The Effect of Paracetamol and Codeine Analgesic Combination on Creatinine Levels in Male Wistar Rats

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ABSTRACT

Background: Pain is one of the most common complaints in patients. Combination of paracetamol and codeine is an alternative analgesic combination in chronic pain management. They belong to a different group of analgesic and have a different mechanism of action. Combination of these drugs gives a better potential in pain management. However, these drugs also have a potential side effect on the kidney.

Methods: An experimental study of post-test only control group design. The sample was 20 Wistar rats, randomized into four groups: control group, a group receiving paracetamol 32 mg/kg body weight, a group receiving codeine 1.9 mg/kg body weight, and a group receiving paracetamol 32 mg/kg body weight and codeine 1.9 mg combination, administered four times a day orally using gastric instillation for 28 days. At the 29th day, blood is collected from retro-orbital vessel to measure the serum creatinine levels.

Results: Obtained from statistical analysis, there is no significant difference in serum creatinine levels in Wistar rats given all treatment group (p > 0.05).

Conclusion: There is no significant difference in serum creatinine levels between the administration of paracetamol and codeine combination compared to the control group.

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1. Introduction

Pain is defined as an unpleasant sensory and or emotional experience, which is associated with actual or potential tissue damage. This sensation delivered to the brain through sensory neuron fibre.1

Until now, pain is one of the most common complaints presented by patients. Approximately 50 million Americans had chronic pain. 2 In England, it is estimated that 8 million people suffer chronic pain and 13% of its population have chronic pain varying from moderate to severe pain for more than six months. 3

Based on the duration, pain can be classified into acute pain and chronic pain. It can also be classified based on the degree of intensity into three groups, which mild pain, moderate pain and severe pain. Acute pain lasts about 1 – 7 days, while chronic pain can last until six months or more. 4

Pain management can be done through pharmacology and non-pharmacology therapy. A non-opioid analgesic such as NSAID and paracetamol or acetaminophen can be used for mild pain. Moderate pain can be treated by non-opioid analgesic as monotherapy or combined with a weak opioid such as hydrocodone and if necessary, adjuvant analgesic can be added. In the case of severe pain, NSAID can be combined with strong opioid and adjuvant analgesic if necessary. 4
Analgesic or painkiller is a substance that in a therapeutic dose, can reduce the pain. Excessive use of analgesic might lead to several side effects, such as digestive tract haemorrhage, hepatotoxicity or increase the risk factor of kidney failure.  

Paracetamol or acetaminophen is a non-opioid analgesic that also provide an antipyretic effect. It is widely used in case of mild until moderate pain. Meanwhile, codeine is a weak opioid analgesic that is widely used for treating post-surgery pain, cancer pain and other severe pain. Codeine has several side effects, such as hypotension, respiratory depression, elevated intracranial pressure and gastric obstruction.

Excessive use of analgesic can induce kidney damage and increase the risk factor of renal failure. There is a various mechanism of kidney injury based on the drug, such as by causing chronic interstitial inflammation and fibrosis, thickening of the vascular wall in the kidney, renal papilla necrosis, and cortex necrosis.

Serum creatinine is a substance produced by catabolism of muscle keratin phosphate. It can be used as a marker to assess renal function. Creatinine serum level will increase in case of renal toxicity or renal injury.

2. Method

2.1 Sample and Treatment

This study uses an experimental research form with the Post-Test Only Control Group Design approach, which uses 20 male Wistar rats as the object of research. Treatment was given for 28 days. The experimental animals were divided into four groups, namely the control and treatment groups (Table 1) where each group consisted of 6 experimental animals with the following criteria:

- Inclusion criteria: (1) male Wistar rats, (2) 2-3 months old, (3) healthy and active, (4) weight 200-250 grams, (5) no anatomical abnormalities.
- Exclusion criteria: Rats die during adaptation and treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>Group control that are given standard food and drinks</td>
</tr>
<tr>
<td>P1</td>
<td>Male Wistar rats that are given standard food and drinks + Paracetamol dose 32 mg/kg body weight four times/day for 28 days</td>
</tr>
<tr>
<td>P2</td>
<td>Male Wistar rats that are given standard food and drinks + codeine dose 1,9 mg/kg body weight four times/day for 28 days</td>
</tr>
<tr>
<td>P3</td>
<td>Male Wistar rats that are given standard food and drinks + combination of paracetamol with dose 32 mg/kg body weight and codeine 1,9 mg/kg body weight four times/day for 28 days</td>
</tr>
</tbody>
</table>

The dosage of paracetamol and codeine used has been converted from human doses to rat doses. The dose calculation for paracetamol is 500 mg x 0,018 x (50 kg: 70 kg) : 200 x 1000 = 32 mg/kg body weight, the dose of codeine 30 mg x 0,018 x (50 kg : 70 kg) : 200 x 1000 = 1,9 mg/kg body weight, and the combination of paracetamol dose 500 mg x 0,018 x (50 kg : 70 kg) : 200 x 1000 = 32 mg/kg body weight and codeine 30 mg x 0,018 x (50 kg : 70 kg) : 200 x 1000 = 1,9 mg/kg body weight.

Before being treated, all Wistar rats were acclimatized first for seven days by being caged and fed the same standard and drink in ad libitum. After that, each group of rats was treated according to the above mentioned for 28 days. On the 29th day, blood samples were taken through Wistar rat retro-orbital blood vessels. Creatinine levels were then measured in the Clinical Pathology laboratory.

