Analgesic Activity Of Piper Nigrum Extract Per Se And Its Interaction With Diclofenac Sodium And Pentazocine In Albino Mice

S Pooja, R Agrawal, P Nyati, V Savita, P Phadnis

Citation

Abstract
Objective: To evaluate the analgesic activity of Piper nigrum extract per se and its interaction with diclofenac sodium and pentazocine in albino mice.

Materials and Method: Healthy albino mice of either sex weighing 25-30 grams were taken and divided into 4 groups of 8 animals each. Peripheral analgesic activity was evaluated by acetic acid induced writhing test, using diclofenac sodium (5 mg/kg), Piper nigrum extract (10 mg/kg) and their combination (5+10 mg/kg) orally. Similarly central analgesic activity was studied using tail flick method and pentazocine (5 mg/kg) orally was used instead of diclofenac sodium.

Results:
Piper nigrum extract alone did not show any significant analgesic activity in tail flick and writhing methods. However results of acetic acid induced writhing model showed that diclofenac sodium reduces writhing 54.90% with respect to control when administered alone, but showed significant decrease in writhes 78.43% with respect to control when Piper nigrum extract was co administered with diclofenac sodium. When Piper nigrum extract combined with pentazocine showed significant increase in tail flick latency in comparison with pentazocine alone and control group (P<0.05).

Conclusion: The findings suggest that the Piper nigrum extract significantly increased the analgesic activity of diclofenac sodium and pentazocine.

INTRODUCTION
Black pepper is one of the oldest and best-known spices in the world. Belonged to Piperaceae family, which is cultivated in damp nutrient rich soil of South India, it is also found in Indonesia, Malaysia, and Brazil. In India it is called “kali mirchi”, which is a common household spice.

Its chief constituents are crystalline alkaloid piperine (5-8.25 %), volatile oils (1-2.3 %) and a resin called Chavicin.1 Black pepper was found to enhance intestinal absorption of methionine and calcium ions.3 Piperine, the best-known compound of pepper was reported to inhibit fatty acid oxidation in rat liver microsomes.3 It is also proved that it increases the bioavailability of theophylline, propanolol and nutrients.4

The present study was planned to investigate whether Piper Nigrum extract has any analgesic activity and if so how does it interact with diclofenac sodium and pentazocine?

MATERIALS AND METHODS
The study was carried out on albino mice (20-30 g), maintained under standard laboratory conditions of food and water before start of the experiment. All experiments were carried out between 0900 and 1700 hr according to the guidelines for the care of laboratory animals.5 Animals were also kept under observation for 7 days, after the completion of experiment to observe acute or sub acute toxicity. The Piper nigrum extract was from Amsar (P) LTD, Indore, India containing 95 % piperine.

DRUGS
All drugs were administrated orally half an hour before the onset of pain stimulus in different models of nociception in
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Vehicle 10 % v/v ethanol (10ml/kg), Diclofenac sodium (5mg/kg) in distilled water (D_a), Diclofenac sodium in 10 % v/v ethanol (D_b), Pentazocine (5mg/kg) in distilled water (P_a), Pentazocine in 10 % v/v ethanol (P_b). Test component Piper nigrum extract (10mg/kg) in 10 % v/v ethanol

STUDY DESIGN
A factorial study design was planned. The animals were divided into four groups, of 8 animals each.

Group A control (vehicle treated) 10 % v/v ethanol, and Group C test component Piper nigrum extract where similar in both the models of nociception.

For exploration of peripheral analgesic activity
Group B standard diclofenac sodium (solution D_b),
Group D combination of diclofenac sodium (solution D_a) and Piper nigrum extract

For exploration of central analgesic activity
Group B standard pentazocine (solution P_b),
Group D combination of pentazocine (solution P_a) and Piper nigrum extract

Piper nigrum extract was given half an hour before administrating standard drugs. Two different solution of diclofenac sodium and pentazocine in distilled water and in 10 % ethanol were used to maintain the ethanol concentration in all the groups and thus to maintain the homogeneity between the groups.

