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Original Research Article

Utilizing Acacia Nilotica aerial parts for peripheral pain relief

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ABSTRACT

Background: Acacia nilotica, a medicinal plant from the Fabaceae family, is found in tropical and subtropical regions. It is used in traditional medicine for various ailments, but despite extensive scientific data, its analgesic properties remain under-researched.

Aims and Objective: Thus, this study aimed to assess the analgesic effects of A. nilotica aerial parts using an acetic-acid induced writhing model in crude ethanolic extract and its fractions (hexane, chloroform, ethyl acetate).

Material and Methods: The study was designed and conducted at PCSIR Labs Complex, Karachi in May-June 2024. The methods and procedures were approved by the Committee for the Ethical Use of Experimental Animals at PCSIR Laboratory Complex, Karachi (IEC/AN-04). The analgesic effect of extracts of A. nilotica were investigated at doses 250 and 500 mg/kg body weight, using acetic-acid induced writhing test in albino mice. Diclofenac sodium was used as standard. Twenty minutes post drug period show that all test groups had dose-dependent analgesic effects, characterized by reduction in the number of writhes.

Results: As compared to control group all test groups exerted a dose-dependent decrease in abdominal constriction. At 500 mg/kg body weight, the extremely significant activity was seen in the n-hexane fraction (76.5%; $p<0.05$) and the chloroform fraction (76.2%; $p<0.05$). Diclofenac sodium exhibited 70.4% ($p<0.05$) writhing response.

Conclusion: The conclusion of present study supports traditional claim of A. nilotica for treating pain and discomfort.

1. Introduction

Pain, being an acute sensation of discomfort, may serve as a potential indicator of physical harm or disorder, and needs individual attention to effectively address and alleviate the stimulus causing the pain.[1] A drug that relives pain without loss of consciousness is known as analgesic or pain killer (tablets, gels, ointments etc)[2] despite the availability of adequate medications, pain and inflammation remain major health problems and are considered the main clinical, social and economic issues.[3] In numerous instances, pain-relieving medications that have been extensively utilized in the past few decades can solely alleviate 50% of the pain experienced by approximately one-third of patients, while also being associated with significant adverse reactions.[4] Peoples mostly from developing countries preferred herbal medicines to cure various health issues due to their easy availability, safety and cost-effectiveness. It is estimated that approximately one quarter of approved modern medicines are derived from herbal substances and still continuous

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research is being going on to introduce effective, safe and economical drugs from plant origin.[5]

Acacia Nilotica (Babool, kikar) is a member of Fabaceae family with many therapeutic properties. It is found in Africa, Middle East and the Indian subcontinent.[6,7] Almost every part of it (seeds, gum, flowers, leaves, bark, roots and pods) has important therapeutic properties and is used to treat diarrhea, dysentery, piles, periodontitis, abdominal pain, sore throat, diabetes, asthma and hypertension.[8,9] There are number of powerful phytochemicals present in A. nilotica having medicinal values, proven by scientific data.[10,11] Although the vast scientific data is reported on this plant but till date, no analgesic study has been conducted to evaluate its aerial parts of A. nilotica. Therefore, the present study used the acetic acid-induced writhing test model to evaluate the analgesic activity of the crude ethanol extract and its derived fractions in albino mice (Figure 1).

Figure 1: Graphical abstract

2. Materials and Methods

2.1. Extraction of plant material

The aerial parts (bark, branches and leaves) of A. nilotica were purchased from the local market. The specimens were kept in the herbarium of the Department of Botany, University of Karachi. A total of 25 kg of plant material was dried in a dryer at 50 °C for three days, ground, sieved and soaked in 50 liters of ethanol for one week. The entire mixture was then filtered through Whatman No.1 filter paper and concentrated using a rotary evaporator at 40-50 °C to obtain a concentrated gelatinous material (about 520 g).

2.2. Fractionation

To yield different fractions, the crude ethanolic extract was suspended in water and then it was extracted successively using a series of organic solvents which were n-hexane, chloroform and ethyl acetate sequentially based on their polarity[12,13] which were used for analyzedanalgesic activity.

2.3. Animal selection

Healthy Swiss albino mice (20-30 g) of both sexes were selected for the study. The animals were maintained in the animal house of PCSIR Laboratory Complex, Karachi, housed individually on a 12 h light-dark cycle for one week prior to the start of the experiment and given free access to food and water. The methods and procedures were approved by the Committee for the Ethical Use of Experimental Animals of PCSIR Laboratory Complex, Karachi (IEC/AN-04).

