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Original Research Article

Anti-nociceptive effect of seed extract of *Acacia tortilis* in rodents

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ABSTRACT

Background: Management of pain is a primary clinical concern for any pathology in medical field. Addiction liability of opioids and troublesome gastrointestinal side effects of NSAIDs leads to intensive research for compound with lesser side effects. The aim of the study to evaluate the anti-nociceptive activity of Acacia Tortilis Seed Extract (ATE) in experimental animals.
Methods: First of all, animals were randomly allocated into four groups of six animals each. In acetic acid induced writhing test model, Group I (NC) served as vehicle control received saline/Tween 80 0.1%, 10ml/kg BW orally, group II (ATE-100) and III (ATE-200) received ATE in dose of 100 and 200mg/kg BW orally respectively and group IV received the standard drug diclofenac sodium in dose of 50 mg/kg BW orally. Group I to IV were same in rest of three experimental models. One additional group of standard drugs (group V) morphine sulfate in dose of 5mg/kg BW subcutaneously (SC) was allocated for screening method hot plate and tail flick tests. In Formalin induced paw licking test, three additional groups (group V) morphine sulfate in dose of 5mg/kg BW SC, group VI- morphine+naloxone (5mg/kg SC +2mg/kg intra-peritoneally (IP) and group VII - ATE+ naloxone (200mg/kg BW orally +2mg/kg BW IP) were also made.
Results: The ATE when administered orally in dose of 100 and 200mg/kg body weight (BW), produced significant analgesic activity ($P < 0.01$) in acetic acid induced writhing syndrome and late phase of formalin test. In the hot plate test in mice and tail flick test in rats, ATE in same doses also showed significant analgesic activity ($P < 0.05$) which is almost equally efficacious to standard drug diclofenac sodium (50mg/kg BW orally) but far less efficacious than morphine sulfate (5mg/kg BW subcutaneous).ATE (200mg/Kg BW orally) activity did not blocked by naloxone (2mg/kg intra-peritoneal).
Conclusions: ATE possesss significant anti-nociceptive activity as evidenced in all the animal models of nociception. It might exert its effect through the peripheral mechanism of analgesic action possibly by interference in biosynthesis, release and/or action of prostaglandins and leukotrienes.

Keywords: Anti-nociceptive effect, *Acacia tortilis*, Formalin test, Hot plate test, Tail flick test, Writhing test

INTRODUCTION

Pain is a component of virtually all clinical pathologies, and management of pain is a primary clinical concern in medical practice. Opioids are one of the most important classes of drugs used in the treatment of pain but depending upon the pain state, other drug classes, such as NSAIDs, antidepressants and anticonvulsants can be

used.[1] Addiction liability of opioids and troublesome side effects of NSAIDs like Gastrointestinal mucosal irritation leads to intensive research for compound with lesser side effects. As far as the Medicinal plants is concerned, they have been used since ancient times to treat variety of diseases and represent a rich source of new chemical entities with diverse pharmacological properties, which are lead compounds for the development of new drugs. Regarding the in vitro studies, they can only partially substitute the animal experiments involving pain as pain is a common phenomenon in all animals especially in vertebrates, which is felt same in human being. Therefore, the present work have been planned to study indigenous plants (*Acacia tortilis*) in experimental animals. Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, first described from Africa by Linnaeus in 1773.

Acacia is one of the plants that have been frequently used as medicine like Acacia Bark has been used in treatment of haemoptysis, gonorrhea, leprosy and headaches.[2] Acacia Catechu has been used for treating fever, diarrhoea, leucorrhoea, piles, erysipelas and throat infection.[3,4] Acacia catechu has been reported to have hypoglycemic antipyretic, hepatoprotective, and digestive properties.[5-7] In recent past, we evaluated hypoglycemic and anti-hyperglycemic activity of Acacia tortilis seed extract in diabetic experimental animals.[8,9]

Till date, no scientific reports exist about the possible anti-nociceptive and anti-inflammatory activity of Acacia tortilis. Hence the present study was designed to evaluate these effects of *Acacia tortilis* Seed Extract (ATE) through screening analgesic models of mice and rats.

