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Analgesic principle from the bark of *Careya arborea*

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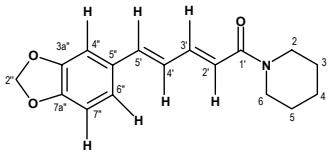
Bioactivity guided isolation of the bark of *Careya arborea* afforded piperine – an alkaloid chemically known as 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine, which was found to possess significant central and peripheral analgesic activity. At oral doses of 10, 20 and 30 mg/kg body weight, piperine exhibited 41 ($p < 0.01$), 45 ($p < 0.01$) and 53% ($p < 0.001$) inhibition of acetic acid induced writhing in mice respectively. At doses of 20 and 30 mg/kg body weight, the compound also showed 31.8 ($p < 0.05$) and 52.4% ($p < 0.01$) prolongation of tail flicking time of mice 30 min after the treatments determined by the radiant heat method.

1. Introduction

Careya arborea (Lecythidaceae, Bengali name Gadila or Kumbi) is a small shrub indigenous to India and widely distributed to other parts of the Indian sub-continent. The bark of *Careya arborea* is used in diarrhea, tuberculosis, skeletal fracture, cough, cold and skin diseases. Powdered bark is employed in snake bite; leaf paste is useful in ulcer and root promotes digestion [1]. Besides, the bark is also used as analgesic, antipyretic and antipruritic in traditional herbal practice [2].

Previous phytochemical investigation of *Careya arborea* showed that the bark contains terpenes and steroids [3]. The leaves have been reported to contain a triterpenoid lactone named careyagenolide, maslinic acid, 2-α-hydroxy ursolic acid [4], sapogenol [5], valoneic acid dilactone [6], ellagic acid, hexacosanol, α-spinosterol, β-sitosterol, quercetin and teraxeryl acetate [7]. The seeds of the plant have been found to contain olean-12,15-diene-3β,21α,22α,28-tetrol [8] and sterols [9].

In continuation of our work on the isolation, purification, characterization of bioactive molecules from the medicinal plants of Bangladesh, we investigated this time the bark of *Careya arborea* which yielded piperine, an alkaloid chemically known as 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine [10]. The structure of the alkaloid was elucidated by high field ^{1}H NMR, ^{13}C NMR, HMQC, COSY and MS techniques. Although piperine is mostly found in the plants of Piperaceae family, this is the first report in the plant *Careya arborea* and probably in the family Lecythidaceae.



2. Investigations, results and discussion

After extraction with distilled methanol, the bark extract of *Careya arborea* was evaluted for analgesic activity by acetic acid induced writhing and radiant heat method in mice [11, 12]. Given orally at doses of 500 and 250 mg/kg body weight, the extract produced 68 ($p < 0.01$) and 76% ($p < 0.01$) inhibition of writhing respectively (Fig. 1). Again thirty minutes after the administration, the extract prolonged the tail flicking time of mice by 42 ($p < 0.01$) and 78% ($p < 0.01$) at the same doses level respectively (Fig. 2).

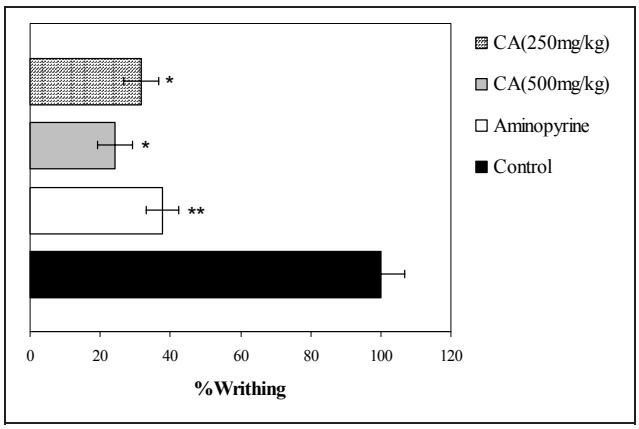


Fig. 1: Effect of crude bark extract of *Careya arborea* (CA) on acetic acid induced writhing of mice. Each bar represents mean ± SE. The number of writhing in control was taken as 100%. $^{**}p < 0.001$, $^{*}p < 0.01$.

