ANTINOCICEPTIVE EFFECT OF THE METHANOLIC EXTRACT OF ROOTS OF ANDRACHNE ASPERA IN THREE MODELS OF NOCICEPTION

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Summary

Adrachne aspera spreng grows at an altitude of 1000 –2400m often in rock crevices. The roots of this plant are traditionally used in for various ailments. Despite its widespread use, this medicinal plant has not been subjected to pharmacological studies. It was the aim of the present study to evaluate the antinociceptive effect of the root extract. The 80% methanolic extract was dissolved in 2.5% v/v tween 80 in normal saline and the antinociceptive effect of different oral doses was assessed in the writhing, the tail flick and the hot plate tests in mice. The extract produced significant antinociception in all the tests as compared to the vehicle (2.5% tween 80 in normal saline (v/v)). The antinociception produced was dose-dependent in the tail flick test. The results of this work, therefore, support the claimed traditional use.

Keywords: *Andrachne aspera,* antinociception, hot plate, tail flick, writhing, methanol extract

Introduction

Currently available analgesics exhibit a variety of adverse reactions (1) necessitating the search for newer agents with better safety margin. In the search for drugs in general and analgesics in particular, applying ethnopharmacological knowledge is of paramount importance at least to developing countries (2). Medicinal plants, which have yielded novel antinociceptive substances, are of especial importance in this regard. The use of the juice or gum from the unripe seedpods of the poppy. *Papaver somniferum* is among the oldest recorded medications. The writings of Theophrastus around 200 B. C., describe the use of opium in medicine. The pharmacist Surturner first isolated an alkaloid from opium in 1803 and named it morphine, an analgesic that has continued to be used to date (3). In addition, white willow bark, *Salix alba*, had been used throughout the world as an antipyretic and analgesic. In fact, the principal constituent salicin led to the synthesis of acetylsalicylic acid (4). The above two examples could justify the importance of medicinal plants as sources of drugs.

Several medicinal plants are traditionally used in Ethiopia for the relief of pain. Among the many, few have been subjected to pharmacological screening for their antinociceptive effects and have shown promising results (5-7). *Andrachne aspera* Spreng is a medicinal plant used in Ethiopia to relieve pain and reduce fever. Despite its widespread use the pharmacological effects have not been studied. It is known in Konso, South Ethiopia by the vernacular name “kora megagna” and belongs to the family Eurphorbiaceae. It grows at an altitude of 1000 –2400m often in rock crevices (8). The present study was therefore conducted to investigate the antinociceptive effect of the roots of *A. aspera* 80% methanol extract using three models of nociception.

Materials and Methods

Plant acquisition

The roots of *A. aspera* were collected from Konso, south west part of Ethiopia in February 2003. Identification was made by a taxonomist and a voucher representative was deposited in the Herbarium of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute (Herbarium number AA-2078).

Preparation of drugs and chemicals

Glacial acetic acid (Carlo Erba Spa Farmitalia, Lot. 0286NI00) was diluted to 0.6% with double distilled water. Tween 80 (BDH, Poole, England, Lot ZA 7088516 647) was diluted with normal saline to make 2.5% v/v solution. Pethidine HCl injection (Amino Ltd. Switzerland, Lot 4906) injection was diluted with normal saline to prepare a solution giving 30mg/kg in 0.2ml.

Preparation of extracts

Air-dried and powdered roots of *A. aspera* (115g) were macerated with 80% methanol (Merck Art. 6009 9678100) for three days. The methanolic extract was then filtered and concentrated under vacuum in rotary evaporator to give 3.7% w/v gummy residue. The

residue was then solublized in the vehicle (2.5% w/v tween 80 in normal saline) to give 300mg/kg, 600mg/kg, and 900mg/kg doses in 1ml.

Administration of substances

The vehicle and the extract of *A. aspera* were administered by oral gavage (1ml/40g, body weight. while pethidine. HCl (30mg/kg, 0.2ml) was administered subcutaneously.

Animals

Adult male Swiss albino mice weighing 25-40gm were used for the experiment. The animals were obtained from Ethiopian Health and Nutrition Research Institute. They were kept in cages and housed in a standard animal house under a 12/12h light-dark cycle. They were fed on pellets and tap water *ad libitum* except during the experiment. Prior to the experimentation they were acclimatized to the test environment for one hour.

