# EVALUATION OF ANALGESIA INDUCED BY MITRAGYNINE, MORPHINE AND PARACETAMOL ON MICE

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***The leaves of Mitragyna speciosa*** *were chewed as a substitute to opium in Thailand and Malaysia. A study was therefore undertaken to compare the antinociceptive activity of morphine and paracetamol to that of mitragynine, a major constituent of fresh leaves of* ***M. speciosa.*** *The tests employed were acetic acid induced writhings, hot tail-flick and cold tail flick. All test drugs were administered orally to mice. Results indicated that mitragynine (200 mg/kg) and morphine (5 mg/kg) reduced writhings from 17.5 ± 2.8 per 5 min to 9.6 ± 0.6 and 7.3 ± 0.6, respectively. Paracetamol (100 mg/kg) did not significantly reduce writhings in mice. All three drugs produced significant analgesia when tested by the hot tail-flick producing peak maximum possible analgesia (MPA)* of *35.1 – 4.8% (morphine), 27.8 ± 2.1% (paracetamol) and 42.8 – 7.2% (mitragynine). Morphine produced significant and marked analgesia when tested by the cold tail-flick technique achieving a peak MPA of 66.2 – 2.4% at 45 min following oral administration. Mitragynine produced a peak MPA of 49.0 – 5.9% at 30 min whilst paracetamol did not appear to be active. Mitragynine may be a potential new analgesic and requires further study.*

## INTRODUCTION

In 1932, Grewal reported that on the isolated ileum the alkaloid from Mi*tragyna speciosa* reduced the amplitude and tone of the smooth muscle. Perry (1980) quoted a report by Field and Quisumbing which stated that Mitragynine has a local anaesthetic effect and that the side-effects include dryness of the mouth, diuresis, constipation, with stools becoming small and dark, loss of appetite and subsequently a reduction in body weight. Jansen and Prast (1988) reported that the alkaloid from M.*speciosa* had opiate-like effects such as analgesia, antitussive and also cause hypothermia in animals. On the other hand, its action was not reversed by Nalorphine though it suppresses the opioid withdrawal syndrome.

It was thought that this novel analgesic action from the alkaloids of *M. speciosa* would be interesting to study since Nalorphine did not seem able to overcome its action (Idid *et al., 1992).* The alkaloid may be useful in the treatment of opiate addiction as a replacement therapy without the attendant dependence if it should act as an opioid agonist-antagonist.

## METHODS

### Preparation of Mitragynine

Mitragynine was extracted from fresh leaves of *M. speciosa* according to the method described by Ikram and Houghton *(1986).*

Preparation of Drug Solutions

Morphine sulphate, paracetamol and mitragynine were dissolved or suspended in *4%* acacia gum.

Antinociceptive Assay

Three types of antinociceptive assays were employed in this study; these were i) Writhings test, ii) Hot tail-flick and iii) Cold tail-flick test.

*(i) Writhing tests* The abdominal constriction test described by Collier *et al. (1968)* was used to measure the antinociceptive actions of morphine, mitragynine and paracetamol. Male or female albino mice weighing between *20-25* g were fasted for *24* hours with water given *ad libitum,* and were pretreated with oral *4%* acacia gum solution *(0. 15* ml/10 g), morphine *(5* mg/kg), paracetamol(100 mg/kg) and mitragynine *(200* mg/kg) 30 minutes prior to intraperitoneal injection of *0. 15* ml/l 0 g of *0.6%* acetic acid to cause a typical stretching response. Writhings or stretchings (abdominal constrictions) were coun-ted for a period of *5* minutes under a double blind observation. The antinociceptive effects of drugs were measured by calculating the mean reduction in the number of abdominal constrictions for each drug, as compared to gum acacia controls.