METHODS

Animals

Healthy *Wister albino rats* weighing 150-250g and *Swiss albino mice* weighing 25-30g of either sex were procured from Central animal house of institute where research was conducted. All the animals were kept in standard polypropylene cages and maintained under standard conditions: temperature (25±2°C), relative humidity (40-55%) and 12:12 light: dark cycle. The animals were fed with standard rodent diet and water ad libitum. The study was approved by the Institutional Animal Ethics Committee of the institute. The animal care and handling were done as per the guidelines set by CPCSEA.

Chemicals and solvents

The following chemicals and solvents were purchased and used as received: Diclofenac sodium (Sigma Aldrich Co., India), Morphine sulfate (BDH Chemicals Ltd., England), Naloxone hydrochloride dihydrate (Sigma Aldrich Co., India), formalin (BDH Chemicals Ltd., England), and acetic acid (BDH Chemicals Ltd., England).

Group allocation

First of all, animals were randomly allocated into four groups of six animals each. In acetic acid induced writhing test model, Group I (NC) served as vehicle control received saline/Tween 80 0.1%, 10ml/kg BW orally, group II (ATE-100) and III (ATE-200) received ATE in dose of 100 and 200mg/kg BW orally respectively and group IV received the standard drug diclofenac sodium in dose of 50mg/kg BW orally. Group I to IV were same in rest of three experimental models. One additional group of standard drug (group V) morphine sulfate in dose of 5mg/kg BW subcutaneously (SC) was allocated for screening method hot plate and tail flick tests. In Formalin induced paw licking test, three additional groups (group V) morphine sulfate in dose of 5 mg/kg BW SC, group VI-morphine+naloxone (5mg/kg SC+2mg/kg intra-peritoneally (IP) and group VII - ATE+ naloxone (200mg/kg BW orally +2mg/kg BW IP) were also made.

Preparation of plant extract

Seeds of *Acacia tortilis* were taken for the study. Dried seeds of the plant were properly grinded and sieved with mesh size 40-60. Extraction of powdered seed was done with distilled water to separate the volatile and non-volatile fraction with the help of klevenger apparatus and heating mental at 100°C. Non-volatile fraction was cooled and further precipitated with the help of ethyl alcohol for isolation of gum and other solutes. Isolated gum was purified with the help of filtration technique followed by ion exchange and freeze-drying process. Finally, the *Acacia tortilis* seed extract (ATE) was stored in refrigerator until use.

Acute oral toxicity Test in Rats

Authors have tested it previously and found that there were no signs of toxicity, behavioral changes and mortality recorded up to a dose of 1000mg/kg body weight of the extract.[8,9]

Acetic acid- induced writhing response in mice

The response to an intra-peritoneal injection of 0.6% acetic acid solution (writhing reflex: one writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb) was studied according to procedures described by Koster et al.[10] Mice were divided into four groups of six animals each. An injection of 0.6% acetic acid aqueous solution (10ml/kg BW) was administered into the peritoneal cavity and the animals were placed in a transparent plastic box. The number of writhes was counted for 15 min starting from 5 min after the acetic acid injection. The vehicle, test and standard drugs (as per group allocation, group I to IV) were administered 40 minute before the acetic acid injection. Percentage inhibition of writhing in comparison to control group was taken as an index of analgesia and was calculated using the following formula:

Inhibition (%) = $[(WR_c - WR_t)/WR_c] \times 100$

Where WR_c is the average number of writhing reflex in the normal control group and WR_t is the average number of writhing in the test groups.

Formalin induced paw licking response in mice

This test was performed according to the method of Reisine and Pasternack.[11] Mice were divided into two sets of seven groups of six animals. vehicle, test and standard drugs (as per group allocation, group I to VII) were administered 1 h prior to formalin injection to animals in the first set (for early phase) and 40 min prior to formalin injection to animals in the second set (for late phase), respectively. Mice were injected subcutaneously with 50µl of 1% formalin in normal saline solution into the right dorsal hind paw. The time animals spent in licking of injected paw was determined during 0-5 min (the first set of mice for early phase) and during 20-30 min (the second set of mice for late phase) after the injection of formalin.

Hot plate test in mice

Experiments were carried out according to method by Parkhouse and Pleuvry.[12] Mice were used and divided into five groups of six animals. For testing, mice were placed on hot plate maintained at 55±0.5ºC. The time that elapsed until occurrence of either a hind paw licking or a jump off from the surface was recorded as the hot plate latency. Before treatment, the reaction time of each mouse (licking of the forepaws or jumping response) was done at 0 and 10 min interval. The average of the two readings was obtained as the initial reaction time. Mice with baseline latencies of <5 s or >30 s were eliminated from the study. The reaction time following the administration of vehicle, test and standard drugs (as per group allocation, group I to V) was measured at 30 minutes, 1 hrs, 2 hrs and 3 hrs.