DETERMINATION OF ANALGESIC ACTIVITY
Antinociceptive activity was assessed by two different models of noiception.

1. Writhing test: For exploration of peripheral analgesic activity

Abdominal constrictions were induced by 1 % v/v glacial acetic acid solution (10ml/kg, i.p.) in mice pretreated with vehicle or one of the test substance. The number of abdominal writhing were measured over 20 min after the injection of acetic acid. Results were expressed as percentage inhibition of abdominal constrictions with respect to control.

2. Tail flick test: For exploration of central analgesic activity

The response was measured using the analgesiometer as described by the D'Armour and Smith. The tail flick latency was obtained thrice before drug administration, and mean was used as pre drug latency. The tail flick latencies were measured at 0, 0.50, 1, 2, and 4 h after the administration of vehicle or drug(s). A cut off time of 10 sec was planned to avoid any tissue damage in the animal. Results of tail flick latencies were expressed in terms of reaction time in seconds.

STATISTICAL ANALYSIS
Group results are expressed as mean ± SEM. One – way ANOVA followed by Tukey’s multiple comparison was applied for multiple comparisons amongst different groups. P < 0.05 was regarded as statistically significant.

RESULTS
ANALGESIC ACTIVITY OF INDIVIDUAL DRUGS

(a) Piper nigrum extract

Piper nigrum extract at a dose of 10 mg/kg did not show any significant effect on tail flick latency also failed to produce any decrease in the number of writhes (P>0.05).

(b) Diclofenac sodium

Diclofenac sodium at a dose of 5 mg/kg produced significant decrease in number of writhes (P<0.05).

Figure 1

Table 1: Effect of extract (PNE) in acetic acid induced writhings in mice

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Dose (p.o.)</th>
<th>Number of writhes</th>
<th>Inhibition with respect to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mg/kg</td>
<td>51 ± 0.71</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>5 mg/kg</td>
<td>23 ± 2.49</td>
<td>54.90</td>
</tr>
<tr>
<td>PNE</td>
<td>10 mg/kg</td>
<td>45.75 ± 1.65</td>
<td>10.59</td>
</tr>
<tr>
<td>Diclofenac + PNE</td>
<td>5 + 10 mg/kg</td>
<td>11 ± 2.64 * t</td>
<td>78.43</td>
</tr>
</tbody>
</table>

One way ANOVA
F = 87.02
P = 0.001

Values are mean ± SEM, n= 8 in each group, df = 3, 28
*P< 0.05 as compared to control
†P< 0.05 as compared to diclofenac group

(c) Pentazocine

In tail flick test, with a dose of 5 mg/kg of pentazocine, significant antinociceptive effect was observed 0.5 hr after
the treatment, reaching peak at 2 h and the effect persisted for the entire test period (P<0.05).

**Figure 2**

Table 2: Effect of (PNE) on tail flick latency in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Pne</th>
<th>Treatment</th>
<th>0.5 hr</th>
<th>1 hr</th>
<th>2 hr</th>
<th>4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mg/kg</td>
<td>3.37±0.32</td>
<td>4.23±0.11</td>
<td>4.36±0.03</td>
<td>4.38±0.03</td>
<td>4.33±0.02</td>
<td></td>
</tr>
<tr>
<td>Pentazocine</td>
<td>5 mg/kg</td>
<td>3.62±0.31</td>
<td>6.18±0.07</td>
<td>7.54±0.04</td>
<td>7.49±0.13</td>
<td>7.49±0.07</td>
<td></td>
</tr>
<tr>
<td>PNE</td>
<td>10 mg/kg</td>
<td>3.75±0.15</td>
<td>5.93±0.17</td>
<td>4.31±0.31</td>
<td>4.65±0.21</td>
<td>4.50±0.13</td>
<td></td>
</tr>
<tr>
<td>Pentazocine-PNE</td>
<td>5±10 mg/kg</td>
<td>3.08±0.09</td>
<td>9.90±0.32</td>
<td>9.23±0.47</td>
<td>9.55±0.97</td>
<td>9.69±0.79</td>
<td></td>
</tr>
</tbody>
</table>

