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The Effect of Paracetamol and Codeine Analgesic Combination on Serum Alanine Aminotransferase and Aspartate Aminotransferase Levels in Male Wistar Rats

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Abstract

BACKGROUND: Paracetamol and codeine are classified as different analgesic categories with different mechanism. The combination of both paracetamol and codeine as an analgesic works synergistically and may give better outcome in pain management in moderate-to-severe degree. However, the combination of those analgesics might bring side effects in liver.

AIM: This study was to determine the effect of analgesic combination of paracetamol and codeine on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of Wistar rats.

METHODS: This study was an experimental study with a pre- and post-test control group design. The study objects were 20 male Wistar rats with certain criteria, which were randomly divided into four groups, that is, control group (C), group with paracetamol therapy alone (32 mg/kgBW), group with codeine therapy alone (1.9 mg/kgBW), and group with combination therapy of paracetamol (32 mg/kgBW) and codeine (1.9 mg/kgBW). Analgesic drugs were administered orally 4 times a day for 28 days with gastric sonde. On the 29th day, blood samples were collected through retro-orbital blood vessels for measuring ALT and AST levels. Statistical tests used were one-way ANOVA and Kruskal–Wallis test.

RESULTS: They showed that there were no differences in ALT levels between C, P1, P2, and P3 in both at baseline and post-treatment. However, there were significant increases in ALT levels after treatment in comparison to baseline in the control group (C) (87.2 ± 18.43 vs. 40.6 ± 5.02; $p < 0.05$), P1 (78.9 ± 8.52 vs. 44.4 ± 1.14; $p < 0.05$), and P3 (86.4 ± 17.22 vs. 44.0 ± 1.00; $p < 0.05$). There were no differences in AST levels between C, P1, P2, and P3 at baseline, but there were significantly higher AST levels in P1, P2, and P3 in comparison to control at post-treatment ($p < 0.05$). There were no differences in AST levels between P1, P2, and P3 at post-treatment ($p > 0.05$). There were also significant increases in AST levels after treatment in comparison to baseline in the control group (C) (93.9 ± 1.10 vs. 37.7 ± 1.69; $p < 0.05$), P1 (97.6 ± 1.85 vs. 36.3 ± 1.22; $p < 0.05$), P2 (97.6 ± 1.70 vs. 37.7 ± 1.73; $p < 0.05$), and P3 (98.6 ± 0.79 vs. 36.4 ± 1.20; $p < 0.05$).

CONCLUSION: The combination therapy of paracetamol and codeine might not bring difference in serum ALT and AST levels compared to paracetamol therapy alone or codeine therapy alone.

Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1], [2]. Pain can be categorized based on its duration, that is, acute pain and chronic pain. Acute pain is the physiological reaction due to short-term tissue damage and will relieve as it heals, while chronic pain is a continuation of acute pain and may last more than 3 months [3].

Pain has high morbidity rates worldwide. It was estimated that 20% of adults suffered from pain and 10% of them suffered from chronic pain each year [4]. Chronic low back pain (cLBP) is one example of chronic pain which is defined as a pain felt on the low back area that lasts for more than 3 months. This pain can be felt as local pain, radicular pain, or both [3], [4]. A study revealed that chronic pain worldwide was around 30.3 ± 11.7% in which the prevalence in Asian ranged from 7.1 to 61% [5], whereas among the Asian geriatric population, the prevalence was higher and ranged from 42% to 90.8% [5], [6], [7]. Meanwhile, European epidemiology study revealed that the 1-month prevalence of moderate-to-severe non-cancer chronic pain was 19% [7], [8].

Pain aggravates the quality of lives of patients and their families [1], [2], [3], [4]. Chronic pain may affect on patient-perceived health status, routine activities, economic pursuits, personal relationships, and was associated with depressive symptoms [8].

Analgesic is a class of drugs that bring effects on reducing pain without losing consciousness [1], [9], [10], [11], [12]. Analgesics are divided into two groups, that is, opioid-narcotic

analgesics and non-opioid analgesics. The pain management based on analgesic ladder which was published by the World Health Organization (WHO) was determined by pain intensity. Pain with mild intensity level can be managed with non-opioid analgesics and/or adjuvants, such as paracetamol or nonsteroidal anti-inflammatory drugs (NSAIDs) or a combination of NSAIDs with adjuvant analgesics. Pain with moderate intensity level can be managed with weak opioids, NSAIDs, or a combination of NSAIDs with adjuvant analgesics or a combination of NSAIDs and analgesic adjuvants with weak opioids. Pain with severe intensity level can be managed with strong opioids, a combination of strong NSAIDs and opioids, or a combination of strong NSAIDs and opioids with adjuvant analgesics [9], [10].

