ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *ALTERNANTHERA BRASILIANA* KUNTZ. IN ANIMAL MODELS OF NOCICEPTION

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Summary

Methanolic extract of leaves of *Alternanthera brasiliana* Kuntz. was studied for its antinociceptive activity in both peripheral and non-narcotic models like acetic acid induced writhing syndrome test and narcotic analgesic models like tail flick and hot plate test. The methanolic extract of *Alternanthera brasiliana* when administered @ 300, 600 and 900mg/ kg body wt. orally, produced significant (P<0.01) analgesic activity in acetic acid induced writhing syndrome. In the hot plate analgesic test in albino mice, *A. brasiliana* treated (@ 300, 600 and 900 mg/ kg body wt.) and the standard drug (Morphine @ 1.5 mg/ kg) treated group, the duration of reaction time (in sec) increased dose dependently and was significantly (P< 0.01) higher. In the tail flick test, the plant extract produced dose dependant increase in reaction time and was also significantly higher (P<0.01) compared to the control group.

The plant possessed significant antinociceptive activity as evidenced in all the animal models of nociception. It might possibly exert its effect through diverse mechanism that may involve both central and peripheral pathways. The preliminary phytochemical investigation revealed the presence of steroids, alkaloids and triterpene in the methanolic extract of leaves of *A. brasiliana* which might be responsible for its antinociceptive activity.

Keywords: *Alternanthera brasiliana*, hot plate, nociception, tail flick test, writhing syndrome.

Introduction

Synthetic drugs are very expensive to develop since for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested, the cost of which ranges from 0.5 to 5 million dollars [1]. About 70-80% of the world's populations rely on non-conventional medicine mainly of herbal sources in their primary health care according to a WHO report. A medicinal plant is factually any plant which in one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of direct therapeutic agents [2]. Medicinal plants are important elements of traditional medicine in virtually all cultures. The idea that certain plants had healing potential was known long before human beings discovered the existence of pathogens [3].

Alternanthera brasiliana Kuntz (Amaranthaceae), an herbaceous plant commonly known in Brazil as Penicillin or Brazilian Joy Weed is a neotropical native species, which grows easily on poor and deforested soil. The plant is used against inflammation, cough and diarrhea in Brazilian popular medicine [4]. The extracts of *A. brasiliana* exhibited antinociceptive effects in mice [5], antimicrobial effect [6] and also anti-herpes simplex virus activity [7]. Aqueous or ethanolic extracts of *A. brasiliana* are able to block human mitogen induced lymphocyte proliferation without any toxic effect [8].

Most of the drugs used at present for analgesic effect are synthetic in nature, prolonged use of which cause many side and toxic effects like respiratory depression, constipation, kidney damage, physical dependence as well as gastrointestinal irritation. As these drugs are not commonly available to the rural folks that constitute the major populace of the world, it is therefore essential that effort should be made to introduce new medicinal plants to develop cheaper drugs. The study, therefore seeks to assess methanolic extract of the leaves of *Alternanthera brasiliana* for analgesic activity in different experimental animal models.

Materials and methods

Plant material

The leaves of the plants were collected from the medicinal garden of the Department of Pharmacology, College of Veterinary Science, Khanapara during the month of Feb - June, 2008. it was identified by Taxonomist of NEIST, Jorhat, Assam and a voucher specimen (AAU/CVSC/PHT/ 02) was deposited.

Preparation of methanol extract

Fresh leaves of the plant were cleaned from extraneous materials, washed, shade dried, powdered mechanically, weighed and stored in air tight container. About 250 g of powdered material was soaked in 1000 ml methanol for 72 hours in beaker and mixture was stirred every 18 hour using a sterile glass rod. Filtrate was obtained 3 times with the help of Whatman filter paper no 1 and the solvent was removed by rotary evaporator (Roteva, Equitron, Medica Instrument Mfg. Co.) under reduced pressure at $<45^0$ C temperature leaving a dark brown residue. It was stored in air tight container at 4^0 C until use. Recovery was 6.12 % (w/w).

