The Analgesic Effect of Pineapple Fruit Juice on Mice

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Abstract

Background: Pain is a feeling stimulated by the nervous system which can be suppressed by giving an analgesic agent. Some studies revealed that pineapples have an analgesic effect. This study aim was to determine analgesic effect of pineapple on mice.

Methods: In this experimental study, the effect was examined by using a writhing method on the 28 male mice. Subjects were divided into 4 groups with 7 mice each. The control group received aquades and other groups received pineapple fruit juice with 20%, 40% and 80% concentration with the dosage of 10 mL/kg/body weight. After 30 minutes, 3% acetic acid was injected intraperitoneally to induce pain. Writhing responses were observed every 5 minutes for 30 minutes.

Results: The result for mean of total writhing reaction was 2.39±0.40, 1.92±0.40, 1.50±2.13, 1.66±0.11 respectively for group 1 to 4. These data indicated a significant decrease of total writhing response in mice with 20%, 40% and 80% concentration compared to control group (p=0.023; p=0.000 and p=0.000 respectively). Most optimal concentration was 40% with the protective percentage equal to 71.8%.

Conclusion: Pineapple fruit juice concentrations (20%, 40%, and 80%) has an analgesic effect with the most optimal concentration of 40%. [AMJ.2014;1(2):100–4]

Key words: Analgesic, pain, pineapple fruit, writhing method

Efek Analgesik Jus Buah Nanas Pada Mencit

Abstrak


Metode: Efek analgesik diperiksa dengan menggunakan metode "writhing" pada 28 mencit jantan. Subyek penelitian dibagi menjadi 4 kelompok masing-masing 7 ekor. Kelompok kontrol menerima aquades dan kelompok lainnya menerima jus buah nanas secara oral dengan konsentrasi 20%, 40% dan 80% dengan dosis 10 mL/kg/berat badan. Setelah 30 menit, asam asetat 3% disuntikkan intra peritoneal untuk menginduksi nyeri. Respons "writhing" diamati setiap 5 menit selama 30 menit.

Hasil: Hasil rata-rata reaksi total "writhing" adalah 2.39 ± 0.40, 1.92 ± 0.40, 1.50 ± 2.13, 1.66 ± 0.11 masing-masing untuk kelompok 1 sampai 4. Data ini menunjukkan penurunan yang signifikan dari jumlah respond "writhing" pada tikus dengan 20%, 40% dan 80% konsentrasi dibandingkan dengan kelompok kontrol (p=0.023; p=0.000; dan p=0.000 masing-masing). Konsentrasi optimal adalah 40% dengan persentase pelindung sebesar 71,8%.

Simpulan: Konsentrasi jus buah nanas (20%, 40% dan 80%) memiliki efek analgesik dengan konsentrasi paling optimal pada 40%. [AMJ.2014;1(2):100–4]

Kata Kunci: Analgesik, nyeri, nanas, writhing

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Introduction

Pain is a feeling stimulated by the nervous system. It is a complex phenomenon that warns an individual about some tissue damage which currently is occurring or has occurred. It happens when there is a damage or injury, the tissue will release some chemical mediators such as bradykinin, and prostaglandin (PGE₂) which will stimulate pain sensation. This sensation can be suppressed by using analgesic drugs such as paracetamol and ibuprofen.

Some plants also exhibit analgesic effects such as pineapple (Ananascomosus). In previous study, pineapple had been proved as a plant that have an analgesic effect due to the presence of bromelain. This active compound which can be found in the root and stem of pineapple, bromelain, is a proteolytic enzyme which has many pharmacological activities such as analgesic agent, anti-inflammatory, prevent edema, and increase tissue permeability of antibiotic drugs.

In this study 3% acetic acid had been used to induce pain sensation on mice. Acetic acid is an irritation agent that when injected intraperitoneally, it usually induces hypersensitivity. The objective of this study was to determine the 20%, 40% and 80% of the pineapple fruit juice concentration which has the protective result as an analgesic effect.

Methods

The study subject used in this experiment were 28 healthy male mice (Mus musculus) with body weight of 20–30 gram each which then were divided into 4 equal groups. The research materials in this study were pineapple fruit juice as the intervention with the concentration of 20%, 40% and 80% for group 2, group 3 and group 4 respectively. To make 20% concentration of pineapple fruit juice, 20 gram pineapple was used. The pineapple was blended finely, filtered to get the juice and added with 100 mL distilled water. For concentration of 40% and 80%, 40 gram and 80 gram of pineapple fruit were used respectively and then the same procedure was applied.

Other materials used were 3% acetic acid as the pain stimulation and aquades as placebo. All mice were left freely for one week as an adaptation period in the laboratory with optimal room temperature without any effort of intervention. Prior to the experiment, all mice must undergo a fasting period for 18 hours with free access of drinking water.

Writhing method or abdominal contraction method was used in this study to evaluate the analgesic effect. For the experimental group, each will receive 20%, 40% and 80% pineapple fruit juice concentration respectively via oral and for the control group, aquades was given orally as a placebo. After 30 minutes, 30% acetic acid was injected intraperitoneally to induce pain. Right after acetic acid was given, writhing response was observed. The reaction was observed for 5 minutes every 30 minutes right after giving acetic acid. The writhing reflexes observed on mice included stretching of the body, retraction of the abdomen followed by withdrawing of the lower limbs backward.

Protective percentage in this study was calculated by using the formula of 100% - (total number of writhing in experimental group / total number of writhing in control group) x 100. For statistical analysis, analysis of variance (ANOVA) was used to see whether significant differences between groups. The test then subjected to Tukey HSD multiple comparison post test.