2.4. Acetic acid-induced writhing test

[1] An acetic acid-induced writhing test was conducted to evaluate the peripheral analgesic effect of the aerial parts and fractions (n-hexane, chloroform and ethyl acetate) of the ethanol extract of A. nilotica at doses of 250 and 500 mg/kg. The animals were divided into ten groups (n=5), of which groups I-VIII were used as test groups, while groups IX and X were considered as standard and control groups. Groups I and II were orally administered with ethanol crude extract at doses of 250 and 500 mg/kg. Groups III and IV were orally administered with n-hexane fraction at doses of 250 and 500 mg/kg. Groups V and VI were orally administered with 250 and 500 mg/kg of the chloroform fraction. Groups VII and VIII were orally administered with 250 and 500 mg/kg of the ethyl acetate fraction. Groups IX and X served as standard and control groups and were orally administered with 5 mg/kg body weight of diclofenac sodium or an equal amount of distilled water. All drugs were administered 30 minutes before the injection of acetic acid. Thirty minutes later, each mouse was injected intraperitoneally with a 1% acetic acid solution (0.1 ml/10 g). Mice were caged individually and the number of abdominal contractions was counted for each mouse over a twenty-minute period five minutes after the intraperitoneal injection of acetic acid (Figure 1). Evidence of analgesia was measured by a reduction in the number of writhes compared to the control group. This was expressed as a percentage of curvature inhibition and was calculated using the following formula:

Inhibition % = C-D/C × 100
Where:
C-Average number of writhing for control group
D-Average number of writhing of test and standard groups

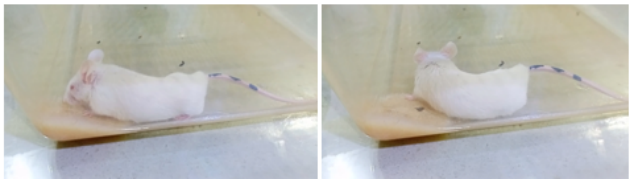

Figure 2: Images showing writhing reflex in mic

2.5. *Statistical analysis*

All numerical data are expressed as mean ±SD. All data were statistically analyzed using Student's ttest. A value of p <0.05 was considered significant compared with the control value.

3. Results

The study was planned and conducted from May to June 2024. Initially, all extracts and fractions were prepared, followed by the implementation of the acid-induced writhing test in mice. In all experimental groups the abdominal constriction and number of writhes was noted for 20 min immediately after the acetic acid injection. As compared to control group all test groups exerted a dose-dependent decrease in abdominal constriction (Table1, Figure3). The maximum protective effect were observed in n-hexane and chloroform fractions at 500 mg/kg body weight that showed significant decrease in writhing response 18.6 and 18.8 with increase in percent of writhing inhibition 76.5% and 76.2%. In overall results, the percentage inhibition of writhes by other test groups at the dose of 250 and 500mg/kg was also found higher than that of Diclofenac sodium (70.4%).

Table 1: Effect of A. nilotica on acetic acid induced writhing in mice

Treatments	Dose (mg/kg)	No. of writhing	% of inhibition
ethanolic crude extract	250	21.4±3.4***	72.9
	500	20.8±1.64***	73.7
n-hexane fraction	250	40.8±7.79**	64.1
	500	18.6±7.79***	67.1
Chloroform fraction	250	22.2±13.4***	48.6
	500	18.8±4.76***	76.2
Ethyl acetate fraction	250	31.2±6.41***	71.9
	500	29±13.17**	76
Diclofenac sodium	5	23.4±9.34***	70.4
D. water	–	79.2±18.2	——-