Phytochemical screening

The methanolic extract of *A. brasiliana* was subjected to preliminary, qualitative phytochemical investigations for the presence of various active principles as per standard method [9].

Determination of LD_{50}

The LD_{50} of *A. brasiliana* was estimated by the employment of up-and-down stair case method in mice [10]. Doses were adjusted by a constant multiplicative factor viz. 4, for this experiment. The dose for each successive animal was adjusted up and down depending on the previous outcome. The acute toxicity and gross effect of crude methanolic extract of *A. brasiliana* was studied in albino mice by using 1/2 LD_{50} dose. A total of six numbers of male albino mice were selected for the experiment. Animals were observed hourly for six hours and again after 24 hours. The parameters for motor activity and gross effect were determined after administration of *A. brasiliana* orally at a dose of 2.5g /kg b. wt.

Chemicals

Morphine sulphate was purchased from Sigma (Poole, UK). Methanol, Acetic acid and Formalin were purchased from Merck Limited (Mumbai- 400 018).

Animals

Healthy adult albino mice of either sex, approximately of same age, weighing between 25-30 g and adult male albino rats weighing between 180-200 g were used for the study. The animals were group housed in polypropylene cages under controlled conditions of temperature (21±2^{0}C), humidity (50±5%), 12/12 hours of light-dark cycle and free access to standard food pellets, water was provided *ad libitum*. All efforts were made to minimize animal suffering and to reduce the number of animal used. The animals were fasted for 14 hours before test to achieve better drug absorption through gastrointestinal tract.

The animals were randomly allocated into five groups of six animals each. Group I served as vehicle control, group II, III and IV received *A. brasiliana* at the dose rate of 300, 600 and 900 mg/kg body weight orally and group V received the standard drug Dolonex D. T. @ 10 mg/ kg body wt., orally in acetic acid induced writhing test and morphine sulphate @ 1.5mg/kg body wt., intra-peritoneally in hot plate and tail flick tests.The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee.

Acetic acid induced writhing test

The intraperitoneal injection of acetic acid results in constriction of abdominal muscle together with stretching of hind limbs known as writhing syndrome. The antinociceptive activity of crude methanolic extract of *A. brasiliana* leaves was studied on chemically induced pain sensation in female non- pregnant albino mice [11]. Methanolic extract of *A. brasiliana* @300, 600 or 900 mg/ kg body wt., Dolonex D. T. @10 mg/ kg body wt. or the vehicle was administered orally 30 minutes prior to intra peritoneal injection of acetic acid (10 ml/ kg of 0.7% v/v solution). Total numbers of stretching episodes for 20 minutes immediately after acetic acid injection in all the groups were recorded and anti-nociception was expressed as the percent reduction in writhing numbers compared between the vehicle treated control and animals pretreated with methanolic extract of *A. brasiliana* or Dolonex D. T.

Screening for analgesic activity of by Eddy's hot plate

In the hot plate test [12], mice of either sex were placed on the hot plate (Rolex) maintained at 55± 0.5^{0}C. The time between placement on the hot plate and the occurrence of licking of the paws, shaking or jumping off from the plate was recorded as response latency. Mice with basal latency of more than 10 sec were not included in the study. The response latencies or reaction time was measured before administration (Basal) and at 30 and 60 min after administration of methanolic extract of *A. brasiliana* (@300, 600 or 900 mg/ kg body wt p.o.), morphine sulphate (1.5mg/kg i.p.) or the vehicle control. A cut-off reaction time was fixed at 20 sec to avoid damage to the paws.

Screening of analgesic activity of by Tail flick method

In this model, Nichrome wire analgesiometer (Rolex) was used. Individually, the tail of rats was placed over the hot wire of the apparatus and the time when the tail is withdrawn was recorded [13]. Five groups of rats (N = 6) were taken. Group I served as vehicle control, group II, III and IV received methanol extract orally at the dose rate of 300, 600 and 900 mg/kg body weight, respectively. Group V received the standard drug morphine sulphate at the dose of 1.5 mg/kg body wt (i.p.). The reaction time of each rat in each group was determined at 0, 30 and 60 minutes following administration of the test compound or the standard drug and compared with the control group.

