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Analgesic principle from *Abutilon indicum*

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Bioactivity guided isolation of *Abutilon indicum* yielded eugenol [4-allyl-2-methoxyphenol], which was found to possess significant analgesic activity. At doses of 10, 30, and 50 mg/kg body weight, eugenol exhibited 21.30 ($p < 0.05$), 42.25 ($p < 0.01$) and 92.96% ($p < 0.001$) inhibition of acetic acid induced writhing in mice. At a dose of 50 mg/kg body weight, eugenol showed 33.40% ($p < 0.05$) prolongation of tail flicking time determined by the radiant heat method.

1. Introduction

Abutilon indicum (Malvaceae, Bengali name karandi or madni) is an erect, woody, gray, velvety, shrubby plant widely distributed in the tropical countries [1]. All parts of the plant are used medicinally. The leaves are useful as a demulcent, a decoction of the leaves is taken internally in bronchitis, catarrhal diarrhea, gonorrhea, inflammation of the bladder, chest affection, arthritis, fever, and as mouthwash in toothache. The bark and the root are used as a diuretic, anthelmintic, pulmonary sedative, and in fever and haematurea. Preliminary pharmacological screening of *Abutilon indicum* indicated significant analgesic activity. The seeds are used as a laxative in piles, and in the treatment of cough. According to the Chinese in Hongkong, the seeds are employed as an emollient and demulcent, the root is used as a diuretic and pulmonary sedative, and the flowers and leaves for local application to boils and ulcers [2]. Previous phytochemical investigation of *Abutilon indicum* showed it to contain saponins, flavonoids, alkaloids, and essential oils [3]. Another phytochemical investigation of this plant found gallic acid, β-sitosterol, β-amyrine, eudesmol, geraniol and caryophylline [4–6]. In addition to gossypetin-8-glucoside, gossypetin-7-glucoside and cyanidine-3-rutinoside, two sesquiterpene lactones named alantolactone and isoalantolactone have also been isolated from *Abutilon indicum* [7, 8].

Our present investigation of *Abutilon indicum* yielded a mixture of triterpenes in trace amounts, significant amounts of essential oils comprising stearic, palmitic, oleic, linoleic, and linolenic acids, in addition to eugenol which is chemically known as 4-allyl-2-methoxyphenol [9]. This is the first report of eugenol in *Abutilon indicum*.

2. Investigations, results and discussion

After extraction with a mixture of solvents consisting of petroleum ether, diethyl ether and methanol in equal proportions, the effect of the whole extract of *Abutilon indicum* on acetic acid induced writhing in mice was investigated to determine its analgesic activity [10]. The extract produced 64 ($P < 0.05$) and 70% ($p < 0.05$) inhibition of writhing at doses of 1 and 3 g/kg body weight respectively (Fig. 1). The extract also exhibited a positive result on tail flicking reflex in mice by the Radiant Heat method [11]. The extract showed 39.48 ($p < 0.05$) and 45.02% ($p < 0.05$) prolongation of tail flicking time after 30 min for oral doses of 1 and 3 g/kg body weight (Fig. 2). Separation of the whole extract by column chromatography yielded four fractions. The effect of all the fractions on acetic acid induced writhing in mice was studied to identify the fraction responsible for the analgesic activity. Among the fractions, AI-2 was found to be the most potent in terms of inhibition of writhing and it produced 56.2% ($p < 0.05$) inhibition of writhing at a dose of 1.5 g/kg (Fig. 3). Further fractionation of AI-2 was performed to find the analgesic principle. Column and TLC separation of AI-2 again gave four fractions among which fraction AI-2.1 produced 43.32% ($p < 0.05$) inhibition of writhing at an oral dose of 150 mg/kg body weight. Writhing inhibition by other three fractions were found to be insignificant (Fig. 4).