2.2 Measurement of Creatinine Level

Blood samples were taken directly from retro-orbital blood vessels, and then creatinine levels were examined by Jaffe examination method without deproteinization. The unit used is Mg/dl.

3. Result

3.1 Data analysis

The data obtained were processed by computer programs and analysed by Shapiro-Wilk normality test, Levene homogeneity test, and One Way ANOVA statistical test.
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Table 2. Creatinine levels in the control group and treatment group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3760</td>
<td>0.04615</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>0.3380</td>
<td>0.03033</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>0.3240</td>
<td>0.03057</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>0.3560</td>
<td>0.03847</td>
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</tbody>
</table>

Table 3. Test for normality and homogeneity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Shapiro – Wilk</th>
<th>Levene Statistic</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.568</td>
<td></td>
</tr>
<tr>
<td>Treatment 1</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>0.492</td>
<td>0.994</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>0.083</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. One Way ANOVA Test

<table>
<thead>
<tr>
<th>Groups</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Treatment 1</td>
<td>0.195*</td>
</tr>
<tr>
<td>Treatment 2</td>
<td></td>
</tr>
<tr>
<td>Treatment 3</td>
<td></td>
</tr>
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</table>

*Significant is \( p<0.05 \)

Based on the result of One Way ANOVA Test, it is obtained that the result is not significant.

4. Discussion

This study compared differences in serum creatinine levels in the administration of paracetamol and codeine orally. The sample of this study was 20 Wistar rats based on predetermined inclusion criteria. Wistar rats then divided into four groups, namely 5 Wistar rats as a control group, 5 Wistar rats with oral paracetamol administration at a dose of 32 mg/kg body weight 4 times per day as a treatment group 1, 5 Wistar rats with oral codeine dose 1.9 mg/kg body weight 4 times per day as treatment group 2 and 5 rats with a combination of oral paracetamol dose of 32 mg/kg body weight and oral codeine dose of 1.9 mg/kg body weight 4 times per day as a treatment group 3. Each group was treated for 28 days.

The results of this study found out that there is no significant difference in creatinine levels between treatment group 1, treatment group 2, treatment group 3 and a control group.

In treatment group 1, which is given standard food and drink and paracetamol dose 32 mg/kg body weight did not give a significant difference in creatinine serum level. Mechanism of renal injury induced by paracetamol is still unclear. One of the hypothesis widely accepted is that paracetamol metabolite, NAPQI, which is produced by oxidation will be reduced by glutathione into mercapturate acid. If there is a high concentration of NAPQI, glutathione concentration will be reduced drastically. This condition will initiate lipid peroxidase which caused cell injury and tubular cells apoptosis.37

Several factors make the result insignificant, such as a dosage of the treatment. Based on previous research by Suchismita Roy, administration of paracetamol dose 550 mg/kg body weight on Wistar rats for 14 days will give nephrotoxic effect in the form of elevated creatinine serum level and degenerative changes on tubular cells in a significant level compared to the control groups which only given standard food and drink.35

Another factor is the duration of the treatment. In this research, the researcher only gives the treatment for 28 days. Based on previous research by Cynthia A. Naughton, administration of paracetamol with the dosage of more than 1 gram per day for two years will increase the risk of chronic interstitial nephropathy.36

In treatment group 2, which is given standard food and drink and codeine dose 1.9 mg/kg body weight did not give a significant difference in creatinine serum level. Renal injury is induced by codeine’s active metabolite, which is morphine.39 Morphine cause degeneration on renal tubular cells by inhibiting glutathione reductase hence free radical will be elevated in cell and leads to cell injury.40

Dosage of codeine administration can be a factor that caused the insignificant result. Based on previous research, administration of high dose codeine (25 mg/kg body weight) and extreme dose codeine (50 mg/kg body weight) for 28 days results in a significant increase of creatinine serum level of male Wistar rats.38

In treatment 3 group, which is given combination of standard food and oral paracetamol dose of 32 mg/kg bodyweight and oral codeine dose of 1.9 mg/kg bodyweight for 28 days also did not give a significant difference in creatinine serum level. This result can be affected by the dosage of administration. Until now, no research measured the effect of a combination of paracetamol and codeine on creatinine serum, however there is previous research conducted that shows 500 mg paracetamol mg combined with 30 mg codeine could give effective analgesic effects and insignificant side effect.41 Administration of 650 mg paracetamol and 60 mg codeine increase drug’s side effects.42

Limitations in this study are that the authors cannot control several factors, including other...
disease factors, as well as intrinsic factors such as resistance and susceptibility of mice. Besides, the lack of research time caused the authors to be unable to vary the duration of drug administration.

5. Conclusion

In this study, it can be concluded that administration of a combination of paracetamol with a dose of 32 mg/kg body weight and codeine dose of 1.9 mg/kg body weight four times a day for 28 days orally did not cause a significant difference in serum creatinine level.

Ethical Approval

Ethical clearance was obtained from Research Ethics Commission Medical Faculty Diponegoro University with the number of 38/EC/H/KEPK/FK-UNDIP/V/2019.

Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions

Writing-original draft preparation, Vinney Natasaputra; writing-review and editing, dr. Taufik Eko Nugroho, M. Si Med, Sp. An

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