



Study on analgesic effects of basil (*Ocimum basilicum* L) extracts on mice

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SUMMARY

Objectives: To study analgesic effects of basil extracts (*Ocimum basilicum* L.) on mice.

Subjects and methods: The experiments were conducted according to heat stimulation (hot plate) and needle slide methods. In each experiment, mice were divided into 4 groups of 10 each, in which the negative control group was given distilled water, the positive one took the control drug, and the others were treated with basil extracts at the doses of 2.4 and 7.2 g/kg/day for 7 consecutive days. Mice in the control groups were drunk codeine phosphate 20 mg/kg or aspirin at a dose of 150 mg/kg depending on being in the hot plate or the needle pricking methods, respectively. The research indicators including response time to heat, pain force and pain response time of an individual mouse before and after drug administration were recorded. This study was conducted between October and November 2021 at Hanoi Medical University, Vietnam.

Results: At oral doses of 2.4 and 7.2 g/kg/day \times 7 days, basil extract showed better central analgesic effects in the treated groups than that in the control group ($p < 0.001$), but these pain relief levels were lower than that of codeine phosphate at a dose of 20 mg/kg/day ($p < 0.001$). In addition, basil extract had better effects on peripheral analgesia in the treated group than that in the control group ($p < 0.05$ and $p < 0.01$) and these effects were equivalent to those of aspirin at a dose of 150 mg/kg/day.

Conclusions: The aqueous extract of basil has both central and peripheral analgesic effects in white mice at the dose regimens of 2.4 and 7.2 g/kg/day \times 7 consecutive days.

Key words: Basil, *Ocimum basilicum* L., extracts, analgesic effects.

INTRODUCTION

Pain is a very common symptom in a number of human bodies' diseases. There are a lot of pharmaceuticals used in the modern era for strong and visceral pain relief such opioids (morphine, pethidine, ...), or as peripheral pain relievers like NSAIDs (indomethacin, diclofenac, ...). Besides, herbal medicines show good analgesic effects and are being applied in traditional medicine in some countries. For example, turmeric is highly effective in treating

stomach pain and ulcers, clove buds contain eugenol which has anesthetic and analgesic effects, and cherries are used to treat pains caused by arthritis and gout, et cetera.

Basil (*Ocimum basilicum* L.) has been considered a precious medicinal plant in many countries around the world. Some African countries use a decoction of the whole basil plant to numb, treat stomach pain, and to relieve pain during childbirth [1], [2], [3]. Basil juice is also used to relieve pain in toothache,

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earache (instilled in the ear to treat tinnitus, and difficulty hearing), treat headaches and pains caused by gout [3]. Furthermore, it works great in treating bacterial infections and repelling mosquitoes [4], [5].

With the objective of developing basil into a medicinal product used in the treatment of pain, this study was conducted to evaluate the central and peripheral analgesic effects of basil aqueous extracts in white mice.

MATERIALS AND METHODS

Time and location

This study was conducted between October and November 2021, at Hanoi Medical University.

Subjects

Sample: The aboveground parts of fresh basil were collected in Yen Xa, Tan Trieu commune, Thanh Tri district, Hanoi capital in May 2021. These collected samples were assessed for species confirmation at the Department of Botany, Hanoi University of Pharmacy before testing. Then, 2.4 kgs of basil were taken, and added 1 liter of water, which was simmered for 30 minutes and then filtered to obtain the first extract. The remaining basil was added to 1 liter of water and simmered for another 30 minutes. After that, the second extract was filtered out. Both the first and second above extracts were mixed and evaporated until 100 ml of the stock basil extract was obtained with a dark greenish yellow color and a basil-solvent ratio of 2.4:1 (corresponding to 240 g of basil /100 ml). This stock basil extract was diluted with different distilled water volumes to obtain extracts of individual concentrations used in the test.

Experimental animals: White mice (*Mus musculus* L.), Swiss strains, selected for the study were provided by National Institute of Hygiene and Epidemiology with the following criteria: both males and females, 4-5 weeks old, weight 20 ± 2 g, mature and healthy. Moreover,

the female ones must be non-pregnant, non-lactating and have never given birth. All mice were stabilized under experimental conditions for 3 days before being included in the experiment.