Fractionation of the whole bark extract by column chromatography using petroleum ether, ether and methanol of varying proportions yielded six fractions and all these were subjected to acetic acid induced writhing test on mice to identify the fraction responsible for the analgesic activity. Among the fractions, CA-4 was found to be the most potent in terms of writhing inhibition and it produced 69% ($p < 0.05$) inhibition of writhing at oral dose of 150 mg/kg body weight (Fig. 3). So further fractionation of CA-4 was done in search of the anlagesic principle(s) responsible for the inhibtion of acetic acid induced

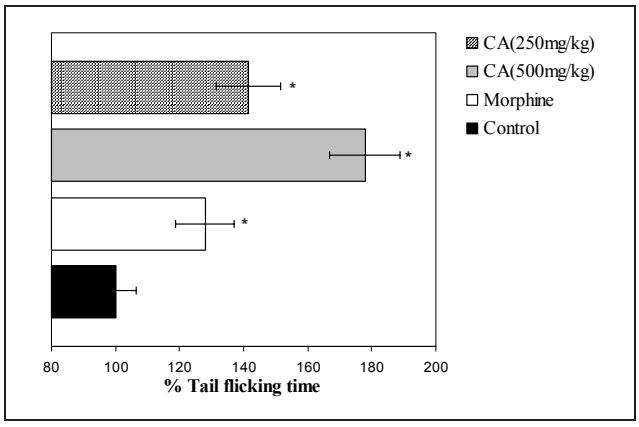


Fig. 2: Effect of crude bark extract of *Careya arborea* (CA) on tail flicking time of mice. Each bar represents mean ± SE. The tail flicking time in the control was taken as 100%. $^{*}p < 0.001$.

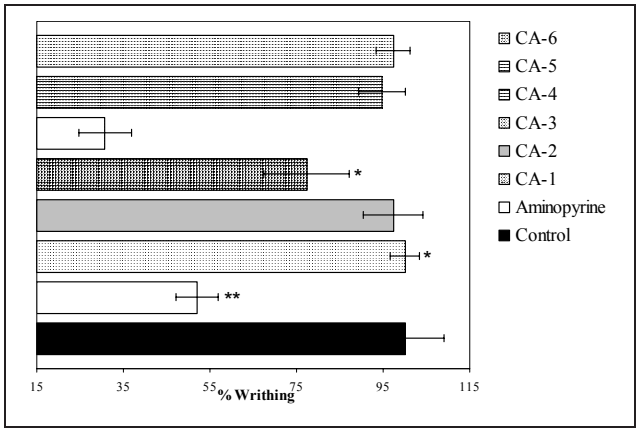


Fig. 3: Effect of different fractions of *Caerya arborea* bark extract on acetic acid induced writhing of mice. Each bar represents mean ± SE. The number of writhing in the control was taken as 100%. $^{**}p < 0.01$, $^{*}p < 0.05$.

writhing. TLC separation of CA-4 again afforded four fractions among which subfraction CA-4.3 showed the maximal 46% ($p < 0.01$) writhing inhibition at oral dose of 100 mg/kg body weight. The writhing inhibition of other subfractions were found to be insignificant (Fig. 4) and were discarded.

Separation of subfraction CA-4.3 afforded three subfractions, which were subjected to acetic acid induced writhing inhibition method and radiant heat method in mice. Among these, the most potent was CA-4.3A which showed 46.7% ($p < 0.001$) writhing inhibition (Fig. 5) and 38.5% ($p < 0.001$) elongation of tail flicking time (Fig. 6) in mice at oral dose of 50 mg/kg body weight. The activities of other two fractions were statistically insignificant and were discarded. The subfraction CA-4.3A was analyzed by spectroscopic techniques and found to contain only piperine in the purest form (53 mg).

The effect of piperine on acetic acid induced writhing and tail flicking time of mice was studied and a significant analgesic activity was observed. At the oral doses of 10, 20 and 30 mg/kg body weight, the compound dose dependently exhibited 38 ($p < 0.01$), 45 ($p < 0.01$) and 53% ($p < 0.001$) inhibition of acetic acid induced writhing in mice respectively (Fig. 7).