Fraction AI-2.1 was further purified by TLC and yielded two sub-fractions namely AI-2.1.1. and AI-2.1.2. Fraction AI-2.1.1 was obtained in very small quantity and seemed

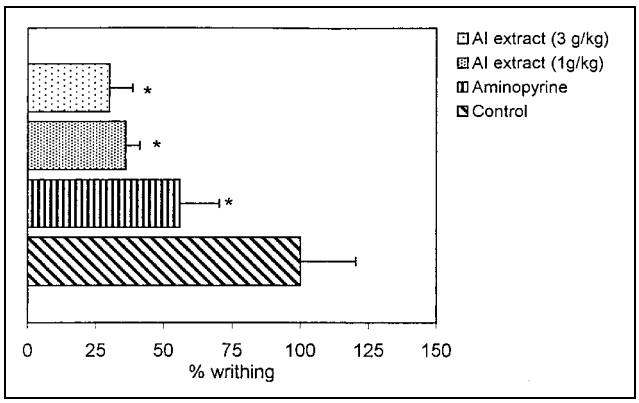


Fig. 1: Analgesic effect of the whole extract of *Abutilon indicum* (AI) on acetic acid induced writhing in mice. Each bar represents the mean ±S.E. The number of squirms in controls was taken as 100%. *$p < 0.05$

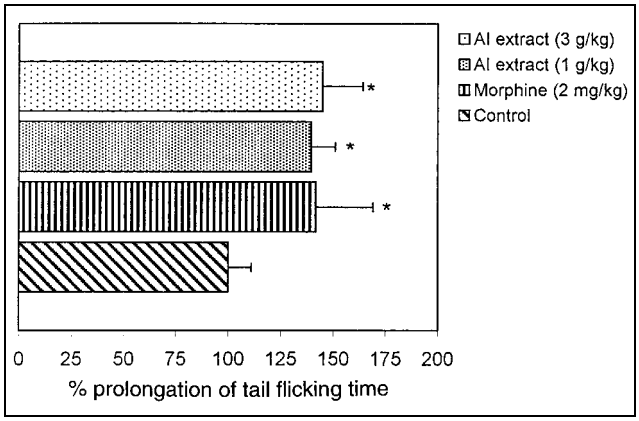


Fig. 2: Effect of whole extract of *Abutilon indicum* (AI) on tail flicking time in mice by radiant heat method. Each bar represents the mean ±S.E. The tail flicking time in controls was taken as 100%. *$p < 0.05$

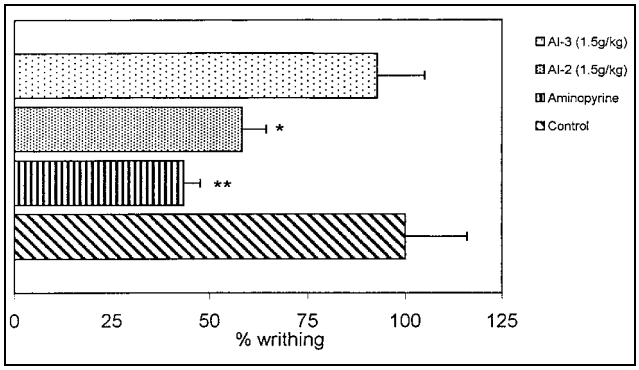


Fig. 3: Analgesic effect of fractions AI-2 and AI-3 on acetic acid induced writhing in mice. Each bar represents the mean ±S.E. The number of squirms in controls was taken as 100%. *$p < 0.05$, **$p < 0.01$

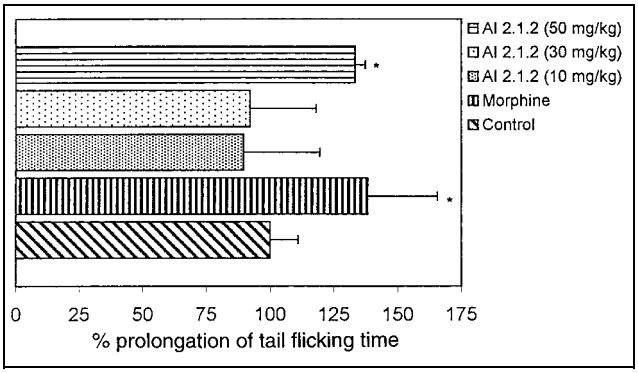


Fig. 6: Effect of AI-2.1.2 (eugenol) on tail flicking time in mice by radiant heat method. Each bar represents the mean ±S.E. The tail flicking time in controls was taken as 100%. *$p < 0.05$

to be an impurity. Fraction AI-2.1.2 was analyzed by spectroscopic techniques and found to contain only eugenol in the purest form (64 mg).

