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A study of the pharmacological actions of Dioclea grandiflora martius ex bentham

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Dioclea grandiflora Mart. ex Bent., known as Mucuna or Mucuna of stone, is a member of the Leguminosae family, popularly used for its possible effects on the Central Nervous System (CNS). Reports mention sedative, tonic, and also antiepileptic properties for this plant. In this paper a screening for CNS action was done with aqueous extracts of the seeds. This study was carried out using some animal models for the study of sedative, hypnotic, anxiolytic, anticonvulsivant and neuroleptic activity. The results showed that Dioclea grandiflora Mart. ex Bent. demonstrates the general profile of a CNS depressant drug. However, the extract did not exhibit any of the specific profiles tested. Moreover, the results suggest a possible toxicity stemming from the popular or folk use of this plant.

UNITERMS: Dioclea grandiflora. Mucuna. Pharmacologic screening.

INTRODUCTION

Various species of the Leguminosae family are frequently used in popular or folk remedies. Dioclea Grandiflora Mart. ex Bent. is a tall climbing plant, with leaves composed of three small oblong leaflets and large seeds. In Brazil it is found in the savannah or scrub of the states of Bahia, Ceará, Paraíba, and Pernambuco (Bentham, 1859 and Maxwell, 1969). It is also known as Mucuna or "Mucuna do caroço", and it is indicated as a folk remedy due to its possible effects on the central nervous system such as: tonic action, calmant and possibly antiepileptic action (Inventário de Plantas Medicinais BA, 1979). However, no systematic study has been made with the intent of confirming these actions. The objective of this paper was to study the pharmacological actions of aqueous extracts of the seeds of Dioclea grandiflora Mart. ex Bent. In order to do this, various specific tests were performed with animal models and the possible sedative, anticonvulsivant, neuroleptic, anxiolytic and hypnotic actions were determined.

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MATERIALS AND METHODS

Botanical Material- The seeds of Dioclea grandiflora Mart. ex Bent. were supplied by the Albano Martins Ltda. company of Sao Paulo, which had them brought from

Bahia state, and were later identified by Professor Luciano Paganucci de Queiroz, curator of the HUEFS herbarium of the State University of Feira de Santana, Bahia, who concluded that they were indeed seeds of the Dioclea grandiflora Mart. ex Bent (hereafter referred to simply as Dioclea grandiflora). In order to do this the shorter size of the hilum of the seeds was considered, this being a differing characteristic, as the other species exhibit a hilum more than 2/3 the circumference of the seed. This distiction is only valid for materials coming from the east of Brazil (Pernambuco to Parana).

Preparation and Liophilization- The seeds were ground using a knife grinder, resulting in a fine powder through tamis no. 180 as recommended by the Pharmacopeia of 1977. Based on non-official sources such as various folk recipes an extract was prepared containing 3.3 g. of powder in 150 ml. of boiling water agitated for 120 minutes. This extract was dried after filtering. The doses of the extract used in the experiments followed the criteria for a pharmcological screening, and represent aproximately 20, 50, 75, and 100 times the doses used by human beings, which is equivalent to 150, 342, 520, and 685 mg/kg respectively.

Animals- Male albino Swiss mice weighing between 30 and 40 g. and male Wistar rats weighing between 300 and 400 g., and 3 to 4 months old were used. These animals were raised in the vivarium of the Department of Psychobiology of the Escola Paulista de Medicina.

1. Preliminary Tests

Measurement of Motor Activity

5 groups of 10 house mice each received by via (i.p.) saline solution and different doses of the extract of Dioclea grandiflora, respectively of 150, 342, 520, and 685 mg/kg. The animals were then individually placed in boxes equipped with photo electric cells in order to register their movements for 60 minutes (Carlini, 1972).

Measurement of Motor Coordination

3 groups of 10 mice, each previously trained, received respectively, saline solution, Diazepam 2.5 mg/kg, and extract of Dioclea grandiflora in a dose of 685 mg/kg (i.p.). They were then subjected to a test by placing them on the spinning bar of a rota-rod apparatus, after 60, 120 and 180 minutes. The time each animal remained on the spinning bar was then evaluated (Carlini and Burgos, 1979).