Intraperitonial administration of acetic acid (0.7%) causes localized inflammation in mice. Following inflammation,

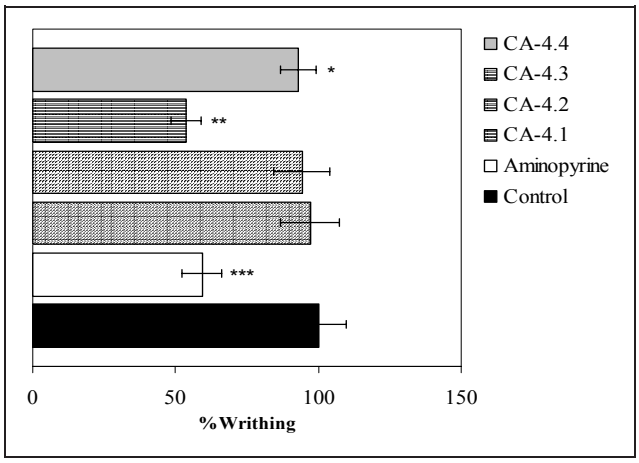


Fig. 4: Effect of sub-fractions of CA-4 on acetic acid induced writhing of mice. Each bar represents mean ± SE. The number of writhing in the control group was taken as 100%. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

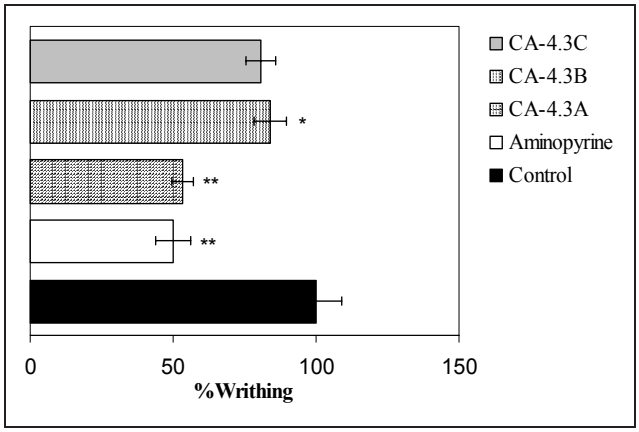


Fig. 5: Effect of sub-fractions of CA-4.3 on acetic acid induced writhing of mice. Each bar represents mean ± SE. The number of writhing in the control was taken as 100%. $^{**}p < 0.001$, $^{*}p < 0.01$.

there is biogenesis of prostaglandins (from cyclooxygenase pathway) and leukotrienes (lipooxygenase pathway). The released prostaglandins, mainly prostacyclin (PGI_2) and to lesser extent prostaglandin-E have been reported to be responsible for pain sensation caused by intraperitoneally administered acetic acid [13]. Aminopyrine, like other NSAIDs, inhibits the prostaglandin synthesis and thus inhibits writhing. As piperine has also been found to inhibit writhing, it can be assumed that the compound acts through the same mechanism of action as that of aminopyrine.

At oral doses of 10, 20 and 30 mg/kg body weight 30 min after administration, piperine showed 23.5 ($p > 0.05$), 31.8 ($p < 0.05$) and 52.4% ($p < 0.001$) prolongation of tail flicking time in mice. Morphine as positive control at a dose of 2 mg/kg body weight showed 43.3% ($p < 0.001$) elongation of tail flicking time after 30 min when compared with the results of control (Fig. 8).

Application of radiant heat in mouse's tail stimulates non-myelinated C fibers, which then excessively release substance P. In the acetic acid induced writhing model, prostaglandings (mainly prostacyclin) stimulate C fiber but in radiant heat method, C fibres are stimulated by radiant heat-serving as noxious stimuli. Opoid analgesics like morphine, pethidine etc. are the agonists of μ, κ and δ receptors which are specific for endogenous opoid like peptides named endorphins, enkephalins etc. These receptors are located in the CNS. After binding to these receptors, opoid analgesics antagonize the action of substance P

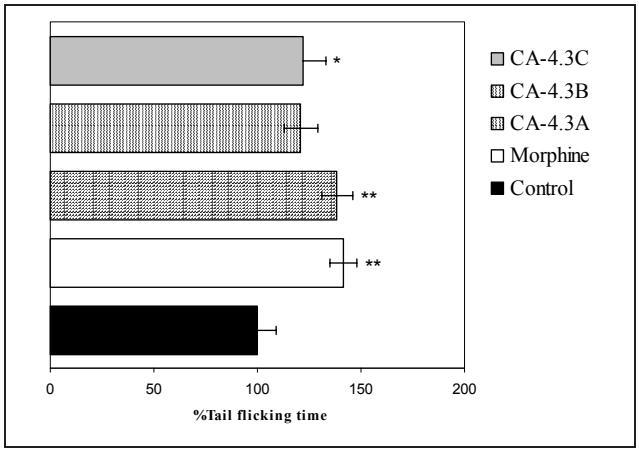


Fig. 6: Effect of sub-fractions of CA-4.3 on tail flicking time of mice. Each bar represents mean ± SE. The tail flicking time in the control as taken as 100%. $^{**}p < 0.001$, $^{*}p < 0.05$.