The effect of the pure compound eugenol on acetic acid induced writhing and tail flicking time was studied by the radiant heat method and a significant analgesic activity was observed. The oral administration of eugenol produced a dose dependent analgesic effect. It showed 21.61 ($p < 0.05$), 42.25 ($p < 0.01$) and 92.96% ($p < 0.001$) inhibition of writhing at oral doses of 10, 30, and 50 mg/kg body wt. respectively (Fig. 5).

Intraperitoneal administration of acetic acid creates inflammation in the peritoneum of the mice as it influences the secretion of prostaglandin. As a result, the animal squirms. Aminopyrine inhibits prostaglandin secretion and thus inhibits writhing. As eugenol also inhibits writhing, we assume that the compound acts through the same mechanism of action as that of aminopyrine.

Eugenol showed 33.40% ($p < 0.01$) prolongation of tail flicking time after 30 min at a dose of 50 mg/kg body wt. But the test sample did not show any significant activity at doses of 10 and 30 mg/kg body wt. after 30 min when compared with the control. Morphine at a dose of 2 mg/kg was used as a positive control which showed 38.26% ($p < 0.05$) prolongation of tail flicking time after 30 min when compared with the control (Fig. 6).

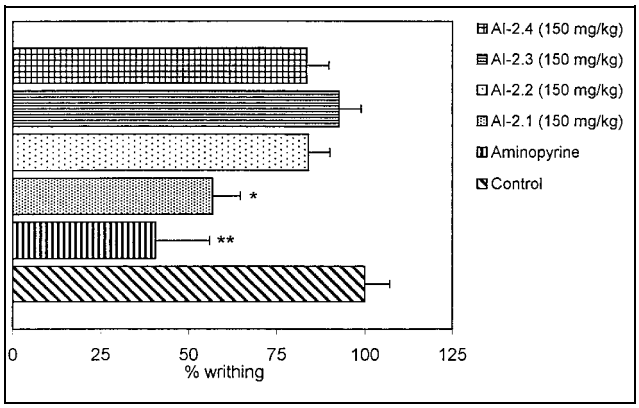


Fig. 4: Analgesic effect of fractions AI-2.1, AI-2.2, AI-2.3 and AI-2.4 on acetic acid induced writhing in mice. Each bar represents the mean ±S.E. The number of squirms in controls was taken as 100%. *$p < 0.05$, **$p < 0.01$

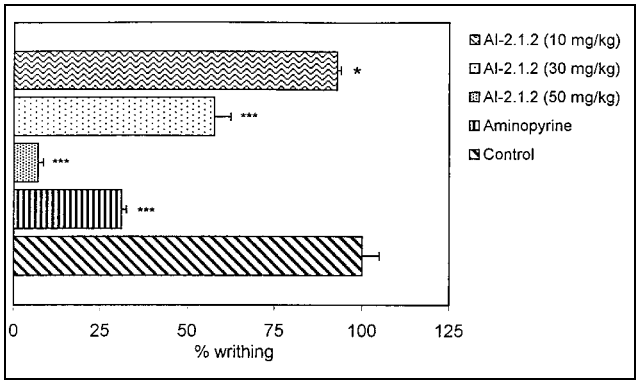


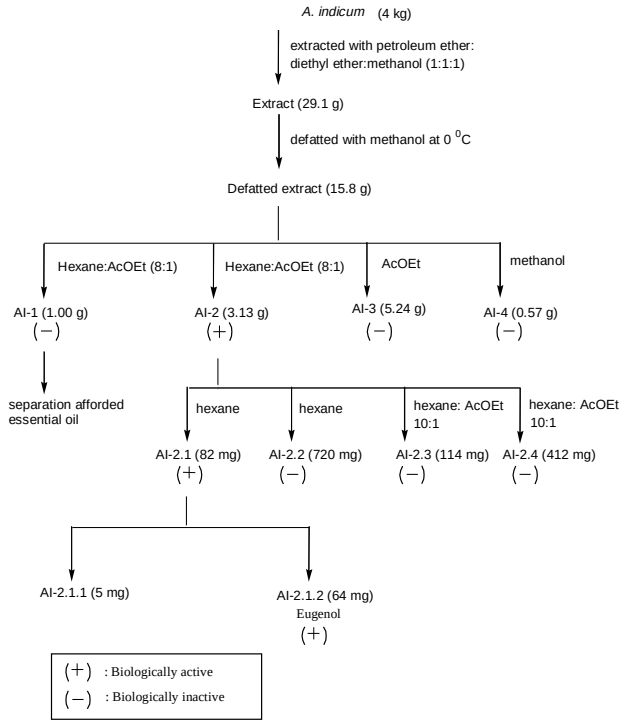
Fig. 5: Analgesic effect of AI-2.1.2 (eugenol) on acetic acid induced writhing in mice. Each bar represents the mean ±S.E. The number of squirms in controls was taken as 100%. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

