Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 1836-1839
Received: 05-05-2018
Accepted: 10-06-2018

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Analgesic activity of hydroethanolic extract of *Alternanthera sessilis* in mice

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Abstract
The present study was conducted to evaluate the analgesic property of hydroethanolic extract of *Alternanthera sessilis* in mice. Hydroethanolic extract of *Alternanthera sessilis* was screened for its analgesic activity by using chemical method (Acetic acid induced writhing) for its peripheral antinociceptive activity and Eddy's hot plate method for its central antinociceptive property at two dose levels (250 mg/kg body weight and 500 mg/kg body weight). The hydroethanolic extract of *Alternanthera sessilis* was found to have analgesic property with varying potencies in Chemical method and Hot plate method. The result was found to be significant in both the methods.

Keywords: *Alternanthera sessilis,* antinociceptive, eddy's hot plate, chemical method

Introduction
Pain is a disabling and unpleasing accompaniment of many diseases and pain management is among the important therapeutic priorities [1]. Pain can be defined as an unpleasant emotional and sensory experience associated with potential tissue damage [2]. Analgesics are the drugs that are used for the treatment or for the reduction of pain. Pain induction mainly either by peripheral mechanisms by the induction of prostanoids, leucotrienes or by centrally modulating agents that acts through various receptors such as serotonergic, dopaminergic, opiates etc.
Based on these therefore, there is the need for the search for bioactive compounds from natural products especially from medicinal plants for use as alternative analgesics with little or no side effects.
Alternanthera sessilis (L.) R.Br. ex DC. (Family: Amaranthaceae) is an aquatic plant commonly grows in many Asian countries including India, Nepal, Sri Lanka, Malaysia, Indonesia, China, and Taiwan [3].
A. sessilis is consumed as vegetable, and also occasionally cultivated to be used as herbal medicine. Traditionally, the leaves of *A. sessilis* are used in eye diseases, skin diseases, wound healing and as an antidote for snake bite [4]. In South Asian countries, the plant is used in the treatment of rheumatism and painful swellings associated with wounds. Decoction of *A. sessilis* is used to cure pain and intestinal inflammation [5]. In some parts of India, poultice of pounded fresh material is used for cure of sprains, burns, anti-inflammatory and eczema [6]. The plant is also used in the treatment of malaria, diarrhoea, dysentery, post-natal complaints, night blindness, and helminthiasis, anthelmintic activity [3].
The plant is found to have diuretic [7], haematinic [8], antioxidant [9], cytotoxic [10], antipyretic [11], hepatoprotective [12], antiulcer [13], antimicrobial, and wound healing [14] properties. The herb is also reported as febrifuge, galactagogue, abortificient, and used in the treatment of indigestion [15]. The plant is reported to contain lupeol, α and β-spinasterol, β-sitosterol, stigmasterol, and campesterol [16, 17].

Materials and Methods
Experimental animals
48 mice of either sex were taken for the experiment. All the animals were kept in the clean polypropylene cages in a small group of 6 rats/cage. A total no of 20 mice of either sex of 22-25 grams were taken for the acute toxicity studies. All the animals were given balanced ration and drinking water ad libitum and were maintained in a standard laboratory condition of (12:12 day and night cycle at an ambient temperature of 22-25˚°C. An acclimatization period of 7 days was given to all the animals before they were subjected to the investigation.

Collection and Identification of plants
Whole plant of *Alternanthera sessilis* was collected from the village area of Kamrup district, Assam. A herbarium specimen was submitted to the regional office, Botanical Survey of India (BSI), Shillong, for authentication. The BSI, authenticated the plant with the letter reference No. BSI/ERC/2016/Plant identification/675.

Processing and Preparation of extract
The leaves of the plant were washed, shade dried, finely ground and stored in air tight container. For preparing hydroethanolic extract, 100 grams of powered leaves of *A. sessilis* were soaked in 70% ethanol, and then kept up to 4 days with intermittent stirring for maximum extraction. After four days the content was filtered with muslin cloth, followed by whatmann filter paper no 1. The extract obtained then further subjected for evaporation at 60◦C with intermittent stirring in hot water bath for a period of 24 hours.

Acute toxicity test
Study for Acute toxicity was carried out as per OECD guidelines 425. Acute toxicity of hydroethanolic extract of leaves of *Alternanthera sessilis* was carried out using groups of six Swiss albino mice by administering a dose 2000 mg/kg, p.o., while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h.

Phytochemistry
Phytochemical tests were carried out with the leaves of hydroethanolic extract of *Alternanthera sessilis* according to standard procedure [18].

Design of the experiment
Analgesic activity of the leaves of the Alternanthera sessilis was estimated by Eddy's hot plate method and by acetic acid induced writhing reflex.

Eddy's hot plate method
Before 7 days of the conducting the experiment, the suitable animals were screened for this test. The main objective of screening the animals were to isolate the animals who are having almost equal response time.
Animals were divided into 4 groups with 6 animals per group. Group I was treated as control. Group II was treated with hydroethanolic extract of leaves of *Alternanthera sessilis* at a dose rate of 250 mg/kg body weight. Group III was treated with hydroethanolic extract of leaves of *Alternanthera sessilis* at a dose rate of 500 mg/kg body weight. Group IV was treated with the standard drug Morphine at a dose rate of 0.50 mg/kg body weight.
Woolfe and MacDonald (1944) originally developed this method [19]. The paws of mice and rats are very sensitive to heat at mild temperature. The endpoint taken is in the form of jumping, withdrawal of the paws or the licking of the paws [20-24]. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5 ◦C. A period of 15 s was kept as cut off period to avoid damage to the paw. Type of response and reaction time were noted using a stopwatch in 0 min, 15 min, 30 min, and 60 minutes. Control rats were treated with normal saline.

