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Comparison of Analgesic Effects of Khat (*Catha edulis* Forsk) Extract, D-Amphetamine and Ibuprofen in Mice

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Abstract

We have compared the analgesic properties of khat (*Catha edulis* Forsk) extract, amphetamine and ibuprofen in mice. After intragastric administration of the drugs analgesia was measured relative to water-injected controls using the hot-plate, the tail-flick, and abdominal-constriction tests.

At the highest doses examined (amphetamine 1·8 mg kg^{-1}, ibuprofen 90 mg kg^{-1}, khat extract 1800 mg kg^{-1}), all three substances produced analgesia, but the order of efficacy varied with the test. Khat and ibuprofen were significantly different from the control in the hot-plate assay at three or more time points post-injection. In the tail-flick test, khat and amphetamine were efficacious; ibuprofen means were somewhat lower but still significantly different from control. Higher doses of the drugs decreased the number of responses in the acetic acid-induced abdominal-constriction assay.

We conclude that khat, like amphetamine and ibuprofen, can relieve pain. Differences in assay results may reflect differences in modes and sites of action, as well as in the type of pain generated by the chemical and thermal stimuli for nociception.

When chewed, the leaves of the khat tree (*Catha edulis* Forsk) release many substances into saliva, including a number of alkaloids. One of these alkaloids, cathinone, is thought to be responsible for at least some effects desired by leaf chewers e.g. euphoria, alertness and anorexia, as well as for undesirable effects, e.g. drug dependence, hypertension and tachycardia (Widler et al 1994). Nencini & Ahmed (1982) reported that cathinone has analgesic properties in mice which they ascribed to monoaminergic and endogenous opioid mechanisms.

Cathinone and its analogues have emerged as drugs of abuse in economically developed nations (Sparago et al 1996), but are generally unavailable elsewhere. However, a huge number of East African and Arabian peoples have khat readily and legally available to them at low cost. Data on the analgesic properties of khat are sparse. Our aim in this study was to compare khat with amphetamine and ibuprofen in quantitative tests of analgesia.

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Amphetamine, which is often compared with cathinone because they share many pharmacologic effects (Kalix 1992), has antinociceptive activity (Görlitz & Frey 1972). Ibuprofen was included because it has unambiguous, clinically significant, analgesic and anti-inflammatory properties (Brooks & Day 1991).

Materials and Methods

Animals

All experiments were performed on mice (albino males, in-house bred, 25–35 g). They were kept 12 to a cage on straw bedding in an animal holding room with a 12-h light–dark cycle. Balanced diet pellets and tap water were continuously available. Changes in pain perception due to drug treatments were determined in a quiet laboratory with ambient illumination and temperature close to those of the holding room. Mice were allowed to acclimate to the testing area for 1 h before the experiments began. Models of

nociception in mice, two thermal and one chemical, were used. The Academic Commission of the Faculty of Medicine, Addis Ababa University approved detailed protocols for the experiments. Criteria used included humane treatment of animals before, during and after experimentation, and restrictions on the number of animals to the minimum required for statistical interpretation.

Treatments

Drugs were injected into the stomach via the mouth of awake mice through a blunted feeding needle fitted to a 1·0-mL syringe. Volumes injected were adjusted to 1·0 mL. Commercially available drugs given by the enteral route of administration, D-amphetamine sulphate (BDH) and ibuprofen sodium (Ciba-Geigy), were dissolved in distilled water. Khat extract (preparation described below) was also mixed with water, continually stirred while filling the syringe, and given via the feeding needle as a fine suspension. The drug doses tested were 1·8, 0·6 and 0·2 mg kg^{-1} amphetamine, 90, 30 and 10 mg kg^{-1} ibuprofen, and 1800, 600 and 200 mg kg^{-1} khat extract.

Khat extract was made from bundles of *Catha edulis* Forsk grown commercially in the Gurage region of central Ethiopia. Bundles were purchased at a local market in Addis Ababa; the extraction process began within 24 h of purchase. The leaves were finely chopped, weighed and placed in an Erlenmeyer flask containing reagent grade chloroform and diethylether (Fisher) in a 1 : 3 v/v ratio. The volume of volatile extractant was 200 mL more than that needed to just cover the minced leaves in the flask. With the flask stoppered, the contents were stirred continuously at room temperature for 24 h. The extractant was decanted, filtered (Whatman No. 1 filter paper), and subjected to vacuum. After the dry residue was weighed, 25% w/w glycerol was mixed into it to prevent it from forming a hard mass. Doses of the extract reported here were adjusted so as to represent only the khat without the glycerol. Weak solutions of glycerol in water (0·5–2%) were found not to be different from water alone in the assays used. Extracts were kept covered and refrigerated.