Appliances

- Aquatron water stills (Bibby sterilin company, UK).
- Sauter scale, accuracy $d = 0.1$ mg (for weighing study drugs).
- Precisa XB 320C digital scale, accuracy $d = 1$ mg (for weighing mice).
- Hot/Cold plate DS37, Ugo Basile, Italy.
- Dynamic Plantar Aesthesiometer 37450 pain meter (Ugo Basile, Italy).
- Jaw-head needles.
- Graduated glass beakers.
- 1 ml syringes (divided in 100 lines).

Chemicals and reference drugs

- Double distilled water.
- Pure codeine phosphate, provided by the National Institute of Drug Quality Control (NIDQC), purity 99.98%, batch No. CP6002.
- Pure aspirin, provided by NIDQC, 0.03% moisture content, lot No. 11968.

Methods

Hot plate method (Heat stimulation method)

Mice were divided into 4 groups in which each one had 10 mice including:

- Group 1 ($n = 10$): (control group) Mice were given distilled water with volumes corresponding to those of reference drug in 7 consecutive days.
- Group 2 ($n = 10$): Mice were given orally with codeine phosphate 20 mg/kg, equivalent to a dose with analgesic effect in humans.
- Group 3 ($n = 10$): Mice were given orally with basil extract at the dose of 2.4 g/kg/day (equivalent to expected dose in humans) in 7 consecutive days.



- Group 4 (n = 10): Mice were given orally with basil extract at the regimen dose of 7.2 g/kg/day in 7 consecutive days.

Before giving drugs or testing samples to mice, each mouse was placed on a hot plate that was maintained at a stable temperature of 56°C by a thermostatic system. The time interval from placing the mouse on the hot plate to the time the mouse flexed its hind legs and licked the paw was recorded. Mice that responded too quickly (before 8 seconds) or too slowly (after 30 seconds) were excluded from the study.

Subsequently, mice were given distilled water, reference drug or testing samples according to the dosage in each group as above, once a day in the morning, for 7 consecutive days. On the last day, 2 hours after taking the study samples, an individual mouse was placed on a hot plate, observed and recorded the mouse's response time to heat as described above. The reaction times to heat before and after using the study sample as well as those among batches were compared. [6]

Needle pricking method

Mice were divided into 4 groups in which each one had 10 mice including:

- Group 1 (n = 10) (control group) Mice were given distilled water with volumes corresponding to those of reference drug in 7 consecutive days.

- Group 2 (n = 10): Mice were given orally with aspirin 150 mg/kg, equivalent to a dose with analgesic effect in humans.

- Group 3 (n = 10): Mice were given basil extract orally at the dose of 2.4 g/kg/day (equivalent to expected dose in humans) in 7 consecutive days.

- Group 4 (n = 10): Mice were given basil extract at the regimen dose of 7.2 g/kg/day in 7 consecutive days.

Before being given the test samples, each

mouse was placed on the pain meter, measuring the force causing its pain and the response time to its pain. A mechanical agent (needle tip) was inserted into the mouse's sole with an initial pain force of 5 grams (to avoid mouse's tissue damage) and a force rate of 0.5 grams per second. The mouse responded by withdrawing its sole from the tip of the needle while the pain force and pain response time of each one was recorded. Then, mice were given water, the reference drug or test samples according to the dosage in each batch as above, once a day in the morning with a volume of 0.1 ml / 10 g for 7 consecutive days. On the last day, 2 hours after taking the sample, an individual mouse was put on the machine, measuring the pain force and pain response time. Comparisons were made to evaluate the differences in pain forces and pain response times between before and after drug administration of each batch and those among groups. [7]

Evaluation criteria

- Heat response time (seconds).
- Pain causing force (grams).
- Pain response time (seconds).

Data processing

Data expressed as mean \pm SD were processed by Excel program (Microsoft) according to the method of medical statistics, using Student's t-test and Fisher's exact test to compare the data before, during and after the test. Also, those data were compared among the control and treated groups. The difference was statistically significant when $p < 0.05$.

RESULTS

Evaluation of pain relief by hot plate method

Before and after 7 days of sample administration, all mice's pain response times were recorded to assess the effect of each test sample. The results are shown in table 1.