Statistical analysis

The results of various parameters were subjected to statistical analysis as per standard statistical method [14].

Results

Phytochemical screening of the methanolic extract of the plant revealed the presence of alkaloid by Wagner's and Dragendroff's test, steroid by Salkowski's and Lieberman Burchardt's test and triterpenes by Salkowski's and Lieberman Burchardt's test. The plant extract was found to be safe up to 5gm/kg body weight, p.o. No acute toxicity was observed at 2.5 g/kg body wt.

The effects of methanolic extract of *Alternanthera brasiliana* on the reaction time in acetic acid induced writhing syndrome test in mice at the dose rate of 300, 600 and 900 mg/kg body weight after single oral administration is presented in Fig 1. The reaction time in hot plate test in mice at different dose rates, after single oral administration is shown in Fig 2. The result of tail flick test in rats after single oral administration of the plant extract at different dose rates is depicted in Fig 3.

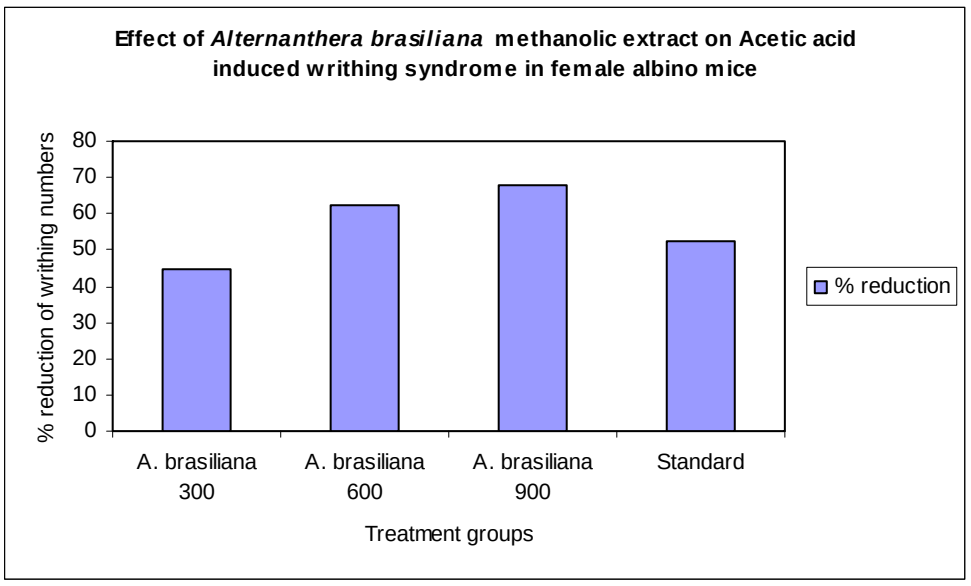

Fig 1. Effect of methanolic extract of leaves of *Alternanthera brasiliana* and the standard drug (Dolonex D. T.) on percent reduction in writhing numbers in acetic acid induced writhing syndrome in female albino mice.

