# A MODIFIED HOT PLATE METHOD

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## ABSTRACT

*Hot plate induced-algesia is selectively inhibited by a central acting analgesic compound like morphine. This experiment investigated the factors involved in the hot plate method, begining with the temperature of the plate. Mice treated with either aspirin, diclofenac, morphine and Cardiospermum halicacabum, Linn. extract were placed on a glass cylinder, in a water bath to determine their response threshold. Each animal was tested four times at hourly interval. The first two times of testing before drug administration were averaged and represented the control threshold. The results show different levels of response in temperature and in time between those mice treated with paracetamol, diclofenac and those treated with morphine. If factors involved in the hot plate testing are known and controlled, the central and peripheral acting analgesic compound may be distinguished by the level of temperature and time of response.*

**Keywords: analgesic, hot plate, *Cardiospermum halicacabum.***

## INTRODUCTION

Among analgesic activity tests: acetic acid writhing test, hot-plate and tail pressure , the acetic acid test is the most commonly used (11: 6: 3).It is used to detect the effect of both central acting analgesic compounds such as morphine and peripheral acting compounds like aspirin. Considering standard drugs used in the hot plate method, morphine sulfate and aspirin, there is no doubt about high level of pain response inhibition by morphine, but the inhibitory effect of aspirin is inconsistently reported. From no activity[1] ,little activity less than 10% [2,3] or even doubled jumping latency [4] has been reported. All these articles used a hot plate of 55± 0.5°C to 60 ± 1°C to assess pain responses, forepaw licking, hind-paw licking and jumping. Some researchers measure latency to pain response when mice elicit any responses [2,3,5] and some record latency to each response[4]. The cut off time on the plate varies from seconds to 2 min and the results of Valencia et al. (1994) showed that eventhough aspirin did not affect licking latency, the drug prolonged jumping latency. This raises the questions of which response latency is latency is sensitive for the action of aspirin ? and how we can demonstrate the effect of peripheral acting analgesic compound by the hot plate method? To meet such objectives, various analgesics with known mechanism of action and unknown action were studied. This experiment, therefore, included an analgesic herbal which grows widely in Khon Kaen, *Cardiospermum halicacabum*. The plant has been used in the form of decoction in the treatment of pain and inflammatory condition in India,Ceylon, China and West Indies[6] .

## MATERIALS AND METHODS

### *Animals*

Female albino mice of 25-35 gm were acclimatized in the glass cylinder for 4 hr before the experiment began.

### *Apparatus*

Water bath, thermometer, surface probe thermister connected temperature recorder and glass cylinder of 12.5 x 24 cm (d x h) were used in the experiments.

### *Chemicals*

Morphine sulfate, paracetamol (Siam Pharmaceutical, Bangkok), diclofenac (Hoechst Pharmaceutical, Bangkok) were injected intraperitonealy at 25, 200 and 40 mg/kg, respectively. *C. halicacabum* extract were fed orally at 0.75, 1.5 and 3.0 g/kg.

### *Methods*

Hot plate apparatus or hot plate analgesia meter is commonly used and set at 55 ± 1 ° C. A few researchers used just a glass flask placed in a water bath or a basin of 56°C water[1,5] .In any laboratory a water bath and a glass flask are readily available, they will be properly used in the hot plate test once the temperature of the glass surface where a mice is placed, is known. This experiment we used a glass cylinder of 12.5 x 24 cm (diameter and height) with initial temperature of 27.7 °C before being placed in a water bath of 72±1 °C. The inner surface temperature of the glass plate, where the mouse was placed, had the temperature increases by 0.2 °C/ sec. Five glass cylinders with known rate of temperature changes within 3 mins were used alternatively. Control threshold of each mouse was taken 2 times, at hourly interval before drug treatment and twice 1-hourly after the drug administration. The

elapse time to behavioral changes such as paw padding(shaking),paw licking and jumping were recorded.

## RESULTS

### 1. The study of pain responses and threshold temperature

The temperature changes in the glass cylinder (Fig. 1) during 0 to 2 min was constant at the rate 0.2 °C /sec and the temperature at 2 min was slightly above 50 °C, so the cut off time was set at 2 min. All mice except 2 (Table1) showed sequencing of responses, paw licking and finally jumping. The temperature which induced these responses was above 40 °C.