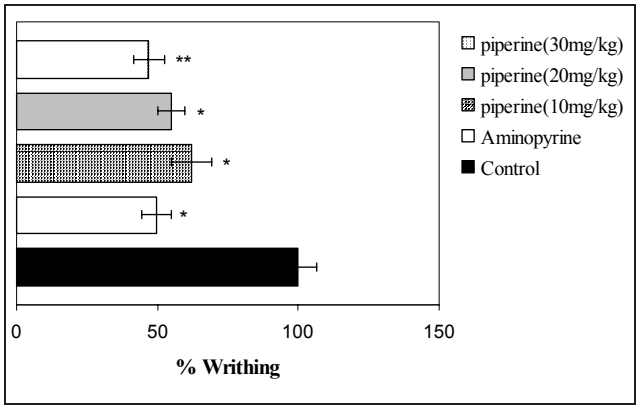


Fig. 7: Effect of piperine on acetic acid induced writhing of mice at different doses. Each bar represents mean ± SE. The number of writhing in the control was taken as 100%. $^{**}p < 0.001$, $^{*}p < 0.01$.

on the CNS by exerting postsynaptic inhibitory actions on interneurons and the output neurons of the spinothalamic tract, which process the nociceptive information to be conveyed to the higher centers of brain [14].

As piperine exhibited good analgesic activity in the radiant heat model, it is likely to act by a central antinociceptive mechanism like that of the opoids.

3. Experimental

3.1. Chemistry

The bark of the plant *Careya arborea* was collected in October, 1999 from Gazipur district in Dhaka. The leaves, flowers and bark of the plant were submitted to the Bangladesh National Herbarium and were positively identified by taxonomists. The coarse powder (3 kg) of the sun-dried bark was macerated with distilled methanol (6 l) for 2 days at room temperature. After maceration the filtrate was concentrated in a rotary vacuum evaporator at 45 °C to give a gummy concentrate (30 g) which was dissolved in methanol (30 ml) and subsequently defatted at refrigeration temperature. The defatted crude extract (23 g) was then fractionated by CC using petroleum ether (60–80 °C), ether and methanol with increasing polarity. The crude fractions obtained from CC were further separated and repeated TLC of one of the fractions ($CH_3OH : Et_2O = 1 : 9$) yielded 53 mg of piperine. The isolation procedure of piperine is shown in the Scheme.

3.2. Pharmacology

Swiss Albino mice of either sex, weighing between 22–26 g were employed as experimental animals. They were bought from the Animal Resource Division of International Centre for Diarrheal Diseases and Research, Bangaldesh (ICDDR,B). The mice were randomly divided into different groups depending on the number of samples and doses to be applied and consisted of 5 mice in each group. All the animals were individually weighed and the dose of the test samples and control materials adjusted accordingly. The animals were kept in the laboratory atmosphere for at least 7 to 10 days for acclimatization prior to any experiment. In the experiments, the test samples for administration were prepared as suspensions with a few drops of Tween-80 (1%) as suspending agent.