2. Evaluation of the Anticonvulsivant Action

Prevention of Electroshock-induced Convulsion

3 groups of 10 mice received, respectively, saline solution, 20 mg/kg of diphenylhydantoin, and extract of 685 mg/kg Dioclea grandiflora (i.p.). Transcorneal electroshock (8 mA/0.2 seconds) was applied 120 minutes after the treatment, being then recorded the numbers of animals which exhibited tonic convulsions and the number of deaths.

Prevention of Pentylenetetrazol-induced Convulsions

4 groups of 10 mice each were treated with saline solution and 3 different doses (342, 520, and 685 mg/kg) of Dioclea grandiflora (i.p.). After 60 minutes 100 mg/kg of pentylenetetrazol was applied (s.c.). The latency times for clonic and tonic convulsions, and the number of deaths were then recorded (Vale and Leite, 1983).

3. Evaluation of Neuroleptic Action

Induction of Catatonia

2 groups of 10 mice each received, respectively, 5 mg/kg of a haloperidol drug (i.p.), and 685 mg/kg of the extract of Dioclea grandiflora (i.p.). The animals were then placed with their front paws supported by a horizontal bar and observed for tonic imobility and palpebral ptosis (Tufik et. cols., 1979)

4. Evaluation of Anxiolytic Action

Anti-pentylenetetrazol Effect

2 groups of 10 mice each received, respectively, saline solution and 685 mg/kg of the extract of Dioclea grandiflora. After 60 minutes the animals received 100 mg/kg of anticardiazol effect (s.c.), after which the latency time for clonic convulsions was observed and recorded.

Punished Response Test

10 rats previously deprived of water were conditioned, reinforcing the behavior of pressing a bar on a Skinner box with water. The animals were then subjected to the Geller and Seifter conflict test (1960), modified in accordance with Almeida and Leite (1990). The animals were trained, after being deprived of water for 20 hours, to perform bar-pressing behavior at a fixed ratio (FR-10) reinforcement scheme . When the animals attained a stable level of responses, obtaining continuously the reinforcement or reward, a luminous stimulus was

introduced, This way each time the animal pressed the bar it received the reward and the luminous signal was activated. After 5 sessions, a shock of 0.7 mA was released if the animal pressed the bar during the luminous signal. The behavior of pressing the bar was thus suppressed. The animals received, at three day intervals, saline solution, 2.0 mg/kg of diazepam or 685 mg/kg of the extract of Dioclea grandiflora (i.p.) one hour before being subjected to the test.

Evaluation of Hypnotic Action

Potentiation of Pentobarbitol-induced Sleep

4 groups of 10 mice each, received, respectively, doses of 150, 342, and 685 mg/kg (i.p.) of the extract of Dioclea grandiflora. The control group was treated by the same via of administration. After 60 minutes, the animals received a dose of 35 mg/kg of sodium pentobarbitol, and then the time between loss and recovery of balance reflexes was determined. This interval was considered to be the sleep time (Savaki et cols., 1976, Masur and Boerngen, 1980).

Statistical Analysis

The data were evaluated through variance analysis (ANOVA) followed by the Student's t-test.

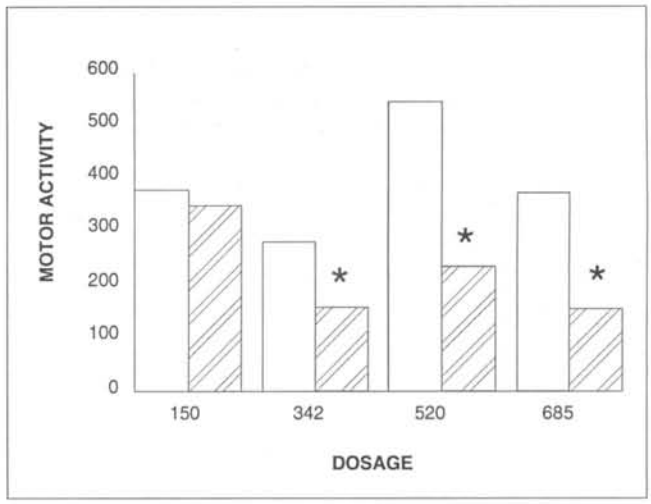

Figure 1 - Locomotor activity of control mice __ or those treated with extract of Dioclea grandiflora _ _ in doses of 150, 342, 520, and 685 mg/kg.
* Statistically different from the control group ($p<0.05$) (Student's t-Test).

Table I
Average times of mice remaining on a spinning bar during the rota-rod apparatus test, after being treated with diazepam or extract of Dioclea grandiflora.