A combination of analgesics may increase the effectiveness of each drug in reducing pain, but is intended to decrease the possibility of the presence of side effects from each or both drugs [10], [12]. One of the analgesic combinations is paracetamol and codeine [10], [12]. Paracetamol is the most commonly used non-narcotic analgesic drug which also has an antipyretic effect. Paracetamol is the drug of choice in patients who cannot be treated with NSAIDs, such as patients with moderate-to-severe pain who with hemophilia, fever, neuralgia, headaches, and others [11], [12], [13], [14]. Meanwhile, codeine is a mild opioid analgesic metabolized by the liver. Codeine is used in pain relief therapy, and it is generally used by pediatric patients. The pharmacological effects of codeine will appear if codeine is metabolized into its active form, namely, morphine [15], [16].

Liver is a body organ that is mainly used for metabolism. Its role on drug metabolism may cause damage to the liver itself. Excessive use of analgesics may cause damage to the liver and provide early signs of hepatotoxic symptoms [16], [17]. Early signs of hepatotoxic are increase of transaminase enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [16], [17]. However, studies in the side effects of combination therapy of paracetamol and codeine on liver function were limited. This study was to determine the effects of analgesic combination of paracetamol and codeine on ALT and AST levels of Wistar rats.

Methods

Samples and treatment

This study was an experimental study with a pre- and post-test control group design approach that using 20 male Wistar rats as the study objects. Wistar rats were divided into four groups. Each group was consist of five, healthy and active, male Wistar rats, aged 2−3 months old, weighted 200−250 g, and without anatomical abnormalities. Treatment was given for 28 days.

These groups were the control group (C) (no treatment) and treatment groups (P1, P2, and P3). All groups were fed with standard food and drink. Group P1 was a group of rats which treated with paracetamol within the dose of 32 mg/kgBW which was given for 4 times a day for 28 days. Group P2 was a group of rats which treated with codeine 1.9 mg/kgBW for 4 times/day for 28 days. Group P3 was a group of rats which treated with a combination of paracetamol (32 mg/kgBW) and codeine (1.9 mg/kgBW) which was given for 4 times a day for 28 days.

The doses of paracetamol and codeine used in this study were the result of human dose conversion to rat dose. The dosage calculation for paracetamol was 500 mg × 0.018 × 3.6 = 32 mg/kgBW. The dosage calculation for codeine was 30 mg × 0.018 × 3.6 = 1.9 mg/kgBW [18].

Before being treated, all Wistar rats were acclimatized, standardized, and fed with same standard foods and beverages for 1 week in *ad libitum*. Each group of Wistar rats was then treated according to the previously mentioned for 28 days. Furthermore, on the 29th day, the blood samples of those Wistar rats were taken through retro-orbital veins. Ethical clearance for animal conduct has been approved by ethics committee of health and medical research of Faculty of Medicine Diponegoro University Semarang.

Measurement of ALT and AST levels

Blood samples were taken directly from retro-orbital blood vessels and examined for the levels of ALT and AST with International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method without pyridoxal phosphate 37°C examination method. ALT and AST levels were measured by automatic analyzer. The unit used was IU/L.

Data analysis

Data were analyzed by computed statistical program. Categorical variables were described with frequency (percentage), while continuous variables were with mean ± standard deviation. The normality of data was analyzed with Shapiro–Wilk normality test. The mean differences of ALT and AST levels were analyzed using one-way ANOVA test if the data were normally distributed and using non-parametric Kruskal–Wallis test if the data were not normally distributed. $p < 0.05$ was considered as statistically significant.