Acetic acid induced writhing response
Animals were divided into 4 groups with 6 animals per group. Group I was treated as control. Group II was treated with hydroethanolic extract of leaves of *Alternanthera sessilis* at a dose rate of 250 mg/kg body weight. Group III was treated with hydroethanolic extract of leaves of *Alternanthera sessilis* at a dose rate of 500 mg/kg body weight. Group IV was treated with the standard drug analgin at a dose rate of 50 mg/kg body weight.
Acetic acid induced writhing method or chemical method was considered for the evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). A solution of acetic acid (3% v/v) in distilled water was prepared. A solution of analgin was prepared in distil water. Animals were kept off fed to avoid food-drug interaction before one night of the experiment till the end of the experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 30 minutes, writhing was induced by intraperitonial injection of 3% acetic acid in volume of 0.1 ml/10g body weight. The writhing responses were recorded up to 30 minutes. Percentage of inhibition was calculated.

Data Analysis
The result were presented as Mean ± SE and analyzed using one way Analysis of Variance (ANOVA). The difference between the means was tested with Post Hoc Dunnett t-test and values of $p < 0.05$ were considered statistically significant.

Result
The phytochemical study of shade dried leaves and hydroethanolic extract of *A. sessilis* revealed the presence of tannins, saponins, flavonoids, steroids and terpenoids.
In Eddy's hot plate method, a significant increase ($p \leq 0.001$) in response time was seen at both the dose rates. The results are comparable to the standard drug used in the test. The result is shown in the Table no. 1.
In chemical method, the acetic acid induced writhing response; a significant reduction ($p \leq 0.001$) in the writhing response is seen at both the dose rates of 250 mg/kg bodyweight and 500 mg/kg bodyweight. The results are comparable to the standard drug analgin. The results are recorded in Table no 2.

Discussion
The writhing response induced by acetic acid is a chemical and sensitive procedure for to evaluate the peripherally acting analgesics [25]. Acetic acid causes pain by liberating endogenous substances such as histamine, serotonin, prostaglandins, substance P and bradykinins. Receptors present in the local peritoneum are postulated to be involved in the writhing response [26]. The method has also been associated with prostanoids in general that is, increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products [27].
The hydroethanolic extract of leaves of *Alternanthera sessilis* could protect the Acetic acid induced pain significantly at both the dose rates i.e. @ 250 mg/kg body weight and at 500 mg/kg body weight which directs the result towards its peripheral analgesic activity. The phytochemical studies revealed that the hydroethanolic extract of the test compound posses flavonoids [28] in it which might have contributed to the analgesic activity of this plant.
The significant increase in pain produced by Eddy's hot plate test in these models suggests involvement of central pain pathways. Pain is found to be centrally modulated via various

complex pathways including opiate, dopaminergic descending noradrenergic and serotonergic systems [29-31].

The hydroethanolic extract of Alternanthera sessilis showed a significant result in protecting the pain modulation in central pathways.

The protection from nociception exhibited by the tests and standards may be through centrally modulating mechanisms involving opiate, dopaminergic descending noradrenergic and serotonergic receptor systems or may be by peripherally inhibiting the prostaglandins, leukotrienes, and other endogenous substances that are key components of causing pain.

Table 1: Response (Mean ± Se) shown by the mice treated with hydroethanolic extract of leaves of *Alternanthera Sessilis* in eddy' hot plate method.

Treatment	0 MIN	15 MIN	30 MIN	45 MIN
Standard	4.50±0.43	5.00±0.52	5.50±0.43	4.50±0.22
250mg/kg	4.83±0.60	11.00±0.73**	12.00±0.37**	12.17±0.31***
500mg/kg	4.33±0.42	12.83±0.79***	13.50±0.72***	13.50±0.22***
Morphine	4.17±0.54	16.67±1.82***	28.00±1.63***	23.33±0.83***

*Implies p ≤0.05 when compared with CCl4, ** implies p ≤0.01 when compared with CCl4, *** implies p≤ 0.001 when compared with control.

Table 2: Response (Mean ± Se) shown by the mice treated with hydroethanolic extract of leaves of *Alternanthera Sessilis* in chemical method

Treatment	Mean ±Se	%Protection
Control	57.83±2.27	-----
250MG/KG	19.33±2.26***	66.58
500MG/KG	17.83±1.35***	69.17
Analgin	16.00 ±1.86***	72.33

*Implies p ≤0.05 when compared with CCl4, ** implies p ≤0.01 when compared with CCl4, *** implies p≤ 0.001 when compared with control.

Conclusion

Considering the results found, it can be concluded that the hydroethanolic extract of the leaves of the *Alternanthera sessilis* possesses antinociceptive property at the dose rate of 250 mg/kg body weight and 500 mg/kg bodyweight. It could protect the pain caused by central modulation as well as by peripheral mechanisms.

Acknowledgement

The authors are thankful to College of Veterinary science, Guwahati for the support of the ongoing research. The authors are thankful to the Department of Pharmacology and Toxicology, C.V. Sc. Khanapara for their support for the research work.

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