Table 1. Effects of basil extracts on heat response times of mice

Groups	Heat response times (seconds, mean ± SD)		p (before-after)
	Before	After	
1: The control, taken distilled water (n = 10)	15.45 ± 0.508	15.75 ± 0.704	> 0.05
2: taken codeine phosphate 20 mg/kg (n = 10)	15.16 ± 0.807	22.50 ± 0.704	2E-14 (< 0.001)
3: taken basil extract 2.4 g/kg/day × 7 days (n = 10)	15.84 ± 0.731	17.08 ± 0.551	0.0004 (< 0.001)
4: taken basil extract 7.2 g/kg/day × 7 days (n = 10)	15.24 ± 0.89	19.07 ± 0.715	3.6 E-09 (< 0.001)
p (1-2), p (1-3), p (1-4), p (2-3), p (2-4), p (3-4)	> 0.05	< 0.001	

The data from table 1 show that the mice's heat response times in the control group did not differ significantly between before and after being given distilled water for 7 days (p values > 0.05). Besides, mice in all 4 groups had similar heat response times before sample administration (p values > 0.05). Nevertheless, after being taken the reference drug or test samples, all mice in groups 2, 3 and 4 had statistically significant increases in response times to heat compared to that before taking codeine phosphate or basil extracts (p values < 0.001). In particular, the mice in the group 2 drunk codeine phosphate 20 mg/kg had the highest increase in reaction time to heat (22.50

± 0.704 seconds), followed by the group 4 drunk basil extract at the regimen dose of 7.2 g/kg/ day × 7 days (19.07 ± 0.715 seconds) and mice in the group 3 receiving basil extract at the regimen dose of 2.4 g/kg/day × 7 days (17.08 ± 0.551 seconds). Finally, the reaction time to heat in the groups 2, 3 and 4 after using the drug or samples at day 7 were higher significantly than that before administration (p values < 0.001).

Evaluation of analgesic effect by the needle pricking procedure

The analgesic effects of basil extract on mice assessed by needle pricking method are shown in table 2.

Table 2. Effects of basil extract on mechanical pain-induced mice

Groups	Pain causing force (grams)		p (force, before-after)	Pain response time (seconds)		p (time, before-after)
	Before	After		Before	After	
1: The control, taken distilled water (n = 10)	7.83 ± 0.403	7.62 ± 0.346	> 0.05	4.73 ± 0.581	4.96 ± 0.381	> 0.05
2: taken aspirin 150 mg/kg	7.75 ± 0.497	9.18 ± 0.437	2E-06 (< 0.001)	4.61 ± 0.458	5.71 ± 0.348	1E-05 (< 0.001)
3: taken basil extract 2.4 g/kg/day × 7 days (n = 10)	7.66 ± 0.484	8.06 ± 0.327	0.044 (< 0.05)	4.71 ± 0.513	5.31 ± 0.443	0.0119 (< 0.05)
4: taken basil extract 7.2 g/kg/day × 7 days (n = 10)	7.78 ± 0.531	8.37 ± 0.529	0.023 (< 0.05)	4.85 ± 0.638	5.55 ± 0.327	0.0064 (< 0.01)
p (1-2), p (1-3), p (1-4)*, p (2-3), p (2-4)*, p (3-4)**	> 0.05	< 0.01 ** > 0.05		> 0.05	< 0.01; * and **	> 0.05



Table 2 shows that before drug administration, pain forces and pain response times of mice in the control and all other groups did not have statistically significant differences (p values > 0.05). Meanwhile, mice in the group 2 had their significantly higher pain forces and pain response times after being taken aspirin 150 mg/kg compared to that before taking the drug (p values < 0.001). Similarly, in both groups 3 and 4 treated orally with basil extract at dose regimens of 2.4 and 7.2 g/kg/day \times 7 consecutive days, mice's pain forces and pain response times increased remarkably after 7 days of dosing compared to that before taking the drug (p values < 0.05 and < 0.01 , respectively). However, these study indexes of mice in groups 3 and 4 were not statistically significant ($p > 0.05$).

