Research Article

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International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Evaluation of Skeletal Muscle Relaxant Activity of Aqueous Extract of Tecoma Stans Areal Parts Sin Albino Rats

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ABSTRACT

Tecoma stans is traditionally used in various diseases because of its medicinal properties. One of its uses is in musculoskeletal disorder. The aim of the study was to evaluate the skeletal muscle relaxant activity of the aqueous extract of *Tecoma stans* areal parts (AETSAP) in albino rats in comparison with diazepam. A total of 20 Swiss albino rats aged 6-7 weeks, of either sex, weighing about 100-150 g, were taken, and after acute toxicity studies two different doses were selected. The animals were divided into four different groups. The first group was kept as the control (normal saline), second as the standard (diazepam) and the remaining two groups as Test I and Test II, and given different doses of the AETSAP. Skeletal muscle relaxant activity (motor coordination) on Rotarod and locomotor activity on photoactometer was performed. Statistical analysis was carried out by using analysis of variance, followed by Dunnett's multiple comparison tests. The result from the Actophotometer test and Rotarod test showed that the extract of AETSAP significantly reduced ($P < 0.05$) the motor coordination of the tested animals. Our data indicates that AETSAP possesses skeletal muscle relaxant activities.

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Keywords: Actophotometer, muscle relaxant, *Tecoma stans*, rotarod, soxhlet apparatus.

INTRODUCTION

Skeletal muscle relaxants are drugs that reduce the muscle tone. They act peripherally at the neuromuscular junction (neuromuscular blockers)/muscle fiber itself or centrally in the cerebrospinal axis to reduce muscle tone or cause paralysis. The neuromuscular blocking agents as adjuncts during general anesthesia to provide

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muscle relaxation for surgery, while centrally acting agents are used for painful muscle spasms and spastic neurological conditions.[1] However, these drugs cause several adverse effects. Thus looking for an effective alternative has always been a priority in this regard.

Tecoma stans (common name yellow bell) also known as yellow trumpet bush belongs o the family bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub two to four meters in height. The leaves are opposite, odd-pinnate, Up to 20 centimeters in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong- lanceolate, 6 to 13 centimeters long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented and borne in short, dense, terminal clusters. The calys is green. 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6 inch long, tan pods that are filled with small, papery winged seeds.[2]

Leaves of *Tecoma stans* contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti diabetic activity. Roots are powerful diuretic and vermifuge [3]. *Tecoma* is not a toxic because this plant is used in latine America as a remedy for diabetes and moreover for feeding cattle and goats in mexico [4]. This work is planned to evaluate the skeletal muscle relaxant ability of the aqueous extract of areal parts of *T.stans*

MATERIALS AND METHODS

Plant Collection & Extraction

The areal parts of *Tecoma stans* were collected in the month of May 2015 from Rasipuram (Namakkal District) Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr.G.V.S.Murthy, Joint Director of the Botanical Survey of India, Southern circle. The areal parts were shade dried and powdered. The aqueous extract of areal parts was prepared using the soxhlet apparatus in the Department of Pharmacology. The extract was dried under vacuum, stored at room temperature, and protected from direct sunlight. The extractive value of *Tecoma stans* flower (AETSAP) was 15.8 GW/W.

Animals

A total of 20 Swiss albino rats aged 8-10 weeks of either sex weighing about 100-150 g were obtained from the Central Animal House. The animals were fed standard pellet diet and with water ad libitum, and were maintained under standard conditions of temperature, humidity, and light (12 h light/12 h dark cycle). The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee; registration number 157/CPCSEA. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

Drugs and Chemicals

Diazepam (Lupin Laboratories Ltd., India), 10 mg/kg and Normal saline (0.9% NaCl solution) were administered in a volume of 10 mL/kg. The extracts were suspended in distilled water and subjected to muscle relaxant activity using the Rotarod apparatus and Actophotometer. The extracts were administered orally (p.o.,) in a volume of 10 mL/kg of body weight, in doses of 100 mg and 200 mg/kg.