*(ii) Hot tail-flick* Male or female albino mice weighing between *20 - 25* g were fasted for *24* hours with water given *ad libitum,* maintained at room temperature and were divided into *4* groups of six mice. Mice were treated with *4%* acacia gum *(0.15* m1/10g), morphine *(5* mg/kg), paracetamol (100 mg/kg) and mitragynine *(200* mg/kg). Antinociceptive effect of the test substances was determined by the hot tail-flick method described by Sewell and Spencer (1976). One to two cm of the tail of mice was immersed in warm water kept constant at 50 °C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociception and was determined before and at 15, 30, 45 and 60 min after the administration of drugs. The maximum reaction time was fixed at 15 seconds. The maximum possible analgesia (MPA) was calculated as:

$$\text{MPA} = \frac{\text{Test reaction time - Saline reaction time}}{\text{15- Saline reaction time}}$$

(iii) *Cold tail-flick* Male or female albino mice weighing between 20 - 25 g were fasted for 24 hours with water given ad *libitum,* maintained at room temperature and were divided into 4 groups of six mice. They were then given the same treatment as in the writhing test. The reaction time of the mice was measured at 15, 30, 45 and 60 min. The antinociceptive activity was determined by the cold tail-flick test described by Pizziketti *et* al. (1985). One to two cm of the tail of mice was immersed in a cold 1: 1 mixture of water and ethylene glycol kept constant at -10 °C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as the mean of the last two readings.

The maximum reaction time was fixed at 30 seconds . The maximum possible analgesia (MPA) was calculated as:

$$MPA = \frac{\text{Test reaction time - Saline reaction time}}{\text{30- Saline reaction time}}$$

## STATISTICAL ANALYSIS

The experimental data were analyzed by two-tailed impaired student's ttest. P <0.05 was considered significant. Data were presented as mean ±SEM of *n* observations.

## RESULTS AND DISCUSSION

Acetic Acid-induced Writhings

Intraperitoneally injected acetic acid produced abdominal constrictions which is characterized by a stretching response. Mean writhings observed in control treated with vehicle (acacia gum) animals over a period of five minutes was 17.5 $\pm$ 2.8 counts. Morphine (5 mg/kg) and mitragynine (200 mg/kg) significantly ($p<0.05$) reduced writhings to 7.3 $\pm$ 0.6 and 9.6 $\pm$ 0.6 counts, respectively (Figure 1). On the other hand, paracetamnol (100 mg/kg), did not significantly reduce writhings induced by acetic acid.

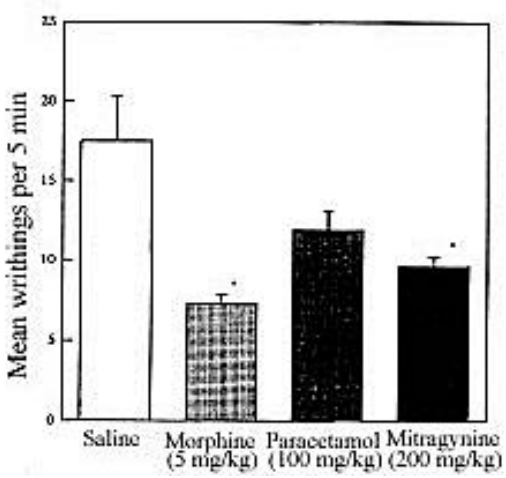


Figure 1. The effect of oral administration of morphine (5 mg/kg), paracetamol (100mg/kg) and mitragynine (200mg/kg) on acetic acid-induced writhings in mice (n=6). * significant at $p<0.05$

pathway involving other mediators than that due to paracetamol. This test also showed that 5 mg/kg of morphine is about equipotent to 200mg/kg of the crude extract. Other investigators have shown that the cold tail- flick method is a selective method able to screen centrally acting

opiate-fike analgesic agents, and is not sensitive to analgesics acting peripherally, such as aspirin, or non-analgesic drugs acting on the central nervous system, such as chlorpromazine (Pizziketti *et al.*, 1985). On the other hand, the hot tail-flick method is shown here as incapable of differentiating between opiate and non-opiate analgesics, or between peripherally acting or centrally acting substances. It is possible that the induction of analgesia by the alkaloid has both a peripheral and a central component. In the report of Jansen and Prast (1988) they found that nalorphine did not antagonize the effect caused by alkaloid from M *speciosa.*