DISCUSSION

Analgesic effect on pain model induced by hot plate method

The hot plate method [6] with temperature as an agent is usually used to evaluate the analgesic effects of drugs that have a central analgesic mechanism such as opioids. In this study, codeine phosphate was appropriate to be selected as a positive control because it itself is an opioid and is metabolized in the body to morphine which is also an opioid.

Table 1 shows that the response times to heat of mice in the control group between before and after being drunk distilled water for 7 days did not change significantly ($p > 0.05$). This proved the machine system was operating stably and the control group had no change in pain response caused by heat. Apart from that, mice in all 4 groups had similar heat response times before taking the drug (p values > 0.05), showing that the mice selected for the study had a relatively uniform pain response. After being taken the drug or test samples, mice in groups 2, 3 and 4 all had statistically significant increases in response time to heat compared to

that before administration (p values < 0.001). In particular, the mice in the group 2 drunk codeine phosphate 20 mg/kg had the highest increase in reaction time to heat (22.50 ± 0.704 seconds), followed by the group 4 and 3 received basil extract at the dose of 7.2 and 2.4 g/kg/day \times 7 days (19.07 ± 0.715 and 17.08 ± 0.551 seconds, respectively). Additionally, the mice's reaction times to heat in the batches on day 7 were higher remarkably than that on day 0 (p values < 0.001).

The above results reveal that basil extract at both doses of 2.4 and 7.2 g/kg/day \times 7 days had good analgesic effects and significantly increased the time for mice to respond to heat pain ($p < 0.001$). Also, at a high dose of 7.2 g/kg/day \times 7 days, basil extract had better analgesic effect than that of regimen dose of 2.4 g/kg/day \times 7 days ($p < 0.001$). Nonetheless, the analgesic effects of basil extract at both the tested doses were still lower than that of codeine phosphate 20 mg/kg ($p < 0.001$).

Our results are also consistent with the results applied in traditional medicine of some countries. Accordingly, the decoction of the whole plant is used as an anesthetic and is highly effective in treating stomach pain, fever, and cough. In addition, basil decoction also worked to relieve pain during labor [1], [2], [3].

Analgesic effect on pain model induced by needle pricking method

The model of pain by pricking the needle using a mechanical agent (needle tip) acting on the mouse's soles to evaluate the analgesic effect of drugs with a peripheral analgesic mechanism such as non-steroidal anti-inflammatory drugs (NSAIDs). In this study, we used Aspirin as a positive control.

Table 2 reveals that mice in the control and all other groups had no statistically significant differences in pain forces and pain response times before sample administration (p values > 0.05). However, after 7 days of drug

administration, mice in the group 2 (being taken aspirin 150 mg/kg), group 3 and 4 (being received basil extracts at regimen doses of 2.4 and 7.2 g/kg/day \times 7 days) had pain forces and response times to pain higher significantly compared to that before taking the drug ($p < 0.001$, < 0.05 and < 0.01 , respectively). Additionally, the pain force and pain response time of mice in two groups drunk basil extract were not statistically significant ($p > 0.05$).

These results are also compatible with that of using basil extract to treat peripheral pains in traditional medicine of some countries, for example, basil juice has a very good effect on toothache, or earache, and is often instilled in the ear to treat tinnitus and difficulty hearing. Basil extract is also used to treat headaches, pain caused by gout, improve gastrointestinal function, and have a mild laxative effect. [3]

In summary, the results from the two above experiments show that basil extract has analgesic effect on both models with two pain agents, temperature and physical. In other words, basil extract has both central and peripheral analgesia with a weaker central analgesic effect than that of Codeine phosphate at a dose of 20mg/kg/day and equivalent peripheral analgesia effect to that of aspirin at a dose of 150 mg/kg/day.

CONCLUSION

We evaluated the analgesic effect of basil extract in white mice with oral regimen doses of 2.4 and 7.2 g/kg/day \times 7 consecutive days. The results revealed that basil extract had central analgesic effects in the experimental groups compared with that of the control group ($p < 0.001$), but these analgesic levels were less than that of codeine phosphate at the dose of 20 mg/kg/day ($p < 0.001$). Furthermore, it had peripheral analgesic effects in the

experimental groups compared with those of the control group ($p < 0.05$ and $p < 0.01$), and this peripheral analgesia was equivalent to that of 150 mg/kg/day of aspirin.

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