Results

ALT levels

Table 1 and Figure 1 presented the ALT levels at baseline (pre-treatment) and post-treatment in the control group (C), group with paracetamol therapy (P1), group with codeine therapy (P2), and group with combination therapy of paracetamol and codeine (P3). They showed that there were no differences in ALT levels between C, P1, P2, and P3 in both at baseline and post-treatment. However, there were significant increases in ALT levels after treatment in comparison to baseline in the control group (C) (87.2 ± 18.43 vs. 40.6 ± 5.02; p = 0.001), P1 (78.9 ± 8.52 vs. 44.4 ± 1.14; p = 0.009), and P3 (86.4 ± 17.22 vs. 44.0 ± 1.00; p = 0.001). Meanwhile, there was also an increase in ALT level after treatment compared to baseline in P2 (63.1 ± 18.57 vs. 45.0 ± 2.64; p = 0.063), although it was not statistically significant.

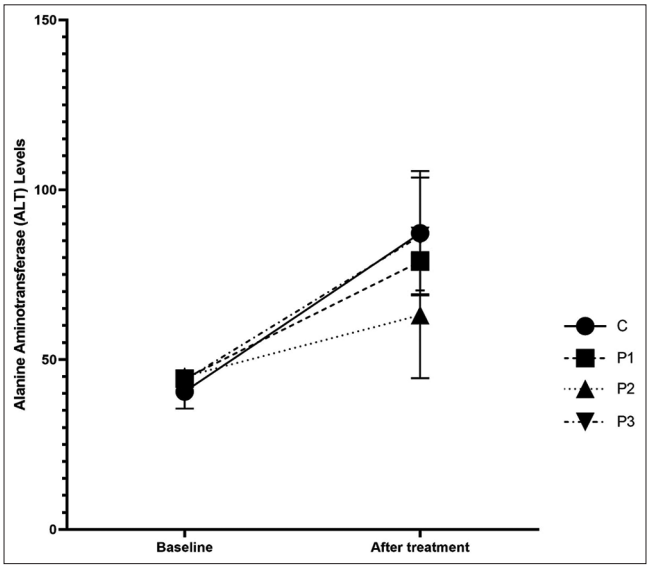

Figure 1. The Level of Alanine Aminotransferase (ALT) at Baseline (Pre-treatment) and Post-treatment between Control (C), Group with Paracetamol Therapy (P1), Group with Codeine Therapy (P2), Group with Combination Therapy of Paracetamol and Codein (P3)

AST levels

Table 2 and Figure 2 showed the level of AST at baseline (pre-treatment) and post-treatment between the control group (C), group with paracetamol therapy (P1), group with codeine therapy (P2), and group with combination therapy of paracetamol and codeine (P3). They showed that there were no differences in AST levels between C, P1, P2, and P3 at baseline. However, there were significantly higher AST levels in P1, P2, and P3 in comparison to control at post-treatment ($p < 0.05$). There were no differences in AST levels between P1, P2, and P3 at post-treatment ($p > 0.05$).

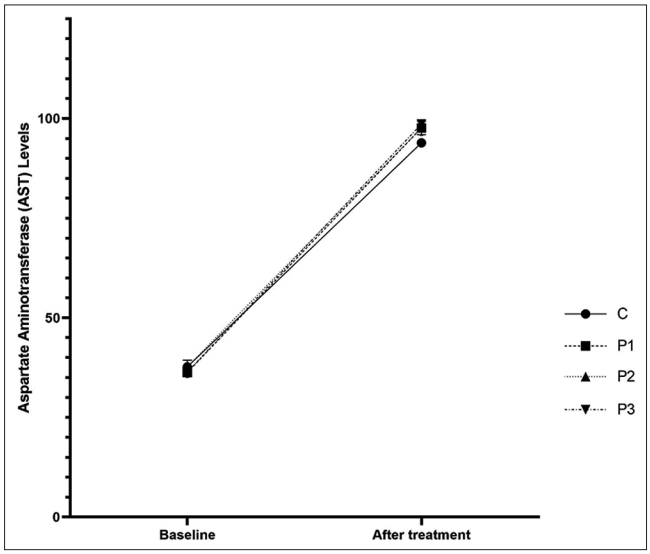

Figure 2. The Level of Aspartate Aminotransferase (AST) at Baseline (Pre-treatment) and Post-treatment between Control (C), Group with Paracetamol Therapy (P1), Group with Codeine Therapy (P2), Group with Combination Therapy of Paracetamol and Codein (P3)

There were also significant increases in AST levels after treatment in comparison to baseline in the control group (C) (93.9 ± 1.10 vs. 37.7 ± 1.69; p = 0.000), P1 (97.6 ± 1.85 vs. 36.3 ± 1.22; p = 0.000), P2 (97.6 ± 1.70 vs. 37.7 ± 1.73; p = 0.000), and P3 (98.6 ± 0.79 vs. 36.4 ± 1.20; p = 0.000).