### 2. Alteration of responses after repeated testing

In Group 1 (Table 2) a high dose of paracetamol was tried in two mice. The treated mice got sick, prostation, but still responded to thermal stimuli. Therefore, the post-treatment data of the two mice was excluded.

The first time of testing, the mice exhibited sequencing of pain responses from paw padding, paw licking and finally jumping. The second time of testing only 2 mice showed paw licking before their jumping response (Table 2). The third and fourth time of testing only jumping was observed. This raises the questions whether they perceived pain or exhibiting learning response (avoidance). Another group (group 2, table 2) of 8 mice was tested under the same condition, allowing mice displayed any pain responses until they showed jumping response and the testing was terminated. The results of group 2-mice were similar to those of group 1-mice. Considering the temperature of the jumping response in group 1- and group 2- mice, as the number of times of testing increased the temperature of jumping was decreased to the level lower than the pain threshold temperature (37 versus 43 °C, respectively). The results indicate that learning avoidance takes part in the response. This disturbance masks the true pain responses, as a consequence, inconsistency of the response temperature or time is observed.

### 3. How to get a more consistent pain response?

In this experiment mice were taken out when they showed any response: paw padding, forepaw- or hindpaw-licking. There was no unified response for a particular mouse, so the pain responses were not categorized (Table 3). The response temperature of four time of testing was similar at 42 to 43 °C. In order to get such consistent and valid results as in Table 3, firstly, the observer must be assured in themselves of judging the pain responses. At least two occurrences of any pain responses must be observed in a short time span. Secondly, the testing should be blind, the observer should not know which animals were treated.

### 4. The temperature elicited pain responses in mice

Eight groups of 10 to 13 mice (totally 83 mice) were tested twice at hourly interval in the blind observation manner. The mice were acclimatized in the glass cylinder for 4 h before testing and those mice showed only jumping response in the first testing were excluded. If the threshold response at first testing was close to the second times as in the Table 4, one can take the average of the two value to represent the control threshold value. The response threshold temperature of mice is

**Table 1** Pain responses expressed as threshold temperature (mean ± SE ) from 8 mice which had sequencing of responses except one elicited only jumping.

| forepaw licking | | | hindpaw licking | | | jumping | |
|---|---|---|---|---|---|---|---|
| °C | # licking | # mice | °C | # licking | # mice | °C | # mice |
| Group 1 | | | | | | | |
| 43.0±0.73 | 3.4±0.81 | 7 | 43.5±1.5 | 4.6±0.81 | 7 | 47.8±1.0 | 8 |
| Group 2 | | | | | | | |
| 43.8±0.9 | 5.1± 0.83 | 7 | 41.5±1.7 | 9.7± 2.76 | 7 | 48.5±1.13 | 8 |