values expressed as mean ± STDEV,**vary significant, ***highly significant

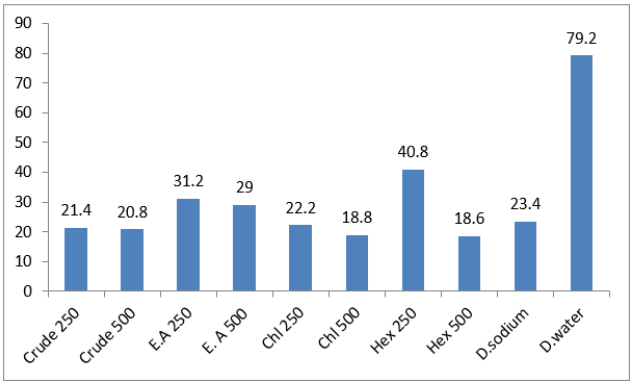

Figure 3: Writhing response in mice

4. Discussion

The peripheral analgesic activity of A. nilotica ethanolic extract and its fractions (n-hexane, chloroform and ethyl acetate) was assessed at doses of 250 and 500mg/kg dose by using acetic acid induced writhing test method. This test is considered a reliable and rapid method for evaluating the peripheral analgesic effect of herbal substances.[14] Intraperitoneal injection of acetic acid produces irritation and stimulation of the abdominal cavity, leading to the synthesis and release of multiple endogenous inflammatory mediators such as histamine, serotonin, bradykin in substance P, and PGs. These endogenous inflammatory mediators trigger chemically induced visceral pain, which is characterized by abdominal muscle contraction, forelimb extension, and body lengthening.[3] The plant's peripheral analgesic properties are indicated by the suppression of the writhing response. The results of current study revealed a significant decrease in writhing reflex in all test and standard group animals. The oral administration of A. nilotica at 250 and 500 mg/kg dose led to a notable reduction in writhes in dose-dependent manner in all groups in comparison to control group (Table 1, Figure 3).

The maximum protective effect was observed with n-hexane followed by chloroform fractions (500 mg/kg b.w.) by showing significant decrease in writhing response 18.6 and 18.8 with increase in percent of writhing inhibition 76.5% ($p<0.05$) and 76.2% ($p<0.05$) respectively. Overall results indicates that there is increase in percent protection in all test groups at both doses while few groups exhibited higher analgesic effects than standard drug Diclofenac sodium70.4% ($p<0.05$) (Table 1, Figure 3). This finding strongly suggests that our test drugs have ability to inhibit peripheral by showing decrease number of writhing reflex in test group animals.

A study conducted by Mamun-Or-Rashid et al.[15]on A. nilcotica bark methanolic extract and ethyl acetate, carbon tetrachloride, hexane, dichloromethane and hexane fraction of at 400mg/kg reported 36%, 27.9%, 37.2%,

29.1% and 39.5% inhibition percentage respectively. Another study conducted on aqueous extract of A. nilotica root reveals the test material significantly inhibited the acetic acid induced writhing reflex in dose dependent manner.[16] Munira et al.[6] also reported their work on A. nilotica seed and seed pods and exhibited dose dependent peripheral analgesic effects. Multiple studies on various parts of A. nilotica reported the presence of medicinally important phytochemical constituents like volatile oil, saponins, sterols, flavonoids, tannins, triterpenoid, phenol, alkaloids, fatty acids and polysaccharides.[17–21] All these phytochemicals are well reported for their analgesic effects.[22–24] The aforementioned findings suggest that the ethanolic extract of A. nilotica and its fractions exhibit a dose-dependent analgesic effect. It can be inferred that as the dosage is escalated, the analgesic efficacy also increases, indicating a rise in the concentration of phytochemical constituents with analgesic properties. Siddiqui et al.[25] reported that acetic acid injected into the peritoneal cavity, it releases prostaglandins, serotonin, and cytokines, which are inflammatory chemicals that excite peripheral pain receptors and generate writhing or painful sensations. The extract's capacity to prevent the production of inflammatory mediators and lessen peripheral pain sensitivity to chemically produced pain may be the cause of the peripheral analgesia seen in this study. These results align with the theories designed to reduce writhing. The results also suggested that A. nilotica possessed analgesic effects and this plant can be utilized in the treatment of various types of pains.

5. Limitations of study

The acetic acid-induced writhing test primarily assesses visceral pain and may not explain other types of pain, which limits its applicability in broader pain research. Additionally, the assessment period is often too short to fully capture the range of analgesic effects or responses.

6. Conclusion

In conclusion, the crude extract of A. nilotica and its fractions (hexane, chloroform, and ethyl acetate) were demonstrated to be a safe, natural remedy for the treatment of pain. A. nilotica has been used traditionally to treat a variety of diseases since ancient times, and experimental studies have confirmed its analgesic activity. However, more thorough research on clinical studies is required to fully understand its medicinal value before it can be established as a standard medication.

7. Authors Contribution

All authors contributed significantly to the reported work, including aspects such as conception, study design, execution, data acquisition, analysis, and interpretation. Each author participated in drafting, revising, or critically reviewing the article and provided final approval for the version to be published.

8. Source of Funding

None.

9. Conflict of Interest

None.

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