllostachys bambusoides* on muscle relaxant activity. Data are means ± S.E.M. of six animals.

DISCUSSION

The objective of this study was to investigate in-depth the skeletal muscle relaxant activity of the chloroform extract of *Phyllostachys bambusoides* leaves. The present results showed that the methanolic extract of *Phyllostachys bambusoides* leaves possess a significant skeletal muscle relaxant activity in experimental rats. At dose of 200 mg/kg it showed highly significant skeletal muscle relaxant activity at 60 min of duration. Preliminary phytochemical screening reveals the presence of glycosides, carbohydrates, flavonoids, tannins and proteins in the plant extract. Therefore, the observed skeletal muscle relaxant activity may be attributed to these compounds. Further studies are in progress to isolate the active constituents responsible for this activity. Since the pharmacological profile of the present investigation of the chloroform extract of *Phyllostachys bambusoides* was similar to that of benzodiazepines, it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, the use of chloroform extract of *Phyllostachys bambusoides* leaves in folkloric medicine may be due to its CNS action.

CONCLUSION

The results of this study provide support for the traditional use of *Phyllostachys bambusoides* an anticonvulsant drug. However, further studies are necessary to find the exact mechanism of skeletal muscle relaxant effect and to isolate the active compound responsible for this pharmacological activity.

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