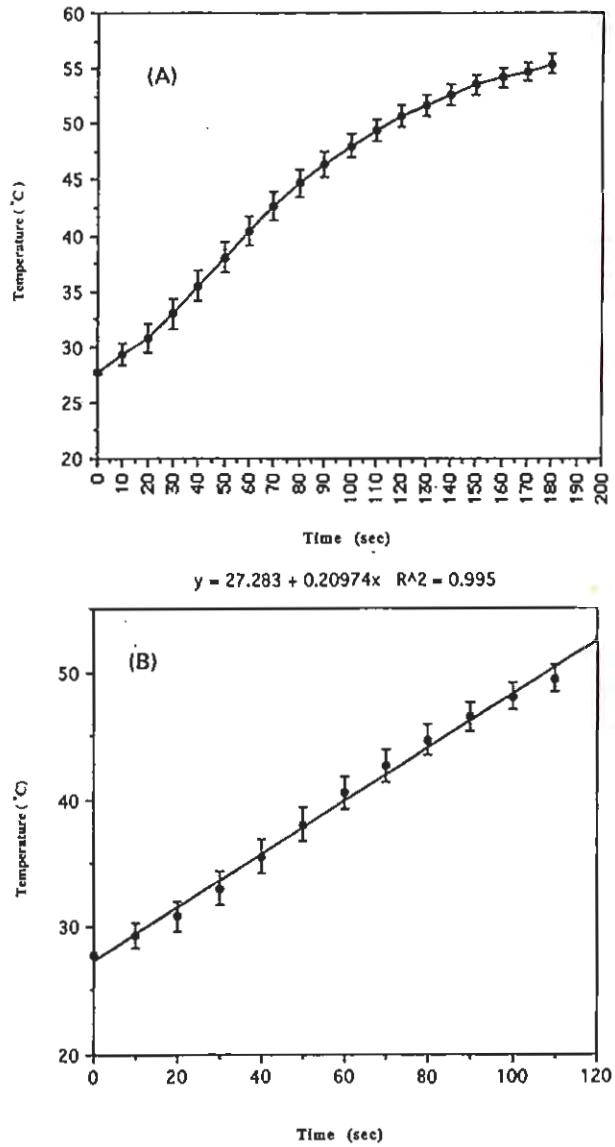


**Fig.1** Temperature at the inner surface of a glass cylinder where a mouse was placed, before (27.7 °C ) and during 3 minutes in 72 °C water bath (A). The glass floor has a constant rate (0.2 °C per second) of increases in temperature during 2 minutes with the goodness-of-fit of the regression line ( $R^2$ ) of 0.995 (B).Each point is the mean ± SE from 9 point of temperature recording.

**Table 2** Transformation of paw licking to jumping during four times testing at hourly interval. Each figure is the ratio of mice elicited the response out of total number of mice tested and the temperature ± SE when the animal exhibited first jump.

Group 1

| Responses | 1st measurement | 2nd | 3rd | 4th |
|---|---|---|---|---|
| forepaw licking | 7/8 | 1/8* | 0/6 | 1/6* |
| hindpaw licking | 7/8 | 1/8* | 0/6 | 1/6* |
| jumping | 8/8 | 8/8 | 6/6 | 6/6 |
| jumping temp. | 47.8±1.0 °C | 42.4± 1.3 °C | 40.8± 1.5 °C | 38.3± 2.5 °C |

* different mice

two mice being treated a high dose of paracetamol were excluded

Group 2

| Responses | 1st measurement | 2nd | 3rd | 4th |
|---|---|---|---|---|
| forepaw licking | 7/8 | 1/8* | 0/8 | 0/8 |
| hindpaw licking | 7/8 | 1/8* | 0/8 | 0/8 |
| jumping | 8/8 | 8/8 | 8/8 | 8/8 |
| jumping temp. | 48.5±1.13 °C | 42.5± 0.81 °C | 40.3±1.52 °C | 37.8±1.63 °C |

**Table 3** A more consistent pain responses in a 4-times testing at hourly interval of 11 mice (mean ± SE).

| | 1st measurement | 2nd | 3rd | 4th |
|---|---|---|---|---|
| pain response | 42.0±1.0 °C | 42.5± 0.91 °C | 43.8±0.83 °C | 42.86± 0.97 °C |
| # mice | 11 | 11 | 11 | 11 |

**Table 4** Pain responses temperature ± SD of 8 groups of 10 to 13 mice taken twice at hourly interval in a blind observation manner.

| | | Response | Temperature °C |
|---|---|---|---|
| Group | # mice | 1st testing | 2nd testing |
| 1 | 10 | 42.0 ± 1.88 | 42.15±1.05 |
| 2 | 10 | 42.1 ± 3.37 | 42.1 ± 2.94 |
| 3 | 10 | 40.0 ± 2.11 | 40.5 ± 3.27 |
| 4 | 10 | 42.4 ± 2.21 | 42.4 ± 2.13 |
| 5 | 10 | 42.2 ± 3.97 | 41.8 ± 1.78 |
| 6 | 10 | 41.5 ± 1.49 | 42.2 ± 1.93 |
| 7 | 10 | 40.0 ± 3.50 | 40.1 ± 2.60 |
| 8 | 13 | 41.5 ± 2.42 | 41.7 ± 2.59 |
| | **Average** | 41.45 ± 2.76 °C | 41.59 ± 2.42 °C |