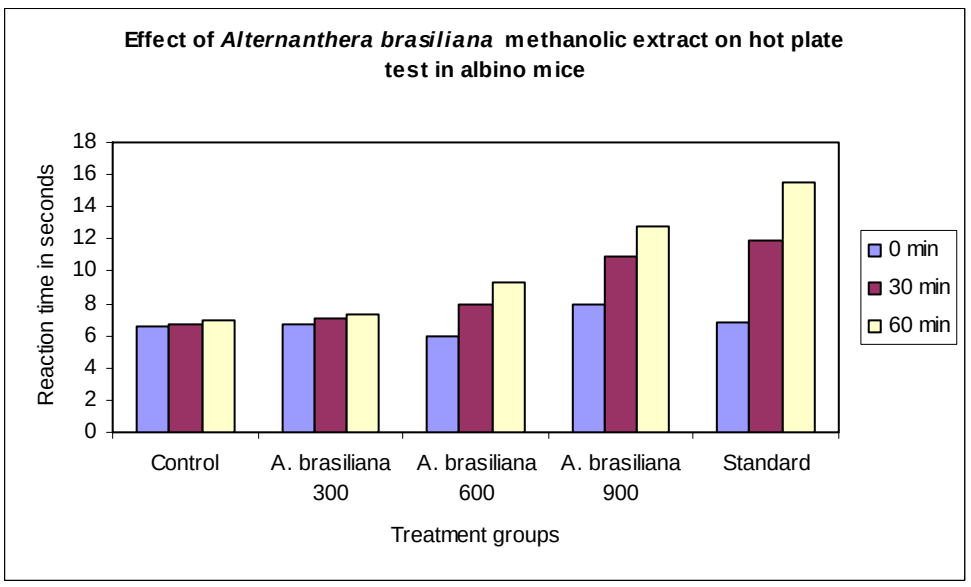

Fig 2. Effect of methanolic extract of leaves of *Alternanthera brasiliana* and the standard drug (Morphine sulphate) on the reaction time in hot plate test in albino mice.

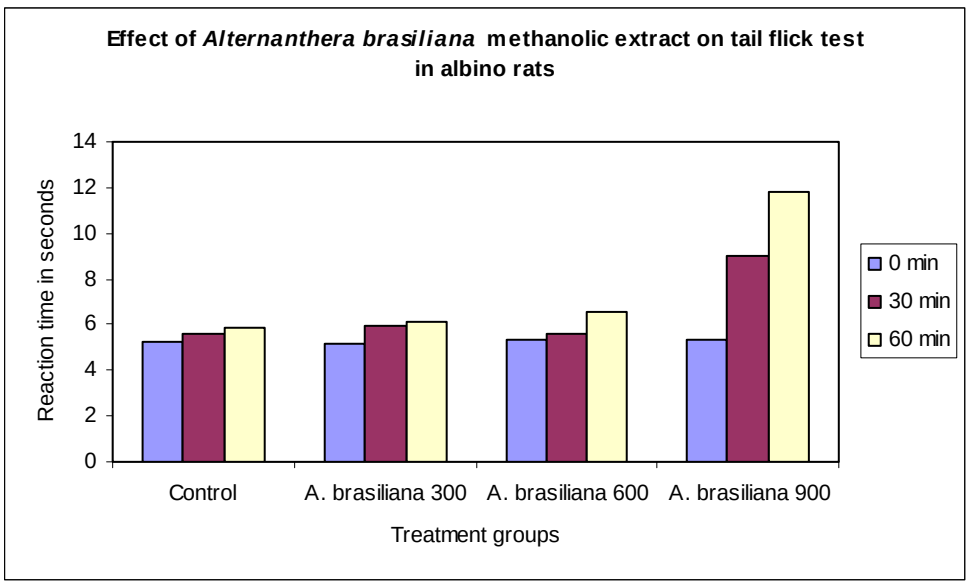

Fig 3. Effect of methanolic extract of leaves of *Alternanthera brasiliana* on the reaction time tail flick test in rats.

Discussion

In this study, the antinociceptive effect of methanolic extract of the leaves of *Alternanthera brasiliana* was evaluated in different experimental models of pain *viz*. non-narcotic model like acetic acid induced writhing syndrome test and narcotic models like hot plate and tail flick test. The low toxicity of the plant observed in this study suggests that the plant extract is relatively safe for consumption and did not affect any of the parameters measured.In the acetic acid induced writhing syndrome test, the percent reduction of writhing number ranged from 44.91% to 67.73% in *A. brasiliana* treated groups during the 20 minutes of observation period, whereas, in case of the standard drug Dolonex D. T. there was 52.66% reduction of the writhing numbers, indicating better analgesic activity of *A. brasiliana* in acetic acid induced writhing syndrome test.