This experiment was done in November 2012 at the Animal Laboratory of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjadjaran, Jatinangor, Bandung, Indonesia.

Results

In this study, the data was analysed by using Shapiro-wilk test to find its distribution and due to data skewness original data of mean of total writhing reaction in mice was transformed into a new data to meet the criteria of parametric test ANOVA. The formula used was ((√ (x+1)). The new data has been recorded in table 1.

Table 2 showed a presence of significant difference in each group with significant value of 0.000 (p<0.05). Post hoc test by using Tukey HSD method was conducted to find out in which group the significant difference can be found (Table 3).

All three experimental groups showed significant reduction in total number of writhing response comparing to control group (Table 3). It means that the analgesic activity was significantly different from the control group (p<0.05), while in three experimental group, there was only a significant difference between group 2 and 3 (p<0.05).

Pineapple fruit juice with concentration of 40% showed the most protective effect as an analgesic than other, which was 71.8%.
Table 1 Mean of total writhing reaction in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean of total writhing reaction in mice</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.39</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>1.92</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>1.50</td>
<td>2.13</td>
</tr>
<tr>
<td>4</td>
<td>1.66</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Note:
Group 1: given aquades
Group 2: given 20% pineapple fruit juice concentration
Group 3: given 40% pineapple fruit juice concentration
Group 4: given 80% pineapple fruit juice concentration

Table 2 Difference Total Writhing Reaction Between Groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of total</td>
<td>2.39</td>
<td>1.92</td>
<td>1.50</td>
<td>1.66</td>
<td>0.000</td>
</tr>
<tr>
<td>writhing reaction in mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Then, it was followed by 80% concentration which was 61.6% and the last one was 20% pineapple fruit juice concentration which was 35.4% (Table 4).

Discussion

Based on its pathophysiology, pain can be classified into three types which are somatic, visceral and neuropathic pain. Somatic pain is usually located at the peripheral, visceral pain involves intra-abdominal organ, and neuropathic pain involves afferent neural pathway.8

This study was conducted to examine the analgesic effect of the pineapple fruit (Ananas comosus) juice on the mice models. The analgesic reactions were measured by the total number of writhing in the mice every 5 minutes for 30 minutes. The mean of total writhing reaction in mice was decreasing from the mean group until group 3 which received 40% concentration of pineapple fruit (Ananas comosus) juice and increased again in the group 4 when the mice in this group had 80% concentration of pineapple fruit juice (Table 1). This condition showed that the pineapple juice had a ceiling effect. Ceiling effect is a condition where at certain dosage, the drugs does not give any therapeutic effect instead this condition can increase the side effect.9 Because of the ceiling effect, 80% concentration of pineapple fruit juice show ed decreasing analgesic effect.

There was a significant difference between control group and experimental groups which received 20%, 40, and 80% pineapple fruit juice concentration (Table 2 and 3). It revealed that at all concentrations, pineapple fruit juice showed its analgesic effect by reducing the bradikinin synthesis. But there was only a significant difference between group 2 and 3 in the experimental groups. Almost all analgesic drugs prevent the PGE₂ formation by inhibiting the activity of cyclooxygenase (COX) which is the enzyme responsible for PGE₂ synthesis.10 In establishing the analgesic effect, bromelain will decrease the bradikinin level released when tissue is damaged. Bradikinin will stimulate the production of prostaglandin while prostaglandin in turn will enhance the sensitivity of bradikinin toward its receptor.2 When bradikinin is low, it also will decrease the PGE₂ synthesis and decrease the pain.11

Moreover, comparison between group 2 (20% concentration of pineapple juice) and group 3 (40% concentration of pineapple juice) revealed a significant difference. From the protective percentage measurement, the result show ed that pineapple fruit juice with concentration of 40% had the most protective result.

Previously, there had been studies upon the effect of bromelain extract of pineapple stems towards the analgesic reaction in mice. The chemical constituent responsible for analgesic effect is known as bromelain which is a proteolytic enzyme. This active compound
Table 3  Post hoc test by Tukey HSD method

<table>
<thead>
<tr>
<th>Group Comparison</th>
<th>P</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.023</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.000</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.042</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.310</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.696</td>
</tr>
</tbody>
</table>

Note:
Group 1: given aquades
Group 2: given 20% pineapple fruit juice concentration
Group 3: given 40% pineapple fruit juice concentration
Group 4: given 80% pineapple fruit juice concentration

Table 4  Protective percentage against 3% acetic acid

<table>
<thead>
<tr>
<th>Group</th>
<th>Total writhing</th>
<th>Protective percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquades</td>
<td>206</td>
<td>0</td>
</tr>
<tr>
<td>20% pineapple juice</td>
<td>133</td>
<td>35.4</td>
</tr>
<tr>
<td>40% pineapple juice</td>
<td>58</td>
<td>71.8</td>
</tr>
<tr>
<td>80% pineapple juice</td>
<td>74</td>
<td>61.6</td>
</tr>
</tbody>
</table>

is present in the stem as well as in the fruit. From the data in this study, we can conclude that the pineapple fruit also has an analgesic activity like the stem. But to ensure the safety issue, several studies should be addressed to reveal the side effect, toxicity, minimum and maximum dosage of pineapple fruit as an analgesic. Other studies can also be conducted to compare the pineapple fruit to other non-opioid analgesic that are commonly used in the community such as paracetamol and ibuprofen.

References