**Table 5** Effects of various analgesic compounds and *C. halicacabum* extract on the latency of pain responses.

| | | Av. Control response time | Mean Time of Response ( sec ± SE ) | | Paired t-test | | % responder | |
|---|---|---|---|---|---|---|---|---|
| group (mg/kg) | n | 0 h | 1 h | 2h | 1h | 2h | 1h | 2h |
| Control | 11 | 50.55±3.90 | 54.55±4.10 | 53.73±4.72 | NS | NS | 0 | 0 |
| Paracetamol 200 | 11 | 55.05±3.92 | 61.45±4.95 | 58.73±4.77 | NS | NS | 27.3 | 27.3 |
| Diclofenac 40 | 11 | 48.59±5.35 | 57.82±5.17 | 56.45±3.54 | p<0.05 | 0.05 | 18 | 0 |
| Morphine 25 | 10 | 48.55±6.24 | 81.20±18.8 | 75.90±10.0 | p<0.01 | 0.05 | 90 | 80 |
| *C. hali.* 0.75 | 10 | 50.70±2.54 | 65.40±3.52 | 59.70±3.63 | p<0.001 | 0.01 | 90 | 50 |
| *C. hali.* 1.5 | 10 | 52.35±4.39 | 57.00±3.70 | 64.20±10.6 | p<0.05 | NS | 0 | 10 |
| *C. hali.* 3.0 | 11 | 46.00±4.75 | 58.60±3.39 | 61.64±6.94 | p<0.05 | 0.05 | 9 | 27 |

* The difference in mean time of response from the control response time was tested by the paired t-test. The responder is the mouse elicited a prolonged response time greater than the group SD.

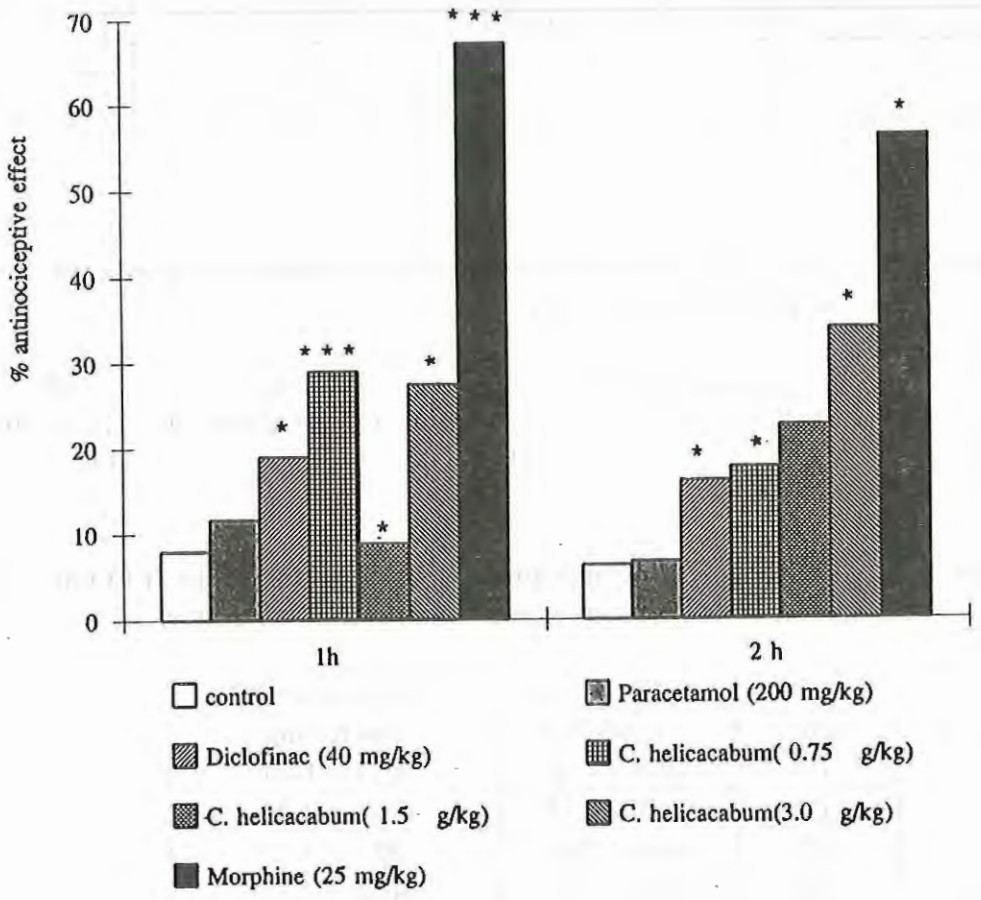


Fig.2 Effect of various analgesic compounds on the pain response time, expressed as % antinociceptive effect.