Group	Dosage mg/kg	time on bar (seconds)
Saline sol.	-	60
Diazepam	2.5	28
Dioclea grand.	685	60

RESULTS

1. Preliminary tests

Measurement of Motor Activity

The results presented in figure 1 show that the extract of Dioclea grandiflora in doses of 150, 342, 520, and 685 mg/kg altered the locomotory activity in a manner proportional to the dose.

Measurement of Motor Coordination

The results presented in table I show that the extract of Dioclea grandiflora in a dose of 685 mg/kg does not produce an alteration in the performance of the animals after 60, 120, and 180 minutes.

2. Evaluation of Anticonvulsivant Action

Prevention of Electroshock-induced Convulsions

Table II shows that the extract of Dioclea grandiflora in a dose of 685 mg/kg does not exercise a protective action with regard to tonic convulsions. There is however a significant protection with regard to the percentage of deaths in relation to the control group.

Table II
The effect of Dioclea grandiflora on transcorneal electroshock-induced convulsions in mice (Average of 10 animals). *(p<0.05)(Student's t-Test).

Group	Dosage g/kg	Tonic convulsions %	Deaths %
Saline sol.	-	100	40
Dioclea grand.	685	100	0*

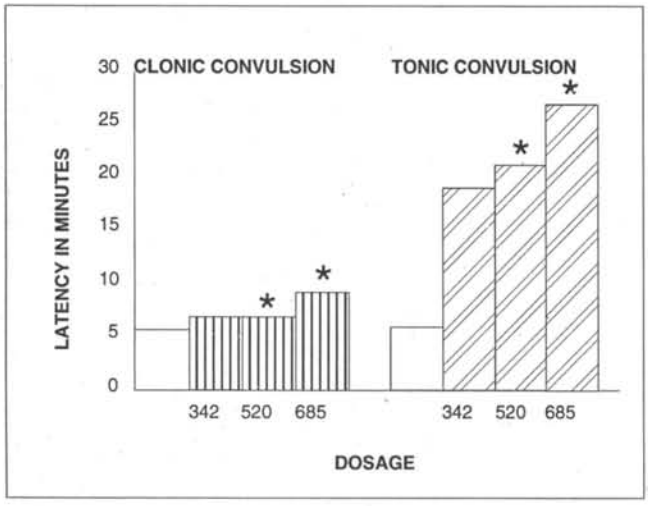

Figure 2 - The effects of different dosages (342, 520, or 685 mg/kg) of the extract of Dioclea grandiflora on pentylenetetrazol-induced convulsions. In the graph are shown the latency times for the beginning of clonic __ and tonic _ _ convulsions in treated mice and in control groups _._ (Average of 10 mice).
*Statistically different from control group (p<0.05) (Student's t-Test)

Prevention of Pentylenetetrazol-induced Convulsions

Figure 2 shows that the extract of Dioclea grandiflora in a dose of 342 mg/kg does not alter the latency for the manifestation of clonic or tonic convulsions. For doses of 520 and 685 mg/kg there is a significant increase in the latency for the manifestation of the two types of convulsions.

3. Evaluation of Neuroleptic Action

Induction of Catatonia

The data obtained show that the extract of Dioclea grandiflora does not induce catatonia even after four hours of treatment.

4. Evaluation of Anxiolyolytic Action

Anti-pentylenetetrazol Effect

The results obtained indicate that the extract of Dioclea grandiflora does not protect the animals from clonic convulsion induced by cardiazol.

Punished Response Test

The data in table III (total supression of response) indicate that the extract of Dioclea grandiflora in a dose of 685 mg/kg does not exhibit antipunishment action.

Table III
Average number of punished and non-punished responses in rats treated with diazepam or extract of Dioclea grandiflora, when subject to the conflict test.

