Anticonvulsant effect of *Indigofera suffruticosa* Mill: Indication of involvement of the GABAergic system

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This objective of this study was to evaluate the anticonvulsant effect of *Indigofera suffruticosa* through the methanol extract of its leaves. Swiss albino mice were subjected to dosages of 100 and 300 mg/kg methanol extract for the study of the anticonvulsant action; the following convulsants were used: pentylenetetrazole (100 mg/kg), picrotoxin (10 mg/kg), strychnine (2 mg/kg), and pilocarpine (600 mg/kg). In the evaluation, 2 mg/kg (ip) diazepam was used as the standard drug in all experimental models and GABA<sub>A</sub> receptor antagonist Ro 15-1788. The methanol extracts presented excellent anticonvulsant activity, increasing the duration of latency for the beginning of the first seizure in all the tests. The extract in a dose of 300 mg/kg showed its anticonvulsant effect similar to diazepam. The results showed an anticonvulsant activity of *I. suffruticosa* which can be, at least partly associated with involvement of GABA-BDZ system according to effect demonstrated with Ro 15-1788.

Key words: *Indigofera suffruticosa*, anticonvulsant activity, experimental models, Ro15-1788, mice.

INTRODUCTION

The use of medicinal plants through ethnopharmacological information shows a broad spectrum of action for the pharmacological study for the various diseases that affect humans (Nasri, 2012). Various diseases affecting humans were studied, as diseases of peripheral origin, such as those affecting the central nervous system, using several experimental models (Alam et al., 2012; Elisabetsky et al., 1999; Almeida et al., 2009). Furthermore, phytochemicals are used in identification of possible therapeutic agents present in plants (Asgarpanah and Ramezanloo, 2012).

Epilepsy is a neurological disorder known, affecting much of the population worldwide (Engel and Pedley, 1998; McNamara, 1999). The essential component of epilepsy is the manifestation of behavioral changes, called seizures classified according to their origins (Jones, 2002). The understanding of the pathophysiology of epilepsy has advanced considerably in recent years, especially in terms of pathophysiology and genetics (Engel and Pedley, 1998). Drug treatment also had a breakthrough with the introduction of new drugs in the 1980s, with improvement in clinical terms.

However, fell short of expectations, up to one third of the patients will continue to experience seizures or side effects related to medication, despite to appropriate the pathophysiology appropriate to receive treatment, without serious adverse side effects (Meldrum and Rogawski, 2007). Moreover, there is still a need to develop new drugs with improved efficacy and safety in patients with epilepsy (Kwan and Brodie, 2004). The herbal ethnopharmacology obtained through the information is
a crucial step in order to identify a plant extract, which is suitable for the treatment of epilepsy (Löcher and Schmidt, 2006). *Indigofera suffruticosa* commonly known as Anil, Guatemalan indigo, Small-leaved indigo (Sierra Leone), West Indian indigo, and wild indigo is a flowering plant in the pea family Fabaceae. Anil is common to the subtropical and tropical Americas, including the Southern United States, the Caribbean, Mexico, Central America, and South America as far South as Northern Argentina (Corrêa, 1969).

These species have been introduced to other parts of the world, and today has a pantropical distribution (Acevedo-Rodriguez et al., 1996). The leaves Anil commonly used as a source for indigo dye, and if mixed with small clays can produce Maya Blue or Azul Maya, a pigment used by the Mesoamerican civilizations. *Indigofera* other species present in their characteristic feature presentation of indigo carmine, such as *Indigofera aspalathoides* M. Vahl ex DC. arrecta Hochst. ex A. Rich., *Indigofera articulata* Gouan, *I. tinctoria* L. In folk medicine of Brazil, the leaves are used as diuretic, antispasmodic, sedative and anti-inflammatory agent (Leite et al., 2003). Embryotoxic effects found as well as an antimicrobial activity (Leite et al., 2004a, b) and a central nervous system (CNS) depressant action using the fluid extract of *I. suffruticosa* (Alejo et al., 1996 a, b).

Methanol extract (ME) is rich in indotin (2, 2'-bis (2, 3-dihydro-3-oxindolilideno) which is useful for coloring of blue.

This study aims to evaluate the anticonvulsant effect of ME of dried leaves of *I. suffruticosa*.

**MATERIALS AND METHODS**

**Plant**

The leaves of *I. suffruticosa* (ME) were collected in the arid region of Pernambuco State, Brazil. The sample were identified and authenticated by Dr. Marlene Carvalho Herbarium of the Biological Sciences Center of Federal University of Pernambuco and a voucher specimen was deposited under number 23076.006792-2008-11

**Animals**

Adult male Swiss mice weighing 25 to 35 g were used. The animals were maintained in a standardized laboratory conditions 22°C, 40 to 50% relative humidity. The mice were maintained in a standardized dark light cycle lights on between 06.00 and 18.00 h. Standard mice pellets and tap water were available ad libitum up to start of the experiments. The experiments were carried out between 08.00 am and 03:00 pm.

