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EVALUATION OF ANTI-NOCICEPTIVE ACTIVITY OF *Acacia sinuata* USING VARIOUS EXPERIMENTAL ANIMAL MODELS

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ABSTRACT:

Pain is the most troublesome event encountered in many diseases and states of injury. Conventional analgesics are useful but have serious draw-backs in terms of their side effects and toxicities hence the search of herbal remedies capable of counteracting pain is going on simultaneously. Traditionally *Acacia sinuata* is reported to possess analgesic activity but literature survey shows that this activity has not been yet evaluated. Hence *Acacia sinuata* was chosen for evaluation of its possible analgesic effect. Ethanolic extract of *Acacia sinuata* pods (ASE) was administered orally in rats and mice at the dose of 200 mg/kg and 400 mg/kg. The tail flick test, hot plate test and acetic acid induced writhing test were used for evaluation of anti-nociceptive activity of ASE using Diclofenac sodium (25 mg/kg) as reference drug. ASE significantly increased the latency for tail flick, jumping time from hot plate and reduced number of writhes induced by acetic acid suggesting good anti-nociceptive activity. Results of the study confirmed the ethno medical use of *Acacia sinuata* in painful conditions. ASE acts probably by inhibiting the release, synthesis and / production of inflammatory mediators like polypeptides, kinins, prostaglandins etc. Further research in this direction would clarify its exact mechanism of action justifying its use as an effective analgesic agent.

Keywords: Pain, Analgesic, *Acacia sinuata,* Anti-nociceptive, Diclofenac sodium.

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INTRODUCTION:

Pain is the troublesome event of many diseases and state of injuries. It has protective function but needs to be managed in order to maintain well-being. As a result of adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over world as alternatives to NSAIDs and opiates [1, 2]. All modern medicines are derived originally from traditional herbal sources. These have evolved to produce conventional medicine, which uses both synthetic drugs and isolated natural compounds. Herbal medicines are popular among the public and improvements in their formulation have resulted in a new generation of phytomedicines that are more potent than before [3]. *Acacia sinuata* is a perennial, woody, large climbing shrub which grows on big trees. Plant pacifies vitiated pitta, skin disease, burning sensation, constipation, calculi, hemorrhoids, vitilligo and eczema**.** It is widely used in treatment of fevers especially that of malaria fever. It helps in clotting of blood and liver related disorders and is effective in jaundice. It is a good anti-inflammatory herb [4-7]. The seeds contain a lactam, (+)-acacia lactam (3,7-dimethyl-7-vinyl-2,5,6,7-tetrahydro-1H-azepin-2-one). The compound is useful as immunosuppressant [8]. The aim of the present study was to investigate the properties of ASE on experimentally induced nociception.

2. MATERIALS AND METHODS:

2.1 Plant Materials

Dried pods of *Acacia sinuata* were collected from local market of Anand, Gujarat and were identified and authenticated by Dr. K.K.Dholwani, Assistant Professor, Department of Pharmacognosy, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, V. V. Nagar, India. A voucher specimen of the plant (voucher no. HSM/Ac-1/1/ARGH-12) is deposited in Pharmacognosy department for future reference.

2.2 **Preparation of Plant Extract** [9]

The dried pods were coarsely powdered and passed from 40# sieve. The pod powder was subjected to cold maceration using petroleum ether to remove fat. Then dried powderwas extracted by soxhlet extraction by using 400 ml of ethanol. In the extraction procedure a total amount of 50 gm powdered pods were used. Then extract was concentrated .The percentage yield of ethanolic extract of *Acacia sinuata*was found to be 11.5%. Texture of extract was gummy and color was reddish brown. The extract was stored in an airtight container in the refrigerator and was used for evaluation of its pharmacological activity throughout work.

2.3 **Phytochemical Analysis** [10]

Ethanolic extract of Acacia sinuata was subjected to standard chemical tests for the detection of different phytochemicals.

2.4 **Drugs and Reagents**

Diclofenac sodium (Novartis India Limited, Mumbai).