Analgesia measured by hot-plate test

The method of O'Callaghan & Holtzman (1975) was used except that a 600-mL glass beaker, instead of a metal box, was placed in the water bath. Temperature, monitored with a mercury thermometer immersed in the water bath, was maintained at 50 ± 0·5°C. The assay endpoint was the first instance of hind-paw licking. Elapsed time between placing the mouse in the beaker and the endpoint was measured with a stopclock (Pye Instruments, UK) to the nearest second. A 60-s cut-off was imposed to prevent tissue damage. Each mouse was tested twice before drug or water administration. The second reading was used as a baseline response for comparison with subsequent treatments (labelled zero dose). Tests were repeated for each mouse 10, 20, 30 and 40 min after drug or water administration.

Analgesia measured by tail-flick test

A radiant heat analgesiometer (Mark 18, Techno Electronics, Lucknow) was used. The technique was based on the concept of D'Amour & Smith (1941), except that the tail of the mouse rested in a groove 1·5 mm above a 63°C wire, rather than under a light beam focussed on the tail from above. Mice were held loosely in a cotton towel during the test. The endpoint was a flick of the tail that moved it away from the heat. Reaction time was measured with a stopclock to the nearest second. A 30-s cut-off was imposed as a protection against tissue damage. Each mouse was tested twice before drug or water administration. Results of the second test were used as baseline responses for subsequent comparison of treatments (labelled zero dose). The test was repeated for each mouse 10, 20, 30 and 40 min after drug or water administration.

Analgesia measured by acetic acid-induced abdominal constrictions

An intraperitoneal injection of acetic acid, 1·0 mL of a 0·6% solution, produced distinctive abdominal constrictions in mice consisting of contraction of muscles in conjunction with hind limb stretching. The response was first proposed as a screen for analgesics by Koster et al (1959). Mice were pre-treated enterally with one of the three drugs dissolved in distilled water or water alone, then placed in a clear perspex box. Abdominal constrictions were counted for 20 min starting 60 min after drug injection and 10 min after acetic acid injection. Antinociception was expressed as reduction in the mean number of constrictions for control (water only) mice vs drug-treated mice. Percent protection was calculated as follows: (control mean − treated mean) × 100/control mean.

Statistical analysis

Results are presented as means ± s.e. Statistically significant differences between treatment groups

were evaluated by analysis of variance followed by Dunnett's multiple comparison test. Probabilities less than 0·05 ($P < 0·05$) were taken as representing significant differences.

Results

The lowest doses tried (0·2 mg kg^{-1} amphetamine, 10 mg kg^{-1} ibuprofen, 200 mg kg^{-1} khat) were consistently below threshold in all the assays. Amphetamine 0·6 mg kg^{-1} and ibuprofen 30 mg kg^{-1} produced significant responses in the abdominal constriction test only. Results from the hot-plate and tail-flick assays were simplified by presenting data only for the highest doses (1·8 mg kg^{-1} amphetamine, 90 mg kg^{-1} ibuprofen, 1800 mg kg^{-1} khat extract).

Table 1 depicts the full range of dose–response relationships in the abdominal constriction assay. Reduction in the number of contractions produced by the highest doses of khat extract, amphetamine or ibuprofen were all significantly different from water only, but not from each other. Amphetamine 0·6 mg kg^{-1} and ibuprofen 30 mg kg^{-1} were also significantly different from control. Potency differences were major, amphetamine being about a thousand times more potent than the extract.

Time-effect curves for the highest drug doses in the hot-plate model are given in Figure 1. Responses of the mice at time zero (no drug) were not different from responses to water only, indicating that the intragastric injections were not analgesic. Ibuprofen appeared to be the most effective of the drugs in this test; significant differences from water controls were obtained at all four post-injection time points. The duration of ibuprofen's analgesic effect in this test was indeterminate. Simple straight line extrapolation of the slope between 20 and 40 min down to the 15 s (control level) of response would suggest a duration of about 70 min, but the curve could be more complex than a straight line.

Three of the four post-injection data points for khat were significantly different from vehicle control (Figure 1), with the response still significantly elevated 40 min post-injection. The response to khat extract was about half that of ibuprofen, but the dose of khat was 20-times that of ibuprofen. Amphetamine 1·8 mg kg^{-1} was not an effective analgesic in the hot-plate test.

In the tail-flick assay, all drugs tested were significantly analgesic (Figure 2). Khat extract seemed to be most active; time to tail flick was doubled at 10 min post-injection, with a peak effect almost triple control (water) around 40 min. A comparison of results for the two thermal assays suggests that the tail-flick test was more sensitive because drug responses were still being identified at a level much above control 40 min after enteral administration.