All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the Brazilian College of Animal Experimentation (COBEA) and the National Institute of Health Guide for Care and use of laboratory animals and approved by the ethical Committee of the Federal University of Pernambuco (UFPE) protocol number 0086207/2010-37.

**Extract preparation**

In obtaining the ME, 500 g of dry material used were previously ground in a forage machine. The extraction was performed using solvents of increasing polarity (hexane, ethyl acetate and methanol). First, we used the hexane at 40°C for 10 min and the solid residue was removed by filtration.

Ethyl acetate was added to the resulting solid. After 24 h of rest, the material was heated and filtered again. To the new solid separated material, the last solvent, methanol was added. After 24 h, methanol was filtered and removed on rotavapor. The material was lyophilized resulting in a yield of 25 g of dark-viscous material that was carefully sealed and stored at -20°C.

**Preliminary phytochemical analysis**

The aqueous, methanolic, hexane and ethyl acetate extracts of the leaves of *I. suffruticosa* obtained by infusion were analyzed by qualitative method (thin layer chromatography on silica gel/UV detection at 365 nm) for the presence of alkaloids, iridoids, saponins, carbohydrates, coumarins, flavonoids, phenol, terpenoids, and indigo carmine, sterol and essentials oils: linalool and pinene. It is extraction was performed by hydrodistillation procedure (Harborne, 1998; Matos, 1988; Galhiane et al., 2006).

**Drugs**

Pentylenetetrazole (PTZ), picrotxin (Picrot), strychnine (Stry), pilocarpine (Piloc), diazepam (DZP), flumazenil (5 mg/kg), and Tween 80 (polyoxyethylene-sorbitan monolate) were purchased from Sigma (USA). In the protocol, the agents were administered intraperitoneally (ip) dose of 0.1 ml/10 g. After the experiments, the animals were sacrificed with an overdose of thiopental and sent for incineration.

**Determination of the lethal dose 50% (LD50)**

The determination of the LD50 was performed using doses of 100, 300, 600, 900 and 2000 mg/kg (ip) in mice by intraperitoneal route (Litchfield and Wilcoxon, 1949).

**Pentylenetetrazol-induced seizures**

In this study, mice used (n = 10) were treated with ME (100 and 300 mg/kg, ip) intraperitoneally in saline + 0.9% Tween 80 solution (0.2%, ip). The positive control was treated with DZP (2 mg/kg, ip). After 60 min of drug administration, the mice were treated with PTZ at a dose of 100 mg/kg (ip) and observed for the first phase of forelimb clonus and the time before the beginning of clonic seizures. The incidence of mortality was noted until 24 h after the injection of PTZ (Kaputlu and Uzbay, 1997).

The effects of the critical GABA<sub>A</sub>-BZD receptor antagonist was also studied, Ro 15-1788 5 mg/kg (i.p.), the anticonvulsant activity of ME was investigated with the system involved in GABA-BDZ. One group with ten mice received Ro 15-1788 5 min before the administration of DZP (2 mg/kg, i.p.). The anticonvulsant activity of DZP and ME in mice pretreated with Ro 15-1788 were assessed and compared with the controls (File and Pellow, 1998).

**Picrotoxin-induced seizures**

The animals were divided into five groups (n = 10). The first group served as control and received saline (0.9%) and with one drop of
Figure 1. Pentylenetetrazol-induced convulsion effect of ME. ME inhibited generalized clonic-tonic convulsions induced by PTZ (100 mg/kg, ip) at doses of 100 and 300 mg/kg (*p<0.5), as in accordance with statistical analysis, using analysis of variance one-way (ANOVA) and followed by a post hoc Duncan’s test. Saline was used in dilution of PTZ.

Figure 2. Effect of Pentylenetetrazol (PTZ) on ME after Ro 15-1788 suppression of seizures evaluated with the ME and DPZ. All data cited are the mean ± S.D of each group (n=10). The data was evaluated by a one-way variance analysis (ANOVA) followed by Turkey test. * p < 0.01.
cremophor while the second group was treated with DZP (2 mg/kg ip).

The remaining groups received a dose of ME, similar to the PTZ test. After 60 min of drug administration, the mice were treated with picrotoxin at a dose of 10 mg/kg (ip). Immediately after the injection of the convulsant drug, mice were individually placed in plastic boxes and observed for the time arrival of clonic seizures (latency), clonic seizures. The incidence of death was not observed until 24 h after injection of picrotoxin. DZP was used as a positive control (Susan et al., 1989).

**Strychnine-induced seizures**

The animals were divided into five groups (n = 10). The first group served as control and received saline (0.9%) with one drop of cremophor while the second group was treated with DZP (2 mg/kg, ip). The remaining groups received a dose of ME. After 60 min of drug administration, the mice were treated with strychnine at a dose of 10 mg/kg (ip). Immediately after the injection of the convulsant drug, mice were individually placed in plastic boxes and observed for the time arrival of tonic-clonic seizures.