Tail flick method in rats

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 were taken. Before treatment, the reaction time of each mouse (flicking of tail) was done at 0 and 10 min interval. The average of the two readings was obtained as the initial reaction time. The reaction time following the administration of vehicle, test and standard drugs (as per group allocation, group I to V) was measured at 30 minutes, 1 hrs, 2 hrs and 3 hrs.

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM) and the differences between control and treated groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. In all cases differences were considered significant if $P < 0.05$. The statistical analysis was carried out using the SPSS program (version 20.0).

RESULTS

Acetic acid- induced writhing response in mice

ATE significantly reduced number of writhes calculated in 15 minutes (Table 1). The protective effect of ATE was dose dependent with 74.71% and 84.83% reduction observed in 100 and 200 mg/kg BW dose respectively which is somewhat less than the standard drug diclofenac sodium (91.37%) (Table 1).

Table 1: Effect of *Acacia tortilis* seed extracts (ATE) on acetic acid- induced writhing response in mice.

Groups	Number of writhes in 15 minutes	Percent inhibition of writhing
Group I (NC)	56.00±0.96	-
Group II (ATE-100)	14.33±0.88***	74.41%
Group III (ATE-200)	8.66±0.66***	84.53%
Group IV (Diclofenac sodium)	4.83±0.47***	91.37%

Values are expressed as Mean±SEM; n=6; Significance levels when compared with Normal Control (NC): * P ≤0.05, ** P≤0.01, *** P≤0.001

Formalin induced paw licking response in mice

Table 2: Effect of *Acacia tortilis* seed extracts (ATE) on formalin induced paw licking response in mice.

Groups	Early phase licking time (in seconds)	Late phase licking time (in seconds)
Group I (NC)	35.16±1.62	34.83±1.44
Group II (ATE-100)	34.83±2.23 (0.93%)	15.50±1.23*** (55.49%)
Group III (ATE-200)	34.00±0.85 (3.29%)	12.33±1.28*** (64.59%)
Group IV (Diclofenac sodium)	32.50±1.14 (7.56%)	8.83±0.79*** (74.64%)
Group V (Morphine sulfate)	3.33±0.42*** (90.52%)	3.00±0.73*** (91.38%)
Group VI (Morphine sulfate +Naloxone)	33.00±0.96 (6.54%)	35.16±1.44 (-0.95%)
Group VII (ATE-200+Naloxone)	34.66±1.33 (1.42%)	14.66±1.44*** (57.51%)

Values are expressed as Mean±SEM; (% reduction) n=6; Significance levels when compared with Normal Control (NC): * P ≤0.05, ** P≤0.01, *** P≤0.001

In Formalin test, ATE exerted its action more efficiently on the second phase (20-30 min) compared to the first

phase (0-5 min). These phases corresponded to neurogenic and inflammatory pains respectively. Diclofenac sodium was significantly active on the second phase whereas morphine acted in both the phases (Table 2). The opioid antagonist naloxone inhibited the action of morphine at both the phases, but the activity of ATE was not interrupted by naloxone (Table 2).

Hot Plate and Tail-flick method

Reaction times to thermal stimulation in both methods (Hot plate and Tail-flick test) were significantly ($p<0.05$) increased after ATE (100 and 200mg/kg BW) administration on dose dependent manner. Peak analgesic effect was observed after 2 hrs of oral administration and remained for more than 3 hrs. Standard drug diclofenac sodium' analgesic effect was comparable to ATE but morphine sulfate was more efficacious in increasing the reaction time as compared to ATE and diclofenac sodium. (Table 3 and 4).

DISCUSSION

In the present study we evaluated the anti-nociceptive effects of ATE by employing various experimental test models. The results indicated that ATE exhibited peripheral anti-nociceptive activity which was comparable with the standard drug diclofenac sodium. As far as efficacy is concerned; it is less than morphine sulfate which is centrally acting analgesics. ATE showed anti-nociceptive effect in acetic acid induced writhing response and formalin induced paw licking response (only on inflammatory phase).The acetic acid-induced writhing test has been used widely for the evaluation of peripheral anti-nociceptive activity.[14]

Table 3: Effect of *Acacia tortilis* seed extracts (ATE) on reaction time in hot plate method in mice.