Discussion

The mechanism of drug metabolism and drug excretion within the body for orally administered drugs will involve the liver metabolism. The liver functions may be assessed with serum ALT, AST, and other parameters. Liver function tests measure the levels of

Table 1: The level of alanine aminotransferase at baseline (pre-treatment) and post-treatment between control (C), group with paracetamol therapy (P1), group with codeine therapy (P2), and group with combination therapy of paracetamol and codeine (P3)

Groups	Baseline (mean ± SD [minimum–maximum])	After treatment (mean ± SD [minimum–maximum])	p (between baseline and after treatment)
Control (C)	40.6 ± 5.02 (35.0–47.0)	87.2 ± 18.43 (71.4–118.4)	0.001*,¶
Treatment 1 (P1)	44.4 ± 1.14 (43.0–46.0)	78.9 ± 8.52 (73.4–94.0)	0.009*,§
Treatment 2 (P2)	45.0 ± 2.64 (41.0–48.0)	63.1 ± 18.57 (43.2–87.2)	0.063¶
Treatment 3 (P3)	44.0 ± 1.00 (43.0–45.0)	86.4 ± 17.22 (68.8–106.4)	0.001*,¶
p (C to P1)	0.138¶	0.347§	
p (C to P2)	0.122¶	0.074¶	
p (C to P3)	0.176¶	0.948¶	
p (P1 to P2)	0.654¶	0.076§	
p (P1 to P3)	0.572¶	0.251§	
p (P2 to P3)	0.452¶	0.074¶	
p	0.121ϕ	0.119Π	

¶Independent *t*-test, ϕone-way ANOVA test, §non-parametric Mann–Whitney U-test, ΠKruskal–Wallis test, *p < 0.05 was considered statistically significant. SD: Standard deviation.

Table 2: The level of aspartate aminotransferase at baseline (pre-treatment) and post-treatment between control (C), group with paracetamol therapy (P1), group with codeine therapy (P2), and group with combination therapy of paracetamol and codeine (P3)

Groups	Baseline (mean ± SD (minimum–maximum)	After treatment (mean ± SD (minimum–maximum)	p
Control (C)	37.7 ± 1.69 (35.6–40.2)	93.9 ± 1.10 (92.1–95.1)	0.000*,¶
Treatment 1 (P1)	36.3 ± 1.22 (34.8–37.8)	97.6 ± 1.85 (95.2–99.9)	0.000*,¶
Treatment 2 (P2)	37.7 ± 1.73 (35.6–40.3)	97.6 ± 1.70 (95.7–99.8)	0.000*,¶
Treatment 3 (P3)	36.4 ± 1.20 (34.8–37.8)	98.6 ± 0.79 (97.5–99.4)	0.000*,¶
p (C to P1)	0.190¶	0.005*,¶	
p (C to P2)	0.999¶	0.003*,¶	
p (C to P3)	0.212¶	0.000*,¶	
p (P1 to P2)	0.195¶	0.959¶	
p (P1 to P3)	0.920¶	0.300¶	
p (P2 to P3)	0.218¶	0.297¶	
p	0.315ϕ	0.000*,ϕ	

¶Independent *t*-test, ϕone-way ANOVA test, *p < 0.05 was considered statistically significant. SD: Standard deviation.

certain enzymes and proteins in blood. Levels that are higher or lower than normal can indicate liver problems [14], [16], [17], [18], [19], [20].

ALT is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and its level increase. Meanwhile, AST is an enzyme that helps metabolize amino acids. Like ALT, AST is normally present in blood at low levels. An increase in serum ALT and/or AST levels may indicate decreased liver function due to liver injury or damage and/or disease [17], [18], [19], [20].