Assessment of antinociception

Screening of the plant extract for antinociceptive effect was done in three tests of nociception: the writhing, tail-flick and hot plate tests. For each test, the animals were categorized into five groups of 6 animals: vehicle treated, extract treated (300mg/kg, 600mg/kg, 900mg/kg) and standard drug treated (pethidine 30mg/kg).

Writhing test

The method used by Connor *et al*. (9) was employed. Mice were pretreated with the test substance or vehicle or standard drug 30 minutes before the administration of acetic acid (0.6%, intraperitoneally, 10ml/kg). Counting of the number of abdominal writhings or stretchings started ten minutes after acetic acid injection and was done for a period of 20 minutes.

Tail flick test

The method used by Connor *et al.* (9) was employed with minor modifications. Animals were restrained in a plastic cylinder with the tail protruding. The tip of the animal's tail was exposed to a focused beam of light from a tail flick analgesiometer (Harvard Tail flick Analgesia meter, Harvard Apparatus, GmbH, Germany). The end point was taken as the time at which the animal responded by vigorously jerking its tail from the path of the beam of light thereby exposing a photocell, which stopped the timer.

Basal latencies were determined twice at intervals of 15 minutes prior to the administration of the various substances. The first reading was discarded. Test latencies were determined at 15, 30, 45, 60 and 90 minutes after test substance, vehicle or standard drug administration. A cut off time was imposed at three times the mean pre-drug latency to prevent tissue damage (10)**.**

Hot plate test

The method used by Connor *et al*. (9) was employed. The animals were gently placed into a glass beaker whose bottom surface was maintained at about 55^{o}C with a thermostat. The time between placing the animals on hot surface and licking of the limbs or jumping by the animals was recorded with a stopwatch as response latency.

Baseline latencies were determined twice at 15-minute intervals and the first reading was discarded. Latencies were then determined at 15, 30, 45, 60 and 90 minutes after test substance, vehicle or standard drug administration. A cut off time of three times the mean pre-drug latency was imposed to minimize tissue damage (10)**.**

Data analysis

For the writhing test, the mean number of writhings and the standard error of the mean were calculated. Data were presented as mean ± Standard error of the mean. For the tail flick and hot plate tests antinociception was quantified according to the method described in the literature (11) as percentage of Maximum Possible Analgesia (%MPA), which was calculated for each animal as

$$\%MPA = \frac{\left(Latency_{test} - Latency_{predrug}\right)}{(Cutoff - Latency_{predrug})} \times 100$$

The mean %MPA was calculated and a plot of mean %MPA versus time was made. Area under the effect (mean %MPA) versus time curve (AUC) from time zero to 90 minutes was calculated for each treatment using the linear trapezoidal rule (12, 13). The AUCs were employed for one-way analysis of variance ($p< 0.05$) followed by Bonferroni *post hoc* t tests. The same statistical tests were used for the writhing test.

Results

Writhing test

One-way ANOVA revealed that there were significant differences between means of the different groups ($F_{4, 25} =2.998$; $p<0.05$). As shown in Table 1, *A. aspera* at all doses produced an apparent decrease in the mean number of writhings as compared to the vehicle. Boneferroni *post hoc* t tests showed that the reduction was significant for the 600mg/kg dose ($p<0.01$). However, there was no significant difference in the reduction of the mean number of writhings caused by the extract at the 600mg/kg dose as compared to that caused by pethidine.

Tail flick test

One-way ANOVA revealed that the there was a statistically significant difference among the means of the different groups ($F_{4, 25}=14.9$, $p<0.0001$). *Post hoc* tests showed that the AUC was significantly higher after administration of 900mg/kg, 600mg/kg, 300mg/kg *A. aspera* extract and pethidine as compared to tween 80 ($p<0.01$). Moreover, effects produced by 900mg/kg and 600mg/kg were each statistically different from that produced by pethidine ($p<0.01$). Significant differences were also observed between the AUCs of 300mg/kg and 600mg/kg and between those of 300mg/kg and 900mg/kg ($p<0.05$).

Table 1. The effect of different doses of 80% methanol extract of roots of *A. aspera* in the writhing test in mice (n=6).

Substance administered	Dose and route of administration	Number of writhings
A. aspera	900mg/kg, oral	24.67±12.26
A. aspera	600mg/kg, oral	12.83±8.55*
A. aspera	300mg/kg, oral	28.33±9.05
Vehicle	1ml/30g, oral	50.33±8.20
Pethidine	30mg/kg subcutaneous	12.0±5.57*

* $p<0.01$

Hot plate test

The effect of treatment was statistically significant ($F_{4, 25}=6.98$; $p<0.001$) as judged by the AUCs. As shown in table 3, *A. aspera* increased the area under the curve at all doses as compared to the vehicle. *Post hoc* t tests did not show any statistical difference among the effects produced by different doses of the extract and between those produced by different doses of *A. aspera* extract and that of pethidine.