2.5 **Selection of Animals**

All animals were selected as specified for the experimentally induced inflammatory animal models. The animals had free access to standard commercial diet and water water *ad libitum* and were acclimatized to standard laboratory conditions (temperature: 25°±2°C) and maintained on 12-hlight: 12-h dark cycle. The protocol (Protocol no: CPCSEA/IAEC/ARCP/ 11-12/ 01) of the study was approved by the Institutional Animal Ethical Committee (IAEC) of A.R.College of Pharmacy as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.6 Analgesic activity

A] Effect of ASE on tail flick time in rats using analgesiometer [11]

Young male Wistar rats (250-300 g body weight) were used. They were placed into individual restrainers leaving the tail hanging out freely. The lower 5 cm portion of the tail was placed on preheated nichrome wire loop (55˚ C) of analgesiometer. The reaction time i.e. time for flicking of tail was determined before and after administration of the test substance. The tail flick time of untreated animals was between 1 and 5.5 seconds.

B] Effect of ASE on reaction time of rats using hot plate [12]

Groups of 6 rats of either sex with an initial weight of 250-300g were used . The hot plate, which is commercially available, consists of a electrically heated surface having controlled temperature of 55° to 56 °C. The animals were placed on the hot plate and the time until animals jump down from the hot plate was recorded using a stop-watch. The latency is recorded before and after 30 min following intraperitoneal or oral administration of the standard or the test compound.

C] Effect of ASE on acetic acid induced writhings in mice [13]

The mice were divided into four groups of six animals each. Briefly, the total number of writhes following intraperitoneal administration of 0.1 ml of 1% (v/v) acetic acid was recorded over a period of 20 min, starting 5 min after acetic acid injection. The animals were pretreated with oral dose of ASE (200 mg/kg and 400 mg/kg) or normal saline, 60 min before administration of acetic acid. Positive group comprised of animals pretreated with Diclofenac sod (25 mg/kg).

3. RESULTS:

3.1 Phytochemical screening of ethanolic extract of *Acacia sinuata*

The ethanolic extract of pods of *Acacia sinuata* showed the presence of alkaloids, carbohydrates, flavanoids, saponins, tannins, phenolic compounds and amino acids.

3.2 Analgesic activity

3.2.1 Effect of ASE on tail flick time in rats using analgesiometer

Exposing tip of tail of rats to radiant heat through heated nichrome wire resulted in flicking of the tail from heat source. Pretreatment of rats with reference analgesic drug Diclofenac sodium (25 mg/kg) and ASE (200 mg/kg and 400 mg/kg) significantly (*P 0.001) increased the reaction time (tail flick time), after 30 minutes of treatment. As shown in Table-1, ASE in the dose of 400 mg/kg produced equivalent analgesic activity as compared to standard drug Diclofenac sodium (25 mg/kg).

Table 1: Effect of ASE on tail flick time in rats using analgesiometer

Sr. no.	Group	Tail flick time (sec)	Increase in tail flick time (sec)	% Increase in tail flick time
1.	Control	2.850±0.112*	-	-
2.	Standard (Diclofenacsodium 25mg/kg)	5.817±0.154*	2.967	51.00%
3.	ASE (200 mg/kg)	5.048±0.374*	2.198	43.54%
4.	ASE (400 mg/kg)	5.835±0.275*	2.985	51.16%

Values are the Mean ± S.E.M. for 6 rats. *P<0.001 statastically significant as compared to control group by One way ANOVA followed by Dunnet`s test.

3.2.2 Effect of ASE on reaction time of rats using hot plate

Rats placed on Eddy`s hot plate preheated to 55˚C developed pain due to thermal stimulus and reacted by jumping down from the hot plate. As compared with the control group, treatment with ASE (200 mg/kg and 400 mg/kg) and reference drug Diclofenac sodium (25 mg/kg) significantly (*P<0.001) increased the reaction time of rats by elevating pain threshold and delaying the jumping time from hot plate. The results of reference drug Diclofenac sodium (25 mg/kg) and ASE (400 mg/kg) are comparable indicating good analgesic activity of ASE. (Table: 2)