Photochemical Characterization

The aqueous extract was subjected to general phytochemical analysis for the presence of carbohydrates, proteins, amino acids, tannins, phenolics, flavonoids, alkaloids, anthraquinones, glycosides, saponins, and steroidal nucleus using the standard methods. [5],[6]

Acute Toxicity

A total of 35 mice were randomly allotted to one control and six treatment groups. The animals were fasted overnight, prior to the experimental procedure. The method of up-and-down staircase was used to determine the dose. [7] The procedure was followed as per the Organization for Economic Co-operation and Development 423 guidelines. The extract in each case was administered orally in three doses: 0.5 g/kg, 1.0 g/kg, and 2 g/kg. The animals were observed for 24 h for signs of toxicity and mortality. In the acute toxicity tests, AETSAP extract treated animals exhibited no alarming signs of toxicity. [8]

Selection of Dose for Pharmacological Screening

The aqueous extract of AETSAP was found to be nontoxic up to a dose of 2000 mg/kg and did not cause death, therefore, it was considered to be safe. Hence, one-tenth of this dose, that is, 200 mg/kg body weight and half of the one-tenth dose,

that is, 100 mg/kg, were used for the elucidation of muscle relaxant activity.

Experimental Design

The animals were divided into four groups of five rats each. The drugs were administered as Group I - Control rats (normal saline 10 mL/kg), Group II - standard (diazepam 10 mg/kg), Group III - *Tecoma stans areal parts*100 mg/kg, Group IV - *Tecoma stans areal parts*200 mg/kg.

The evaluation of skeletal muscle relaxant activity (motor coordination):

The rats were divided into four groups consisting of five animals each. Group I served as the control, which received normal saline 10 mL/kg, Group II received the standard drug diazepam, at a dose of 10 mg/kg, p.o., Group III and IV received the aqueous extract of AETSAP orally at a dose of 100 and 200 mg/kg. The animals remained on Rotarod (25 rpm) for 5 min or more after successive trials were included in the study. After the administration of control, standard, and test material, the fall off time from the rotating rod was noted after 30 min. The difference in the fall off time from the rotating rod between the control and the treated rats was taken as an index of muscle relaxation. [9]

Locomotor Activity

The spontaneous locomotor activity was assessed with the help of a photoactometer. [10] Each animal was observed for a period of 5 min in a square closed field arena (30 cm × 30 cm × 30 cm) equipped with six photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by means of a six digits counter. To see the locomotor activity, the Actophotometer was turned on and each mouse was placed individually in the activity cage for 5 min. The basal activity score for all the animals was noted. Control normal saline, standard diazepam, and two different doses of aqueous extract of AETSAP were given orally and after 1-h of re-testing, the activity score for 5 min was observed. The difference in the activity, before and after drug administration, was noted. The percentage decrease in motor activity was calculated.

Central versus Peripheral Skeletal Muscle Relaxant Property

To elucidate exact site of action, three groups with three rats in each was selected and AETSAP 100 mg/kg and 200 mg/kg was injected directly into the thigh muscles of the above said two groups and control group received distilled water intramuscularly. The locomotor activity was observed with actophotometer before 5 min and 30 min after injection.

STATISTICAL ANALYSIS

The results were expressed as a mean ± standard deviation. Statistical analysis was carried out by using the analysis of variance followed by Dunnett's multiple comparison tests using a Primer of Biostatistics (Stanton A, Glantz; Primer for Windows. McGraw-Hill, Inc., Version 5.0) (2011). $P < 0.05$ was considered significant.

RESULTS

Phytochemical Screening

The preliminary phytochemical analysis of the flower extracts of *N. oleander* showed the presence of carbohydrates, phenols, saponins, tannins, and alkaloids but devoid of steroids. The phytochemical results of *N. oleander* were in conformity with other studies [11],[12] who pointed this species as one of the most poisonous plants, which contain numerous toxic compounds such as alkaloids and triterpenes. All the extracts were stored in a clean glass bottles for further pharmacological studies.

Toxicity Study

A preliminary acute oral toxicity study, the flower extract produced no adverse effects at dose 2000 mg/kg and did not cause any death up to a dose of 3000 mg/kg in mice.

Rotarod Test

For muscle relaxation, in this test, (AETSAP 100 mg/kg and 200 mg/kg) showed highly significant reduction in the time spent by the animals on the revolving rod when compared to the control ($P < 0.000$). The standard drug (diazepam) also showed a highly significant effect when compared to the control ($P < 0.000$). However, two different doses of AENOL (100 and 200 mg/kg p.o.,) showed a dose-dependent increase in muscle relaxation, that is, 186.8 ± 8.04** and 35.4 ± 3.84**, respectively, when compared to the control [Table 1]. Maximum muscle relaxation was observed with 200 mg/kg of aqueous extract of AETSAP. The result from the Rotarod test showed that the extract significantly reduced the motor coordination of the tested animals.