Group	Dosage mg/kg	Non-punished responses	Punished responses
Saline sol.	-	261	14
Diazepam	20	251	32
Dioclea grand.	685	-	-

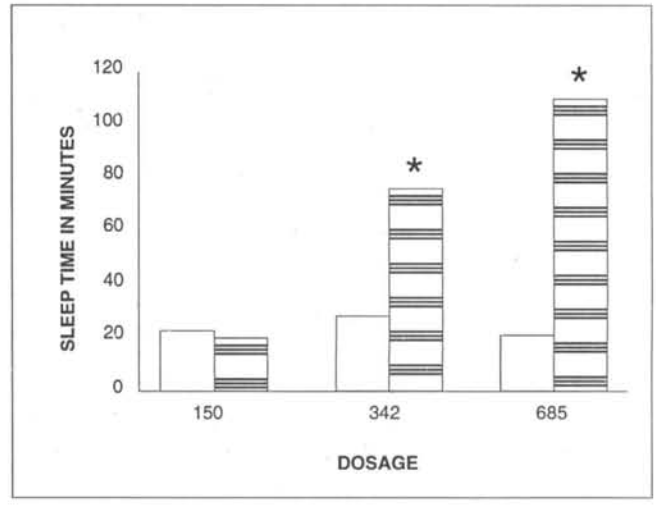

Figure 3 - Pentylenetetrazol-induced sleep in mice in a control group __ or in mice treated with extract of Dioclea grandiflora _ _, in doses of 150, 342 and 685 mg/kg (Average of 10 mice).
*Statistically different from control group (p<0.05) (Student's t-Test).

5. Evaluation of Hypnotic Action

Potentiation of Pentobarbitol-induced Sleep

The results presented in figure 3 indicate that the extract of Dioclea grandiflora in a dose of 150 mg/kg does not alter the time of sleep induced by pentobarbitol. However, in doses of 342 and 685 mg/kg the sleep time is potentiated by this hypnotic substance.

CONCLUSIONS

The initial studies of the action mechanisms of Dioclea grandiflora Mart. ex Bent indicate a probable depressant effect on the level of the central nervous system, with major or minor potency, which could be related to the quantity of the active principle or ingredient present in the preparations used. In determining the possible psychopharmacologic actions of the plant, with regard to folk use (Inventario, 1979), we observed that the therapeutic effects attributed to it were not confirmed through experiment. An anticonvulsivant action, which would justify its use as and antiepileptic, was not observed, although it should be noted that there was a significant protection with regard to the latency time of the manifestation of convulsion, as well as the protection shown with regard to the number of deaths in the animals tested. This fact, however, does not justify its use as an anticonvulsivant. In verifying the possible neuroleptic effect, we didn't obtain a positive response in the animals with regard to catatonia, a typical effect of neuroleptic substances. These observations were further confirmed in studying the anxiolytic effect, where again no evidence was found of a protective action with regard to clonic convulsion. We know that a drug with anxiolytic properties induces cessation of this type of convulsion in certain circumstances, as well as an increase in the number of responses to the conflict test. On the other hand, we saw that Dioclea grandiflora increased the sleep time in specific tests, the time obtained being proportional to the concentration used. It is not possible to conclude, however, that such a response is related to a hypnotic effect. On the other hand, this observation suggests a probable induction of a hepatotoxic effect. The possible toxicity of Dioclea grandiflora Mart. ex Bent. is further suggested by the total supression of responses in the conflict test. Data in the literature show that adverse reactions like dermatitis, palpitations, headache and hallucinations were observed in humans, after consuming seeds of Mucuna Pruriens (Bull. WHO, 1984, and MMRWR, 1985).

Our results indicate a possible medical warning or alert in regard to the therapeutic use of Dioclea grandiflora Mart. ex Bent., with further, more specific tests of toxicity being indicated in order for its use to be justified.

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This study is dedicated to Dr. Armando Silva Ramos Filho (in memoriam).

Resumo

Objetivo: A Dioclea grandiflora Mart. ex Bent., também conhecida como Mucunã ou Mucunã de caroço, é uma espécie da família Leguminosae que tem sido usada popularmente em decorrência de seus possíveis efeitos no SNC. Os relatos populares atribuem à planta ação tônica, calmante e possivelmente antiepiléptica. Com o objetivo de comprovar essas propriedades, procurou-se determinar com estudos em animais, em ações psicofarmacológicas utilizando-se para tanto, as sementes da planta.

Material e Métodos: Foram realizados testes preliminares: medida de atividade e coordenação motora, e avaliadas as ações anticonvulsivante, neuroléptica, ansiolítica e hipnótica.

Resultados e Conclusão: Os resultados sugerem que a Dioclea grandiflora Mart. ex Bent.. possui um perfil de droga depressora, não apresentando entretanto, as ações psicofarmacológicas esperadas. Os nossos dados sugerem ainda, um possível efeito tóxico o que representa um sinal de alerta quanto ao uso popular.

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