Groups	Reaction time (in seconds)				
	Initial control	After 30 min	After 1 hr	After 2 hr	After 3 hr
Group I (NC)	4.33±0.42	4.33±0.28	4.33±0.42	4.28±0.32	4.33±0.78
Group II (ATE-100)	4.66±0.49	5.28±0.16*	666±0.23**	6.66±0.25**	5.23±0.21*
Group III (ATE-200)	4.73±0.83	5.66±0.26**	6.78±0.43**	8.28±0.36**	5.18±0.30*
Group IV (Diclofenac sodium)	4.66±0.49	6.83±0.37***	8.83±0.79***	8.66±0.36***	6.66±0.33*
Group V (Morphine sulfate)	4.83±0.74	8.83±0.82***	12.33±1.28***	14.66±1.44***	13.67±0.18***

Values are expressed as Mean±SEM; n=6; Significance levels when compared with Normal Control (NC): * P ≤0.05, ** P≤0.01, *** P≤0.001

Table 4: Effect of *Acacia tortilis* seed extracts (ATE) in tail-flick reaction method in rats.

Groups	Tail flick reaction time (in seconds)				
	Control	After 30 min	After 1 hr	After 2 hr	After 3 hr
Group I (NC)	4.66±0.49	4.23±0.15	4.28±0.22	4.13±0.15	4.18±0.23
Group II (ATE-100)	4.83±0.74	5.45±0.30*	6.00±0.35**	6.99±0.36**	6.33±0.39**
Group III (ATE-200)	4.66±0.49	5.57±0.25*	6.70±1.02**	9.00±0.37***	6.55±0.20**
Group IV (Diclofenac sodium)	4.83±0.74	6.77±0.37**	7.00±0.33**	9.99±1.44***	6.58±0.32**
Group V (Morphine sulfate)	4.33±0.33	7.33±0.23***	9.77±0.19***	13.28±0.16***	12.33±1.08***

Values are expressed as Mean±SEM; n=6; Significance levels when compared with Normal Control (NC): * P ≤0.05, ** P≤0.01, *** P≤0.001

This method is simple, reliable and also provides rapid evaluation of anti-nociceptive activity.[15,16] The pain mechanism of abdominal writhing induced by acetic acid involves the process of release of arachidonic acid metabolites prostaglandins and leukotrienes via cyclooxygenase and lipo-oxygenase pathway and other endogenous pain mediators, such as histamine, serotonin, bradykinin, and substance P that sensitize pain nerve endings.[17-19] In the first 30 min after acetic acid injection, the levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ was found to be increased in animal.[20] The ability of the ATE to attenuate the acetic acid-induced writhing in mice suggests that they possess peripherally mediated anti-nociceptive activity which might be due to its possible interference in the arachidonic acid metabolites pathway particularly in biosynthesis, release and/or action of prostaglandins and leukotrienes. This assumption was also strengthen by the results of formalin test where ATE showed anti-nociceptive effect only on second phase of the formalin test. The formalin test possessed two distinctive phases which reflected different types of pain. The earlier phase reflected direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflected pain from inflammation hence it was implying its anti-inflammatory effect by interfering during synthesis and/or release of PGs and/or other pain mediators.[21] Naloxone nullified the effect of morphine through receptor antagonism of opioid receptors μ κ and δ whereas anti-nociceptive activity of ATE was not inhibited by naloxone,

indicated that ATE did not act through the spinal opioid receptors.

The results of the rest of two models (hot plate and tail flick test) drew attention about the possibility of additional central anti-nociceptive mechanism for ATE. Since both are specific central anti-nociceptive tests [12] and ATE increased the response time significantly, indicated central analgesic mechanism in neurogenic pain. But it had been confirmed by the formalin test that ATE did not show its activity through the opioid receptors hence additional central anti-nociceptive mechanism through modulation of neurotransmitters in spinal or supra-spinal level may be possible. There may be possibility of diverse mechanism of anti-nociception as it was not as efficacious as Morphine as analgesics but comparable with the diclofenc sodium.

The findings of the study indicate that the seed of *Acacia tortilis* possess antinociceptive, anti-inflammatory activity. Further studies are required to substantiate the above observation and establish the potential mechanism of action of these activities.

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