The incidence of death was observed until 24 h after injection of strychnine (20%). DZP was used as a positive control (Kaputlu and Uzbay, 1997).

**Pilocarpine-induced seizures**

Animals were divided into five groups (n = 10). The first group served as control and received saline (0.9%) with one drop of cremophor, while the second group was treated with DZP (2 mg/kg, ip).

The remaining groups received a dose of ME, similar to the PTZ test. After 60 min of drug administration, the mice were treated with pilocarpine at a dose of 600 mg/kg (ip). Immediately after the injection of the convulsant drug, mice were individually placed in plastic boxes and observed for the time arrival of clonic seizures (latency), clonic seizures. The incidence of death was not observed until 24 h after injection of pilocarpine. DZP was used as a positive control (Graciela et al., 2009).

**Statistical analysis**

Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Duncan's test p<0.5 considered significant. All data were expressed as mean ± standard deviation (SD) using the program Graph Pad Prism, version 5.00.

**RESULTS**

**LD50**

The extract showed a low toxicity with an LD50 of 1600 mg/kg (ip) in mice. The therapeutic dose of the extract was based on the percentage of 10 to 40% of LD50.

**Identification of compounds in the ME of I. suffruticosa**

ME showed the presence of alkaloids, flavonoids, steroids, proteins, carbohydrates, indigo carmine and essential oils (Linalool and Pinene) according to the method used.
Figure 4. Effect of Strychnine (Stry) induced convulsion was evaluated with the ME. All data cited are the mean ± SD, of each group (n=10). The data were evaluated by a one-way variance analysis (ANOVA) followed by Turkey test. *p < 0.01.

Figure 5. Effect of Pilocarpine induced convulsion was evaluated with the ME. All data cited are the mean ± SD of each group (n=10). The data were evaluated by a one-way variance analysis (ANOVA) followed by Turkey test (*p<0.01).

DISCUSSION

The results presented here show that ME exhibits an LD50 of 1600 mg/kg (ip) in mice and did not induce significant changes in individual behavioral and physiological parameters and showed a slight decrease in spontaneous locomotor activity and an increase in breathing frequency (data not shown).
In the phytochemical analysis conducted by us, ME showed the presence of alkaloids, flavonoids, steroids, proteins, carbohydrates, indigo carmine and essential oils (linalool and Pinene). The seizures induced by pentylenetetrazole acts through the chloride channel of the GABAergic system (Meldrum, 2002). ME produced protection against PTZ-induced seizures (p<0.01). Picrotoxin acts as a competitive antagonist for the receptor for GABA chloride channel. Infusions of picrotoxin have stimulating effects and cause seizures. High-dose pilocarpine induced seizures in rats after systemic administration and/or intracerebral (Lechoslaw et al., 1989). The picrotoxin-induced seizure was protected by ME in doses of 100 and 300 mg/kg promoting prevention of seizures.

Strychnine-sensitive glycine receptors located chiefly in the brainstem and spinal cord is the first mediators of synaptic inhibition. ME promoted inhibition of seizures at doses of 100 and 300 mg/kg (*p < 0.05). Treatment with high dose of pilocarpine hydrochloride, a muscarinic cholinergic agonist, induces convulsions in rodents after intracerebral or systemic administration. The amygdala, thalamus, olfactory cortex, hippocampus, neocortex and substantia nigra are the most sensitive regions to epilepsy-related damage after seizures produced by pilocarpine (Tuski et al., 1989; Andre et al., 2009). ME caused block seizures induced by pilocarpine in doses of 100 and 300 mg/kg (*p < 0.01). Diazepam used on all models served as an anticonvulsant.

The identification of flavonoids and essential oils (linalool) of the ME is probably a strong indication that these compounds involved in the anticonvulsant activity of ME (Almeida et al., 2009; Ibrahim et al., 2012; Rasilingam et al., 2009; Elisabetsky, 1995 a, b, c). A significant observation was the effect of Ro 15-1788 on the PTZ and ME (300 mg/kg, ip) in mice (Albertson and Walby, 1986; Darragh et al., 1983; Susan et al., 1989). During the process seizure occurs an increase of reactive oxygen species (ROS) promoting cell damage (Packer, 1997; Evans, 2000).

Flavonoids have the ability to inhibit the formation of ROS. They are large molecules with antioxidant power, which can act in concert with other endogenous antioxidant systems in neuronal cells (Pietta, 2000). Moreover, previous studies have shown the antioxidant activity of flavonoids, in particular during induction of convulsion pilocarpine and other agents (Pietta, 2000).

The presence of linalool can contribute to the inhibition of reactive oxygen species, by inhibiting their formation in neuronal cells in addition to their probable action on the GABA receptor system.

**Conclusion**

The results showed that anticonvulsant activity of ME could be associated with the GABAergic system involvement in the presence of flavonoids, and linalool.

The observations were based on the literature on the action Ro 15-1788 on the GABAergic system. This study was able to increase the knowledge about the use of this plant as a CNS depressant. Probably, an influence on the GABA-BDZ receptor complex.

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**REFERENCES**


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