Hot Tail-flick Response

Throughout the 60 min observation, animals pretreated with acacia gum did not show significant effect on the latent period of tail-flick response. The antinociceptive effect of morphine was evident within 15 min following oral administration. The mean possible analgesia (MPA) increased from 3.9 ± 3.7 to 35.1 ± 4.8 % which remained elevated above the basal levels throughout the observation period (Figure 2 and Table 1). Likewise, paracetamol also exhibited significant antinociception which began at 15 min following oral administration and the effect remained significant throughout the 60 min observation period ($p<0.05$). The MPA calculated for paracetamol increased to 27.8+ 2. 1 %. Similarly, the antinociceptive effect of mitragynine was also observed at 15 mins following oral administration and the effect remained significant throughout the 60 min observation period. The MPA calculated for mitragynine increased to 42.8 ± 7.2

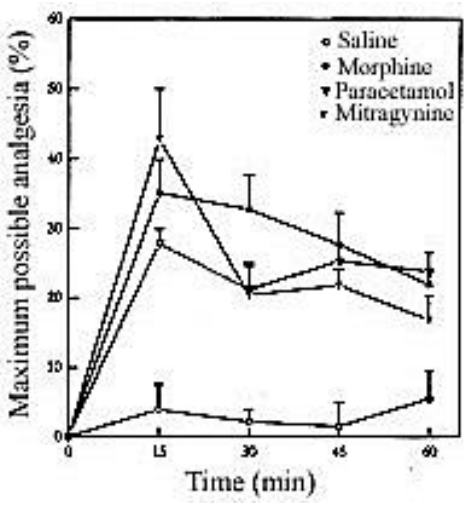


Figure 2. The effect of oral administration of morphine (5 mg/kg), paracetamol (100mg/kg) and mitragynine (200mg/kg) on maximum possible analgesia measured by the hot tail-flick method in mice (n=6).

Cold Tail-flick Response

While animal groups treated with gum acacia and paracetamol showed no significant effect on the latent period of tail-flick response, the antinociceptive effect of morphine was evident only after 30 min, reaching its peak at 45 min and this effect remained significant until the 60 min ($p<0.05$) test period. The peak MPA value calculated at 45 min was 66.2 ± 2.4 % compared to 0.1 ± 7.2 %

induced by the control acacia gum (Figure 3, Table 1). On the other hand, the antinociceptive effect of mitragynine was evident after 15 min reaching its peak at 30 min and this effect remained significant until the 45 min ($p<0.05$) test period. The peak MPA calculated at 30 min was 49.0 ± 5.9 %.

Table 1. MPA values calculated for morphine, Mitragynine and paracetamol using hot tail flick test (1) and cold tail flick (2)

| **Drug** | **1.MPA Value** ( %) | **2. MPA Value** (%) |
|---|---|---|
| Saline | 5.5 ± 3.9 | 2.2 ± 6.5 |
| Morphine (5 mg/kg) | 35.1±4.8 | 66.2 ±2.4 |
| Mitragynine (200mg/kg) | 42.8±4.2 | 49.0±5.9 |
| Paracetamol (100 mg/kg) | 27.8 ±2.1 | 21.2±9.0 |

Reports of the various uses of *Mitraynine* in traditional medicine in the countries of the South East Asia region (Jansen & Prast, 1988; Suwanlert, 1974) spurred research into the pharmacological aspects of its chemical constituents. More than twenty-five alkaloids have been identified from this plant (Ikram, 1885) but of these the major ones (in terms of % yield) are three indoles i.e. mitragynine, speciogynine and paynanthine and two oxindoles i.e. mitraphylline and speciofoline.