Discussion

Using three well known screening assays, we found that khat extract, ibuprofen and amphetamine have

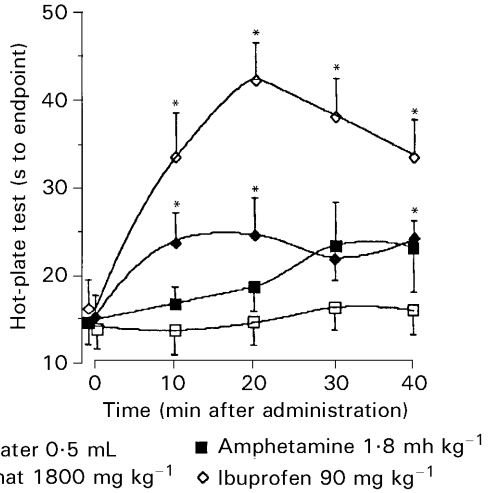

Figure 1. Analgesic effects of khat extract, amphetamine and ibuprofen in the mouse hot-plate test. Values shown are means ± s.e. for n = 6 mice. Drugs and the water vehicle were administered by intragastric injection. Values at time zero were obtained just before the mice were injected. Means for drugs significantly different ($P < 0·05$) from water at the times on the abscissa are marked with an asterisk.

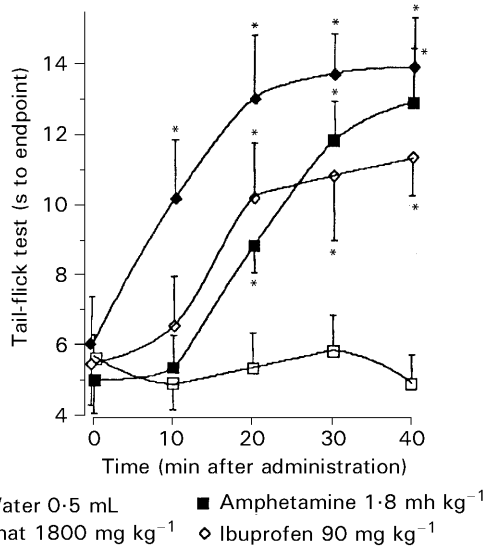

Figure 2. Analgesic effects of khat extract, amphetamine, and ibuprofen in the mouse tail-flick test. Values shown are means ± s.e. for n = 6 mice. Drugs and the water vehicle were administered by intragastric injection. Values at time zero were obtained just before the mice were injected. Means for drugs significantly different ($P < 0·05$) from water at the times on the abscissa are marked with an asterisk.

Table 1. Abdominal constriction responses to intraperitoneal injections of acetic acid (1·0 mL of a 0·6% solution) in mice pretreated enterally with compounds evaluated for antinociception. Abdominal constrictions were counted for 20 min.

Treatment	Dose ($mg\,kg^{-1}$)	n	Abdominal constrictions	% Protection
Water (control)	–	20	105·2 ± 12	–
Khat extract	200	6	87·5 ± 14	16
	600	6	76·1 ± 12	27
	1800	6	55·6 ± 10*	46
Amphetamine	0·2	6	82·9 ± 16	21
	0·6	6	69·3 ± 8*	34
	1·8	6	41·5 ± 9*	60
Ibuprofen	10	6	113·8 ± 17	0
	30	6	48·1 ± 9*	55
	90	6	33·9 ± 6*	67

Results are means ± s.e. $*P < 0·05$ compared with control.

analgesic properties as detected in one chemical and two thermal modes of nociception, however the orders of effectiveness varied with the assay. For example, ibuprofen was clearly a better analgesic in the hot-plate, but not the tail-flick, test. Other investigators have encountered similar experimental inconsistencies. Trentin et al (1997) reported that a plant extract evaluated for analgesic properties significantly affected abdominal constrictions and tests of paw oedema in mice, but was not different from control in the hot-plate and tail-flick assays. Apparently, variations between tests in the order of efficacy represent differences in the modes of action of the drugs and the types of pain elicited by the tests.

A useful way of classifying pain for purposes of studying and comparing analgesics is to distinguish between visceral and somatic types (Dewey et al 1994). Visceral pain is perceived as a diffuse, burning sensation mediated by polymodal C fibres. Somatic pain is localized, sharp and mediated by high-threshold, fast conducting A-delta fibres. Presumably, acetic acid-induced abdominal constriction represents a type of visceral pain, whereas tail flick occurs in response to a reflexive, somatic type of pain. The hot-plate test, which combines features of localized sharp pain with sensations of incipient paw pad oedema, may be an example of a mixture of pain types. By this line of reasoning and consistent with our observations, ibuprofen, which has distinct analgesic and anti-inflammatory properties as a consequence of inhibition of prostaglandin synthesis (Brooks & Day 1991), would be more effective in the abdominal constriction and hot-plate tests. Alternatively, the effectiveness of khat and amphetamine in our tail-flick assays could indicate analgesia brought about by inhibition of pain processing in the spinal cord. Ibuprofen, whose mechanism is independent of neurotransmission, would be expected to provide lesser efficacy in the tail-flick test of analgesia.

Uncertainties about modes of action of various khat alkaloids and of khat itself should not obscure the fact that a khat extract was shown to exert analgesic effects in mice, albeit at high doses relative to ibuprofen and amphetamine. In this regard, the crude leaf may have modest, but real, utility as a pain reliever in traditional herbal medicine. Moreover, modifications of phenylpropylamine structures of khatamines merit further investigation for possible analgesic properties.

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