Table 1: Effect of AETSAP on locomotor activity in actophotometer and muscle coordination on the Rotarod apparatus.

Group	Treatment	Actophotometer score in 5 min before	After 60 minutes of administration	% of reduction	Time spent on revolving rod
I	NS 10ml/kg	152.1±2.58	-	0	317±16.62
II	Diazepam10mg/kg	216.7±7.66	13.32±2.16**	93	14.15±2.21**
III	AETSAP 100mg/kg	193.4±4.65	40.01±1.57**	78.79	185.7±7.03**
IV	AETSAP 200mg/kg	179.1±5.76	24.4±2.06**	85.21	34.3±2.77**

***P<0.000.* All values are expressed as mean±SD. SD=Standard deviation, AETSAP= aqueous extract of *Tecoma stans* areal parts

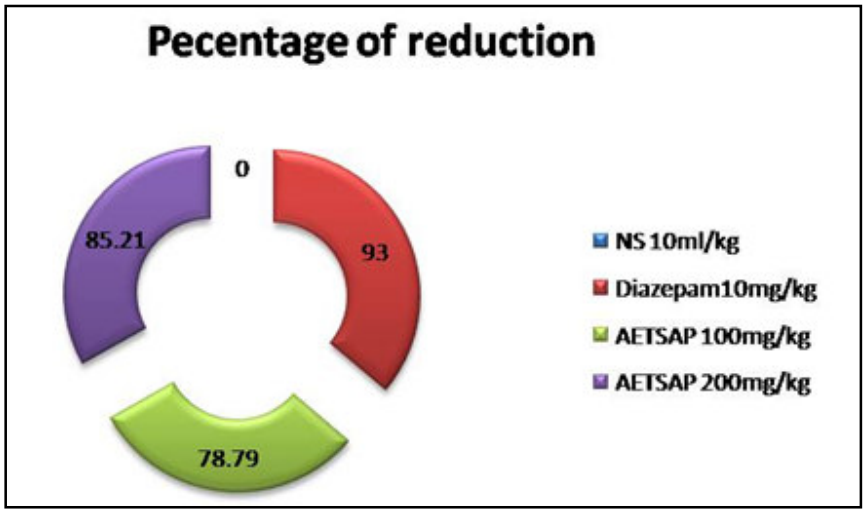

Figure 1: Effect of AETSAP on percentage reduction in locomotor activity using actophotometer
Actophotometer:

Test for locomotor activity: The percentage of reduction in the locomotor activity with diazepam (10 mg/kg, p.o.,) after 30 min was 93.87, that is, there was a highly significant ($P < 0.000$) decrease in locomotor activity compared to the control, whereas, two different doses of AETSAP (100, and 200 mg/kg, p.o.,) showed a dose-dependent decrease in the locomotor activity, that is, 41 ± 1.58** and 25.4 ± 2.07**, respectively, when compared to the control. Maximum muscle relaxation was observed with 200 mg/kg of aqueous extract of AETSAP. The values were highly significant ($P < 0.000$) [Table 1]. In second experiment, where the drugs were given directly into the muscle, there was no statistically significant decrease in the locomotor activity with two different doses of AETSAP (100, and 200 mg/kg, i.m.) that is, 188.8 ± 4.2 and 179.1 ± 3.92, respectively when compared to control group ($P > 0.05$) [Table 2].[Figure:1& 2]

Table 2: Effect of AETSAP on locomotor activity in actophotometer after giving the extract directly into the muscle

Group	Treatment	Actophotometer score 5 min before	Actophotometer score after 5 min	After 30 minutes of administration	% of reduction after 30 Min	p
I	NS 10ml/kg	166.1±3.37	165.7±2.21	163.5±2.21	1.55	0.613
II	AETSAP 100mg/kg	190.7±3.42	189.1±3.21	187.4±3.19	1.57	0.706
III	AETSAP 200mg/kg	181.0±3.11	179.7±3.21	178.2±2.32	1.65	0.680

P>0.05, All values are expressed as mean±SD. SD=Standard deviation, AETSAP= aqueous extract of *Tecoma stans* areal parts