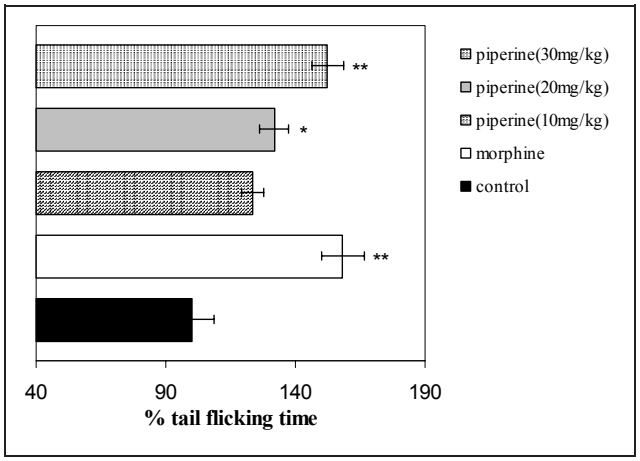
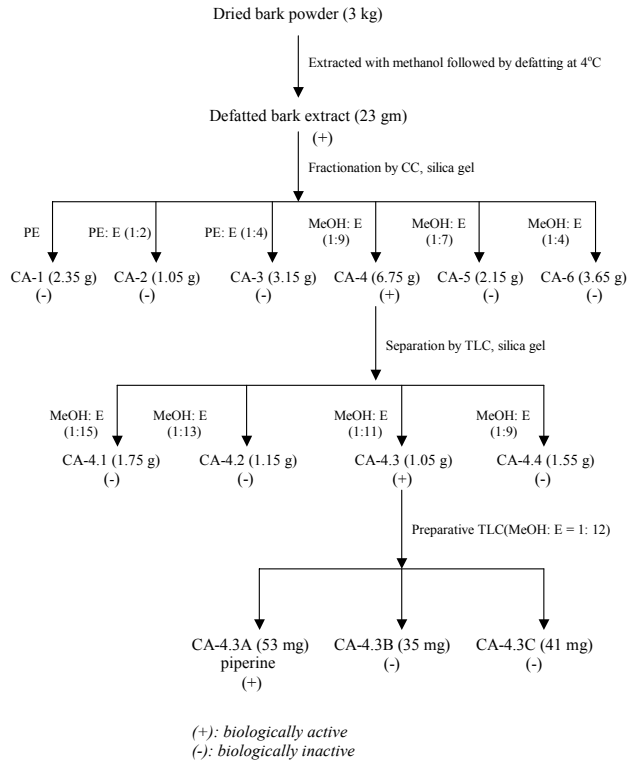


Fig. 8: Effect piperine on tail flicking time of mice at different doses. Each bar represents mean ± SE. The tail flicking time in the control group was taken as 100%. $^{**}p < 0.001$, $^{*}p < 0.05$.

Scheme



3.2.1. Analgesic activity in the acetic acid induced writhing model [11]

Acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a result, the animals squirm their body at regular intervals out of pain. This squirm or contraction of the body is termed as "writhing". As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to reduce the number of writhing of animals within a given time frame and with respect to the control group. As positive control, any standard NSAID like acetylsalicylic acid or aminopyrine can be used. The writhing inhibition of positive control was taken as standard and compared with those of test samples and control.

At zero hour test samples, control (1% Tween-80 solution) and aminopyrine at a dose of 50 mg/kg body weight were administered orally by means of a long needle with a ball-shaped end. After 40 min acetic acid (0.7%) at a dose of 0.1 ml/10 g body wt. was administered intraperitoneally to each of the animals of all the groups. The 40 min interval between the oral administration of test materials and intraperitoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five min after the administration of acetic acid, the number of squirms or writhing were counted for each mouse for 15 min.

Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Two half writhings were taken as one full writhing. Accordingly half of the total writhings were taken to convert all writhings to full writhing or real writhings.

3.2.2. Analgesic activity in the radiant heat model [12]

The animals were orally fed with the test materials at specified doses. After 40 min, each animal received morphine (2 mg/kg body weight) subcutaneously and was placed in an analgesiometer.

A constant thermal stress was applied to the tail of the mice. The thermal stress acts as noxious stimulus to mice. When the stimulus exceeded the pain threshold, mouse showed a quick withdrawal of its tail and this is known as the tail flicking response of mice. The time to evoke tail flicking response in mice is known as the latency of tail flicking response. Compounds with analgesic activity prolong the latency of tail flicking response.

By this test discrimination was made between centrally acting morphine-like analgesics and non-opiate analgesics.
A Medicraft analgesiometer (Mark-N, Medicraft, India) was employed for this experiment. Mice were kept into cages leaving the proximal third of their tail exposed over a holder having a thin wire. In order to make the wire hot, current was allowed to pass through the wire at a low intensity (5 amperes). Within a few seconds, the animal flicked its tail aside or tries to escape. The time for the reflex to occur was measured. The animals were submitted to the same testing procedure after thirty, sixty and eventually one-twenty minutes.

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