$$\% \text{ antinociceptive effect} = \frac{\text{mean treated time - mean control time}}{\text{mean control time}}$$

*p<0.05, ** p< 0.01 and ***p<0.01 significant level by paired t-test in Table 5

41.45 ± 2.76 °C (SD). A significant ($p < 0.05$) increase in the response temperature of 10 mice-group is less than 1 SD, determined by student t-test of homogenous and heterogenous variance (eg. 42.2 ± 3.97 versus X ± 1.05°C). The responses temperature higher than the mean control threshold by 1 SD is considered positive response or antinociceptive response. These can also be applied to the response expressed in time (or latency to any pain responses) if an animal are tested on the same cylinder throughout the experiment.

### 5. Effects of paracetamol, diclofenac, morphine and *C. halicacabum* extract on the pain response time

The pain response time was recorded twice before and after drug treatment. The average control response and the response time after 1 and 2 h of drug treatments: 25 mg/kg morphine sulfate, 200 mg/kg paracetamol,40mg/kg diclofenac and 0.75,1.5 and 3.0 g/kg *C. halicacabum* extract are shown in Table 5. In spite of the antinociceptive effect of paracetamol and diclofenac (Fig. 2) was low (10-20 %) there was some mice (18-27%) responder while there was none in the control group. A higher antinociceptive effect of 20-30 % with a few mice responders was observed in *C.* responders in the 0.75 mg/kg *C. halicacabum* treated mice. It was surprising to notice a high percentage of responders in the 0.75 mg/kg *C. halicacabum* treated group. Among the three concentration levels,0.75 mg/kg dose has the lowest viscosity. The highest antinociceptive (50-60%) and responders (90%) was observed in the morphine treated group.

## DISCUSSION

Temperature of hot plate used in analgesic testing varies from 48 to 60 °C, but the commonly used temperature is 55 °C[2-4] .This experiment shows that mice exhibit pain responses to the temperature of 42 °C (41.45 ± 2.76).Paw shaking,fore-paw licking and hindpaw licking can be observed before the occurrence of jumping response. Unfortunately, the sequence is not unified even in the same mouse, therefore, the pain responses can not be categorized but clustered as "pain responses". To make sure that the elicited response is pain induced ,a mouse exhibit any response not the jumping was selected in the test without knowing how much trouble the jumping mouse can cause. Table 1 and 2 show that if the cut off-response is jumping, there is the transformation of other pain response into jumping which may be not the pain response since the temperature caused jumping is much lower than the pain threshold temperature. It seemed that the transformation of paw shaking and licking to jumping can be reduced by experimental manipulation (Table 3). Taking the mice off once any pain response was observed. Further studies are need to confirm this finding. In the hot plate method, a researcher usually tests a mice twice at 10 min interval before and once at 30 min or 1 h after drug administration (all in the references section). If what we have observed is true that the experimental manipulation is capable of delaying jumping response, one can perform a number of tests before jumping avoidance becomes the main problem of the testing.

The results in this experiment are in concordance to the knowledge that peripheral acting analgesics (paracetamol and diclofenac) exerts a low level effect and central acting analgesics (morphine)

exert a high level of effect in the hot plate method. Significant or insignificant effects of peripheral acting analgesics can be possibly reported since the deviation is derived from the number of responded mice and level of the elicited response. Once factors confounded in the response time such as the different rate of temperature changes in the 5 cylinders used and jumping mice can be controlled, the deviation of the response time (SD) is subsequently reduced. This experiment used standard drugs, both paracetamol, diclofenac in conjuction with morphine to evaluate the analgesic level of a tested compound. *C..halicacabum* extract has low analgesic activity similar to diclofenac but gains high responders similar to morphine. A greater analgesic activity than a high dose of diclofenac interest us to find out how we can use activity level and percentage of responders to roughly distinguish narcotic analgesic from non narcotic analgesics.

If response time is what we want to record, various factors such as rate of temperature rise in all cylinders used, initial temperature of the glass cylinder, jumping mice and testing manipulation need to be studied again in order to explain clearly how these factors affect the response and how can we minimize these factor effects.

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