Table 2. The effect of different doses of *A. aspera* and that of controls on area under effect (Mean %MPA) versus time curves in the tail flick test in mice (n=6).

Substance administered	Dose and route of administration	AUC (mean ± SEM)
A. aspera	900mg/kg, oral	8219.85±30.15**
A. aspera	600mg/kg, oral	6785.395±474.53**
A. aspera	300mg/kg, oral	4407.96±923.31*
Vehicle	1ml/30g, oral	1757.33±823.89
Pethidine	30mg/kg, subcutaneous	4584.61±880.37*

* $p<0.01$, **$p<0.001$

Discussion

The writhing test is a standard test for pain sensitive to opiates as well as non-opiates. The associated nociceptive response is believed to involve the release of endogenous substances, such as bradykinin and prostanoids, which stimulate nociceptive endings (14). The observation that the extract reduced the mean number of writhings suggests that it may have inhibitory effects peripherally on the formation and/or action of these algogenic substances.

The tail flick test, which is considered to be a spinal reflex, is efficient for revealing the activity of opioid analgesics, but not of partial opioid agonists (10). As the extract was shown to significantly delay the reaction time at all the doses tested, it may be speculated to have opioid like action.

The third test employed was the hot plate test. This test produces two behavioral components- paw licking and jumping, both of which are considered to be supraspinally-integrated responses. Both the paw licking behavior and the jumping reaction are abolished by opioids explaining why pethidine was effective. The plate was maintained at 55^{o}C, and at this temperature only opioid like agents are active (10). The effectiveness of the methanolic extract of the root of *A. aspera observed in the present study* could be due to an opioid action.

Table 3. The effect of different doses of *A. aspera* and that of controls on area under effect (Mean % MPA) versus time curves in the hot plate test in mice (n=6).

Substance administered	Dose and route of administration	AUC (mean ± SEM)
A. aspera	900mg/kg, oral	3456.84±920.82**
A. aspera	600mg/kg, oral.	2741.56±425.52**
A. aspera	300mg/kg, oral.	1100.54±462.5*
Vehicle	1ml/30g, oral.	-404.38±531.66
Pethidine	30mg/kg, oral	1493.85±293.01*

* $p<0.05$, ** $p<0.001$

As can be seen from Table 3, the 90 minute AUC of tween 80 was negative in the hot plate test but not in the tail flick test. The reason for this difference could be that the tail flick test involves exposing a small area of the tail to a noxious stimulus (15), whereas all the four limbs and even the tail of the animal are exposed to noxious stimuli in the hot

plate test. Therefore, the magnitude of nociceptive stimulus is higher in the hot plate test than in the tail flick test. As the extract produced antinociception in all the tests, it can be inferred that the compound(s) in the extract may have both a peripheral and central effect *A. aspera* was shown to contain alkaloids like andrachcinine and andrachcinidine, (+)-allosedridine, (-)-8-epi-8-ethylnorlobelol and (-)-8-epihalosaline (16), and many alkaloids have been shown to possess antinociceptive effects (17). Although further work is needed, the antinociceptive activity may reside in the alkaloids.

From the present study, it can be concluded that the methanolic extract of *A. aspera* possesses antinociceptive effects supporting its folklore use in Ethiopia. These effects were comparable to that of pethidine indicating opioid like actions. The mechanism (s) of analgesic action, however, should further be investigated. Evaluation of the extract for its antipyretic and anti-inflammatory effects may reveal the mechanism behind the antinociceptive effect.. Further work also needs to be done to test the extracts in other species or other models of nociception. Toxicity studies and further isolation and characterization of the active components responsible for the antinociceptive effect need to be carried out as well.

Acknowledgments

The authors acknowledge the financial support from the School of Graduate studies of Addis Ababa University and the Department of Drug Research, EHNRI for laboratory space to complete part of this work. We are grateful to Agere Yigezu, Etetu Mamo, Tejitu Lema and Bethelhem Tefera of the Faculty of Medicine, Addis Ababa University and to the staff of the Department of Drug Research, EHNRI for their unreserved cooperation.

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