Since we aimed to determine the effect of the analgesic combination of paracetamol and codeine on ALT and AST levels, our study showed that there were no differences in ALT levels between C, P1, P2, and P3 in both at baseline and post-treatment. However there were increases in ALT levels after treatment in comparison to baseline in P1, P2, and P3. In overall, this finding revealed that the combination therapy of paracetamol and codeine therapy might not bring difference in serum ALT levels compared to without therapy, paracetamol therapy alone, or codeine therapy alone.

Our study also showed that there were no differences in AST levels between C, P1, P2, and P3 at baseline. However, there were significantly higher AST levels in P1, P2, and P3 in comparison to control at post-treatment. However, there were no differences in AST levels between P1, P2, and P3 at post-treatment. This finding revealed that although paracetamol therapy alone, codeine therapy alone, or combination therapy of paracetamol and codeine might increase the AST levels at post-treatment compared to at baseline, the combination therapy of paracetamol and codeine therapy might not bring difference in serum AST levels compared to paracetamol therapy alone or codeine therapy alone.

Paracetamol is an active metabolite of phenacetin which has an analgesic and antipyretic effects. This antipyretic effect caused by the aminobenzene group and can cause hepatotoxicity. The primary metabolic pathway for paracetamol is hepatic glucuronidation, most of which are conjugated by glucuronic acid and sulfuric acid that bring a relatively non-toxic metabolite. A small amount of the drug (<5% of paracetamol) is metabolized through hydroxylation by enzymes cytochrome P-450 pathway such as CYP3A4 and CYP2E1, into highly reactive intermediate and potentially harmful metabolites N-acetyl-p-benzoquinone imine (NAPQI) [11], [12], [13], [14].

Metabolites N-acetyl-p-benzoquinone imine (NAPQI) is a hepatotoxic by product produced during the xenobiotic metabolism of the analgesic paracetamol and is a strong biochemical oxidizer. In excessive doses of paracetamol, NAPQI is not effectively detoxified and becomes a dangerous and hepatotoxic metabolite causing severe liver damage and fulminant liver failure [17], [18], [19], [20], [21].

Codeine is a mild opioid analgesic drug which is used as a pain relief therapy and also generally used by pediatric patients which metabolized by the liver. Codeine metabolism is occurred in the liver, digestive tract, and in the kidneys and will be active after conversion to the active form which is morphine [22].

Our study showed that there was no difference on ALT levels in group with paracetamol therapy or in group with codeine therapy compared to the control group, but there was a higher AST levels in group with paracetamol therapy or in group with codeine therapy compared to the control group at post-treatment. We could not clearly explain yet why there was difference in AST levels but not in ALT levels. Several factors might influence these findings, one of them was the drug doses used in this study which were within therapeutic doses, so that they did not bring impact on ALT levels. At reasonable doses given in this study, those drugs have not reached the maximum doses as well as they might have not given toxicity to the liver of male Wistar rats.

Our study revealed that there were comparable serum ALT and AST levels between the groups with combination therapy of paracetamol and codeine, the group with paracetamol alone, and the group with codeine alone. Our study showed that paracetamol therapy was quite safe. This was in line with the study from Peacock *et al*. which showed that a single dose of intravenous paracetamol was as effective and safe as oral paracetamol in reducing endotoxin-induced fever with low side effects [13]. Wininger *et al*. also showed that intravenous paracetamol revealed good analgesic efficacy compared with placebo and were well tolerated in patients after abdominal laparoscopic surgery [23].

Our study did not mean to prevent us to use combination therapy in clinical practice. Although our findings showed that the administration of combination of paracetamol and codeine was safe and did not show differences compared to paracetamol alone and codeine alone, it still need a monitoring process including the potential side effects on the liver. Other considerations should also be taken in administering combination therapy, such as a history of alcohol consumption, chronic liver infection, or other hepatotoxic drugs usage.

There were several limitations on this study as the authors could not control several factors, including external factors, such as the environment and nutrition; and internal factors, such as genetic and rats' stamina. Further studies need to be done such as the effect of a combination therapy of paracetamol and codeine administration with varying dose and length of exposure to patients with chronic pain and equipped with histopathological image of the liver. Epidemiological studies regarding safe doses of the combined use of paracetamol and codeine as an analgesic option are also needed.

Conclusion

The combination therapy of paracetamol and codeine might not bring difference in serum ALT and AST levels compared to paracetamol therapy alone or codeine therapy alone. If confirmed by further studies, this combination might be potential for the management of pain.

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