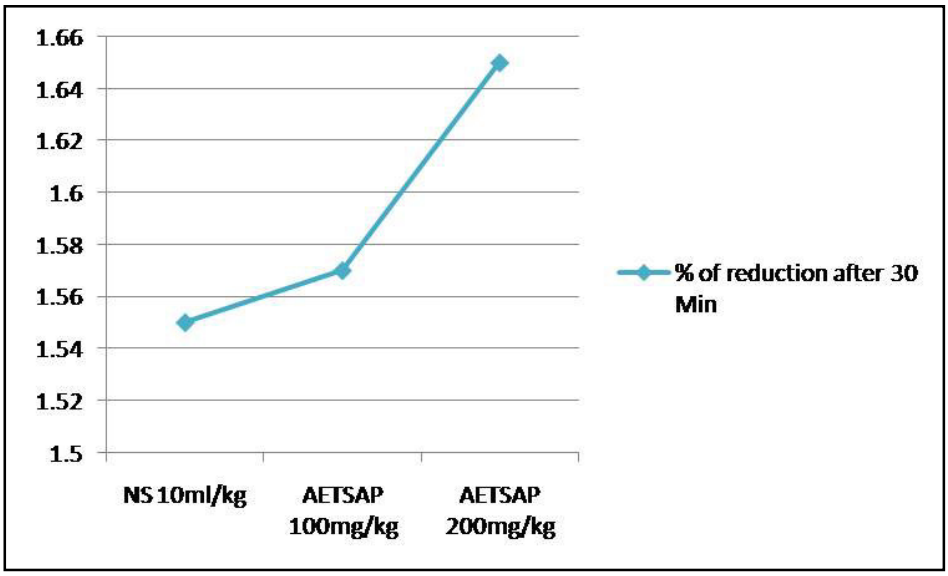

Figure 2: % of reduction after 30 Min of AETSAP administration in locomotor activity using actophotometer after giving the extract directly into the muscle

DISCUSSION

In recent years, the herbal medicines have been extensively used in various diseases because of their safety profile. *T.Stans* is one of them and used against various disorders in indigenous system of medicine, especially for arthritis. The leaves and the flowers are cardiotonic, diaphoretic and diuretic, emetic, expectorant, used in the treatment of scabies, and to reduce swellings. It has also being reported to have antibacterial and antidiabetic activities. [13] The bark contains toxic glucosides, rosaginin and nerrin, volatile oil, fixed oil, etc. [14] The objective of the present study was to investigate the effect of aqueous extracts of the AETSAP on muscle relaxant activity in experimental animals like albino rats. Actophotometer is widely used screening method for evaluating the locomotor activity and antianxiety activity in rodents and Rotarod for muscle relaxation. Anxiety is associated with increased muscle tone along with the other symptoms such as restlessness or feeling keyed up or on edge, easily fatigued difficulty in concentration, irritability, increased muscle tension, and sleep disturbance. The reduction of spontaneous motor activity could be attributed to the sedative effect of the extract. The present study

showed a dose-dependent increase in muscle relaxation with different doses of AETSAP. Decrease in locomotion implies depression effect on the central nervous system and also it has been well-known that an augment in the concentration of gamma-aminobutyric acid (GABA) may lead to CNS depressant effect. This suggests that AETSAP may act via GABA receptors. Phytochemical analysis of AETSAP revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, terpenoids, flavonoids, reducing sugars, and saponins. [15] The muscle relaxant activity observed with AETSAP may be due to the presence of flavonoids, alkaloids, and terpenoids in the plant extract. One study [16] on AETSAP revealed the presence of various compounds such as alkaloids, saponins, flavones, triterpenoids, steroids, tannins, and amino acids. The results of present study suggest the muscle relaxant activity of alcoholic extract of AETSAP at the doses of 100 and 200 mg/kg. The observed muscle relaxant effect of AETSAP may be due to the agonistic effect on GABA/benzodiazepine receptor complex. [17] Studies on this species pointed, as one of the most poisonous plants, which contain numerous toxic compounds such as alkaloids and triterpenes. [14] The standard reference drug diazepam, which acts as an anxiolytic (at low doses), anticonvulsant and also produces sedation, and a myorelaxant effect at higher doses. [18] In this case, diazepam at a dose of 10 mg/kg body weight showed a significant lack in motor coordination and muscle relaxant activity in animals and animals treated with the extract showed muscle relaxation and reduced motor activity. These effects of AETSAP could be due to the interaction of flavones, triterpenoids, steroids (chemical constituents of the plant) with the GABA/benzodiazepine receptor complex in the brain. [19] In another study [20] in Rotarod motor co-ordination test, AENOL at 100 mg/kg, i.p., significantly ($P <$ 0.05-0.01) reduced the endurance. As the phyto constituents of AETSAP and *N. oleander* leaves are same. Our study is in correlation with our previous study. Moreover, also with another study. [21] However, further extensive phytochemical analysis and research is necessary to identify the exact constituents and elucidation of its possible mechanism of action underlying the myorelaxant activity of AETSAP.