Table 2: Effect of ASE on reaction time of rats using hot plate

Sr. no.	Group	Jump time from hot plate (sec)	Increase in jump time (sec)	% Increase in jump time
1.	Control	2.972±0.094*	-	-
2.	Standard (Diclofenac sodium 25mg/kg)	5.903±0.099*	2.931	49.65%
3.	ASE (200 mg/kg)	4.552±0.296*	1.580	34.71%
4.	ASE (400 mg/kg)	5.028±0.150*	2.056	40.89%

Values are the Mean ± S.E.M. for 6 rats. *P<0.001 statistically significant as compared to control group by One way ANOVA followed by Dunnet`s test.

3.3.3 Effect of ASE on acetic acid induced writhings in mice

Results of anti-nociceptive activity of ASE assessed using acetic acid induced writhing test in swiss albino mice are presented in Table-3. Mice were pretreated with ASE (200 mg/kg and 400 mg/kg) or Diclofenac sodium (25 mg/kg) p.o. 60 min before administration of acetic acid. It was observed that ASE (200 mg/kg and 400 mg/kg) produced significant (*P<0.01) and dose dependant inhibition of writhings to the extent of 34.46% and 51.94% respectively as compared to control group. The anti-nociceptive effect produced by ASE high dose (400 mg/kg) was found to be comparable with the effect shown by standard drug Diclofenac sodium (25 mg/kg), which reduced number of writhings by 59.71% as compared to control group.

Table 3: Efffect of ASE on acetic acid induced writhings in mice

Sr.no.	Group	No. of writhings	% Inhibition of writhings
1.	Control	34.33±1.282*	-
2.	Standard (Diclofenac sodium 25mg/kg)	13.83±0.703*	59.71%
3.	ASE (200 mg/kg)	22.50±0.671*	34.46%
4.	ASE (400 mg/kg)	16.50±0.619*	51.94%

Values are Mean ± S.E.M. for 6 guinea pigs. *P<0.01 statastically significant as compared control group by one way ANOVA followed by Dunnet`s test.

4. DISCUSSION

In an attempt to evaluate anti-nociceptive activity, three classical models were selected. The hotplate and tail flick model has been found suitable to investigate central anti-nociceptive activity because of several advantages, particularly the sensitivity to anti-nociceptives and limited tissue damage [14]. Proinflammatory mediators like PGs and bradykinins (BKs) were suggested to play an important role in analgesia [15]. Abdominal constriction induced by acetic acid is a classical model to investigate peripheral anti-nociceptive activity. Abdominal constriction evoked by acetic acid is dependent on the production and release of proinflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-8 (IL-8) from resident peritoneal macrophages and mast cells [16]. Interestingly, the obtained results confirmed that ASE (200 mg/kg and 400 mg/kg) has both peripheral and central analgesic effect which was comparable with reference drug Diclofenac sod (25

mg/kg). Although the results of the present study are inconclusive, they tend to suggest that ASE probably produces its anti-nociceptive effect by inhibiting the release, synthesis and/or production of inflammatory mediators including polypeptide kinins, PGs and so forth, like Diclofenac sodium [17].

Phytochemical screening of ethanolic extract of *Acacia sinuata* pods using different methods described in the methodology revealed presence of various alkaloids [17, 18], flavanoids[19], steroids[20], saponins[19] and glycosides[17]. It has been reported that these phytoconstituents present in other plants are responsible for their anti-inflammatory and anti-nociceptive activity. Hence, it is important to isolate the bioactive principle responsible for this activity.

5. CONCLUSION

In conclusion, our findings demonstrate that ethanolic extract of *Acacia sinuata* pod possesses favorable anti-nociceptive activity probably by inhibition of PGs synthesis and/or release. Although, neither the precise mechanism of action nor the exact chemical constituents responsible for observed pharmacological actions of ASE could be established, yet the results confirmed the traditional/ethnomedical use of *Acacia sinuata* in the treatment of painful conditions. Further, detail study is required for isolation of active constituents responsible for anti-nociceptive activity and to determine it`s possible mechanism of anti- nociceptive action.

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