In the hot plate analgesic test in albino mice, in *A. brasiliana* treated (@ 300, 600 and 900 mg/ kg body wt.) as well as the standard group (morphine @ 1.5 mg/ kg), the duration of reaction time (in sec) increased significantly and dose dependently at 60 min. However, in the control group the duration of reaction time did not increase. Reaction time at 60 min of observation in *A. brasiliana* (@ 600 mg/ kg (9.267 sec), *A. brasiliana* (@ 900 mg/ kg (12.737 sec) as well as morphine @1.5 mg/kg (15.568 sec) treated groups was significantly ($P< 0.01$) higher compared to the control group (6.895 sec), indicating dose dependant antinociceptive activity of the test plant.

In the tail flick test, the reaction time increased significantly ($P< 0.01$) and dose dependently when *A. brasiliana* was administered. However, in the control group, there was no significant increase in the reaction time. At higher dose, the duration of reaction time was more compared to the lower dose, indicating dose dependant analgesic activity of *A. brasiliana*.

Acetic acid causes inflammatory pain by inducing capillary permeability [15] and liberating endogenous substances that excite pain nerve endings [16]. The intensity of the antinociceptive effect of methanolic extract of *A. brasiliana* @ 600 and 900 mg/ kg was higher to that of the standard drug (Dolonex D.T.) in acetic acid induced abdominal constrictions in mice. Non steroid anti-inflammatory drugs (NSAIDs) can inhibit COX in peripheral tissues and therefore, interfere with the mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic effect of methanolic extract of leaves of *A. brasiliana* could be probably due to blocade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Dolonex D.T. and other NSAIDs. Antinociceptive effect of *A. brasiliana* was also reported by Macedo and his co-workers in mice [5].

The hot plate test is considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species [17]. The methanolic extract of *Alternanthera brasiliana* had antinociceptive activity in hot plate test that may be in part mediated by opioid receptors.

The hot plate and tail flick are the most common tests of nociception that are based on a phasic stimulus of high intensity. The nociceptive experience is short lasting and it is well accepted that agonists of mu-opioid receptors produce analgesia in acute pain models [18]. Therefore, it is believed that substances that are effective in tail flick exert their effects predominantly through mu-opioid receptors. These findings indicate that the methanolic extract may extort sufficiently opioid- like compounds out of the plant which are responsible for its analgesic activity.

Acetyl-11-keto-beta-boswellic acid (AKBA) is a pentacyclic triterpenic acid present in the acidic extract of the *Boswellia serrata* gum resin, is a novel highly specific inhibitor of 5-lipoxygenase, the key enzyme for leukotriene biosynthesis. Leukotriene as well as peptide- leukotrienes results in an increase in vascular permeability and chemo taxis of polymorphonuclear leucocytes as well as release of mediators from leucocytes, which sensitize nociceptors [19, 20]. As the plant under study also contain triterpene as one of its phytoconstituent, so it may act through inhibition of leukotriene biosynthesis. The presence of alkaloid in the plant extract supports the claim that this compound have antinociceptive property since, alkaloid, flavonoids and saponins have been found in other natural products with analgesic and anti-inflammatory properties [21, 22]. It may also be related partly to the presence of steroids that have been shown to exert analgesic effects in animal models of nociception [23, 24].

In the present investigation, methanolic extract of leaves of *Alternanthera brasiliana* was studied for its nociceptive activity in both peripheral (acetic acid induced writhing syndrome) and central algesic models (tail flick method and hot plate test). The plant extract administered orally exhibited antinociceptive activity in all the animal models of nociception and might possibly exert its effect through diverse mechanism that may involve both central and peripheral pathways. Present data supports the traditional use of *Alternanthera brasiliana* as an analgesic. Further pharmacodynamic investigations are required to understand the precise mechanism of antinociception exhibited by the methanolic extract of leaves of *Alternanthera brasiliana*.

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