CONCLUSIONS

The study suggests that the aqueous extract of Tecoma stans areal parts has muscle relaxant action. As the comparison is done with centrally acting benzodiazepine group of drug diazepam, it is assumed that the muscle relaxation and reduced motor activity effects of AETSAP could be due to the interaction of isoflavonoids of the plant with the GABA/benzodiazepine receptor complex in brain as the absence of muscle relaxation was observed when the extracts are given directly into the muscles. Still extensive research is needed to synthesize new molecule with muscle relaxation property from T.stans.

REFERENCES

1. Tripati KD. Essentials of Medical Pharmacology. 8th ed. New Delhi: JayPee Brother Medical Publishers; 2018. p. 373-85.
2. Parrotta J A Healing plants of Pennisular india. CABI publishing 2001;701-02.
3. K.N.V Rao et al. establishment of two varieties in tecoma stans ofindian origin pharmacolognostically and pharmacologically. J.Phytology 2010;2:92-102.
4. Khare.CP Indian medicinal plants and illustrated dictionary. Springer science publishers, new delhi.2007.
5. Kokate CK. A Text Book of Practical Pharmacognosy. 10 th ed. New Delhi: Vallabh Prakashan; 1994. p. 112-20.
6. Pathak S, Multani AS, Narayan S, Kumar V, Newman RA. Anvirzel, an extract of Nerium oleander, induces cell death in human but not murine cancer cells. Anticancer Drugs 2000;11:455-63.
7. Jayaraman J. Laboratory Manual in Biochemistry. 1 st ed. New Delhi: New Age International (p) Ltd.; 1981. p. 13.
8. Ghosh MN. Fundamentals of Experimental Pharmacology. 2 nd ed. Kolkata: Scientific Book Agency; 1984. p. 156.
9. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, et al. Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. Food Chem Toxicol 1995;33:223-31.

10. Al-Naggar TB, Gómez-Serranillos MP, Carretero ME, Villar AM. Neuropharmacological activity of Nigella sativa L. extracts. J Ethnopharmacol 2003;88:63-8.
11. Harbone JB. Phytochemical Methods. 2 nd ed. London: Academic Press; 1983.
12. Goetz RJ, Jordan TN, McCain W, Su J, Nancy Y. "Oleander". Indiana Plants Poisonous to Livestock and Pets. India: Cooperative Extension Service, Purdue University; 1998.
13. Mostaqul Huq M, Jabbar A, Rashid MA, Hasan CM. A novel antibacterial and cardiac steroid from the roots of Nerium oleander. Fitoterapia 1999;70:5-9.
14. Hadizadeh I, Peivastegan B, Kolahi M. Antifungal activity of nettle (Urtica dioica L.), colocynth (Citrullus colocynthis L. Schrad), oleander (Nerium oleander L.) and konar (Ziziphus spina-christi L.) extracts on plants pathogenic fungi. Pak J Biol Sci 2009;12:58-63.
15. Leewanich P, Tohda M, Matsumoto K, Subhadhirasakul S, Takayama H, Aimi N, et al. Behavioral studies on alkaloids extracted from the leaves of Hunteria zeylanica. Biol Pharm Bull 1996;19:394-9.
16. Satti AA, Edriss AE. Phytochemical analyses of three sudanese plants for their constituents of bioactive compounds. Int J Sci Nat 2013;4:169-73.
17. Vikas G, Payal M. Phytochemical and pharmacological potential of Nerium oleander: A review. IJPSR 2010;1:21-7.
18. Thippeswamy BS, Mishra B, Veerapur VP, Gupta G. Anxiolytic activity of Nymphaea alba Linn. in mice as experimental models of anxiety. Indian J Pharmacol 2011;43:50-5.
19. Kumar V. Neuropsychopharmacological studies on Indian Hypericum perforatum Linn. Ph.D. thesis. Varanasi: Banaras Hindu University; 2000.
20. Onaivi ES, Maguire PA, Tsai NF, Davies MF, Loew GH. Comparison of behavioral and central BDZ binding profile in three rat lines. Pharmacol Biochem Behav 1992;43:825-31.
21. Singhal KG, Gupta GD. Some central nervous system activities of Nerium oleander Linn (Kaner) flower extract. Trop J Pharm Res 2011;10:455.