3. Experimental

3.1. Chemistry

The plant was collected at the flowering stage from Dhaka during the last week of November 1997. The plant was taxonomically identified by the experts of the National Herbarium of Bangladesh and the Department of Botany, University of Dhaka. The coarse powder (4 kg) of the sun dried plant was extracted with a mixture of solvents comprising petroleum ether, diethyl ether and methanol in equal parts. The extract was evaporated to

Scheme



dryness and defatted. The resulting extract was separated by CC (silica gel) using hexane, ethylacetate and methanol with increasing polarity. The crude fractions thus obtained from CC were subsequently separated and purified by repeated TLC to give 64 mg eugenol. Eugenol, the compound under investigation, was characterized and identified by comparing its R_f value and spectral data with those of the reference compound. The isolation procedure is shown in the Scheme.

3.2. Pharmacology

Swiss-albino mice weighing between 22–35 g were employed as experimental animals. Experimental mice were randomly divided into different groups depending on the number of samples and doses to be applied with 4 mice in each group. In each group 4 different treatments were received by the 4 mice of each group i.e. control, positive control and two doses of samples. Each mouse was individually weighed and the doses of the test samples and control material were adjusted accordingly. The mice were kept at room temperature under conditions of a natural light and dark schedule (approximately twelve hours in light and twelve hours in darkness). The animals were kept for at least one week in the animal house to become adapted to the environment before being employed in any experiment.
In the experiments, the test samples were prepared as a suspension made with a few drops of Tween®-80 (1%) as a suspending agent.

3.2.1. Acetic acid induced writhing method

The analgesic activity of different fractions of the extract of *Abutilon indicum* and the pure compound eugenol was determined by the acetic acid induced writhing inhibition method [10]. In this method, acetic acid (0.7%) at a dose of 0.1 ml/10 g body wt. was administered intraperitoneally to the test animals to create a pain sensation which was demonstrated by the squirms produced by the animals. The squirms or contractions of the body are termed writhing. Each occurence of writhing is counted and taken as an indication of pain sensation. A standard analgesic (aminopyrine), control and test samples were given orally at zero hour. An interval of 40 min was given to ensure full absorption of the substance administered. Then acetic acid was administered intraperitoneally. After an interval of 5 min for absorption of acetic acid, the number of writhings was counted for 15 min. In each group of 4 mice, two were kept as control and positive control. Aminopyrine was administered as a positive control at a dose of 50 mg/kg body wt. If the sample has analgesic activity, the animal which received it will give a lower number of writhings than the controls i.e. samples which possess analgesic activity inhibit writhing. The writhing inhibition of aminopyrine was taken as standard and compared with the test sample and control. Two half writhings were taken as one full writhing. That is why half of the total writhings were taken to convert all writhings to full writhings or real writhings.

3.2.2. Radiant-heat method

In this method, the analgesic activity of drugs is determined by measuring drug-induced changes in the sensitivity of mice or rats to heat stress applied to their tails [11].
A Medicraft Analgesiometer Mask-N was employed for this experiment. Mice were placed in cages with the proximal third of the tail exposed over a holder containing a thin wire. Current was allowed to pass through the wire at low intensity (5 ampere) to make it hot. Within a few seconds the animal flicks its tail aside. The time between the application of heat and the tail flicking reflex was measured.

Acknowledgement: We thank Dr. H. Fujimoto, Lab of Natural Product Chemistry, Faculty of Pharmaceutical Sciences of Chiba University for recording some of the NMR spectra during this work. We express our sincere gratitude to JSPS for giving the financial support to conduct a part of the research work in Japan.

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Received July 28, 1999
Accepted September 3, 1999

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