In a previous study (unpublished report) we had detected the analgesic property of the crude alkaloidal extract using the hot-plate method of Ankier (1974) and the tail-flick method of Sewell and Spencer (1976). The effect showed similarities to analgesia caused by morphine but its effect was not antagonized by Naloxone in the hot-plate test. This is interesting because although paracetamol also exhibited a significant effect in the hot tail-flick test, our additional experiment using the cold tail-flick test further separated the analgesic efficacy of the alkaloidal extract and morphine from that of paracetamol. The failure of paracetamol to exhibit antinociceptive effect in the cold tail-flick test suggests that analgesia induced by morphine and the extract is by a different pathway involving other mediators than that due to paracetamol. This test also showed that 5 mg/kg of morphine is about equipotent to 200mg/kg of the crude extract. Other investigators have shown that the cold tail- flick method is a selective method able to screen centrally acting opiate-fike analgesic agents, and is not sensitive to analgesics acting peripherally, such as aspirin, or non-analgesic drugs acting on the central nervous system, such as chlorpromazine (Pizziketti *et al.*, 1985).

On the other hand, the hot tail-flick method is shown here as incapable of differentiating between opiate and non-opiate analgesics, or between peripherally acting or centrally acting substances. It is possible that the induction of analgesia by the alkaloid has both a peripheral and a central component. In the report of Jansen and Prast (1988) they found that nalorphine did not antagonize the effect caused by alkaloid from M *speciosa.*

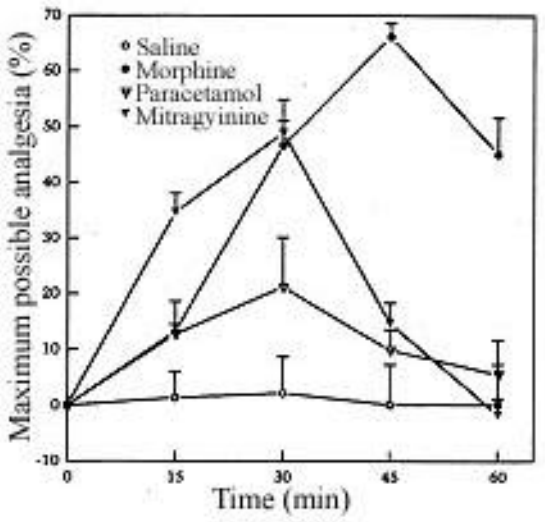


Figure 3. The effect of oral administration of morphine (5 mg/kg), paracetamol (I 00mg/kg) and mitragynine (200mg/kg) on maximum possible analgesia measured by the cold tail-flick method in mice (n=6).

Although it is recognized that the acetic acid writhing test is a useful measure of analgesic activity, the method often shows positive responses even to non-analgesic compounds such as central nervous system depressants (Hendershot and Forsaith, 1959). Consistent anti-nociceptive activity of the M.speciosa extract in all three methods used in the present study is in contrast with the typical antinocipceptive activity exhibited by paracetamol, which shows significant activity only in the hot tail-flick test. The failure of paracetamol to inhibit acetic acid induced writhings may imply that suppression of writhings may only be achieved by analgesic agents of higher efficacy or at higher doses of paracetamol. Antinociceptive effects of morphine and the alkaloidal extract were evident from the fact that both of these substances were markedly effective in reducing the responses in all three tests viz the writhing and the tail-flick tests. Thus the alkaloid from M.speciosa has definite analgesic properties but further study must be carried out to determine in which particular alkaloid this effect resides.

Our present study indicates that the alkaloidal extract has analgesic properties similar to opioid and, similar to morphine is effective in the acetic-acid writhing test, the hot tail-flick and the cold tail-flick tests. We have also shown that aracetamol is only effective in the hot tail-flick test.

## ACKNOWLEDGEMENT

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