One way ANOVA P<0.05 when compared to control group
P<0.01 when compared to treatments group

**ANALGESIC ACTIVITY OF DRUG COMBINATIONS**

Piper nigrum extract and diclofenac sodium

Diclofenac sodium in combination with Piper nigrum extract produced significant decrease in number of writhes when compared to control value or either of the treatment alone (P<0.05). [Table 1]

Piper nigrum extract and pentazocine

Pentazocine in combination with Piper nigrum extract produced significant increase in tail flick latency as compared to control or either of the treatment alone (P<0.05). [Table2]

No significant toxicity or mortality was observed during the observation period of seven days after the completion of experiment.

**DISCUSSION**

Writhing and tail flick methods are the most common test for evaluating the analgesic efficacy of drugs/compound in rodents. The abdominal constriction response induced by glacial acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The number of writhing observed during a 20 min period in control group was 51 ± 0.71 which correspond with the finding of other workers. Piper nigrum extract per se did not show any significant reduction in number of writhes at a dose of 10mg/kg, but when it was combined with diclofenac it significantly reduced the number of writhes greater than that was observed with diclofenac alone group (P< 0.001). We also observed that animals in combination group showed delayed onset of writhes (after 8 -9 min) as compared to other groups in which onset of writhes was within 5 min.

Tail flick method which is used for evaluating centrally acting analgesic effects of drugs showed no increase in latency when Piper nigrum extract alone was administrated. While in combination with pentazocine increased the pain threshold significantly (P <0.01) when compared to pentazocine alone group.

A previous study done with nimesulide in albino mice shows a better therapeutic index and increased effect of nimesulide when co administered with Piperine. It is also claimed that piperine has some anti inflammatory activity and anti convulsant activity.

Piperine has been reported to enhance the bioavailability of drugs like phenytoin, propanalol, rifampicin in humans. This occurs as a result of a nonspecific and noncompetitive inhibitor of the metabolic enzymes.

The result of the present study showed that Piper nigrum extract in dose of 10 mg/kg when combined with diclofenac sodium and pentazocine significantly improves analgesic activity of both the drugs.

In our experiment piperine is enhancing the activity of diclofenac and pentazocine the probable mechanisms of this enhancement may be:

Inhibition of metabolism: Piperine may inhibit different cytochrome P450 isofoms, UDP-glucuronotransferase, hepatic arylhydrocarbon hydroxylase and other enzymes involved in drug and xenobiotic metabolisms. Piperine has also been found to inhibit CYP3A4 an enzyme important for the metabolism and transport of xenobiotics.

Improvement in absorption: Piperine improving the absorption of drugs, perhaps by increasing GI blood flow or by increasing permeability of intestinal cells by stimulating gamma-glutamyl transpeptidase activity and increased amino acid uptake. The absorption may also be enhanced due to its emulsifying action in the gut.

Inhibition of efflux: Piperine also inhibits the efflux mechanism perhaps by inhibiting p- glycoprotein, the ‘pump' protein that removes substances from cells. This efflux
inhibition promotes the stay of drug within the target cells for a longer period.\textsuperscript{19}

Interference with solubilizer attachment: Some solubilizers such as glucuronic acid linked to the drug to enhance drug elimination. Piperine may inhibit this attachment as it has been shown to reduce the endogeneous UDP-glucuronic acid content of the cells.\textsuperscript{20}

Further studies are needed to reveal the exact mechanism of action responsible for the enhanced activity of diclofenac sodium and pentazocine. However the study adds to our concept that piperine can enhance the activity of diclofenac and pentazocine. So the addition of piperine may reduce the required dose of these drugs that may help in reduction of their toxicity. Hence we recommend further studies to acknowledge their facts.

ACKNOWLEDGEMENTS

The author is grateful to Mr. N. Peter Amsar (P) LTD, Indore

Technical assistance of Dr. L. Lakhwani and Dr. G. Pawar is gratefully acknowledged.

The authors would also like to thank Drs. A. Jaiswal and S. Kuchya for their guidance and support.

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