ORIGINAL ARTICLE

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

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ABSTRACT

Background: Combination of paracetamol and opioid morphine is effective to relieve moderate to severe pain. However, these agents may have potential side effects on the liver as both are metabolized in the liver.
Aim: To investigate the effect of paracetamol and morphine analgesic combination on serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in male Wistar rats.
Method: This was an experimental study using Post-Test Only Control Group Design. The samples were 20 male wistar rats randomized into 4 groups; group I as the control group, group II receiving paracetamol 9 mg, group III receiving morphine 0.18 mg, and group IV receiving paracetamol 9 mg and morphine 0.18 mg. Drugs were administered through oral gastric instillation 3 times a day for 14 days. Blood samples were collected at the 15th day through retro-orbital vessel to measure the AST and ALT levels. The data were analyzed using One-Way ANOVA test and Post-Hoc test.
Result: There were significant differences in AST (p=0.022) and ALT levels (p=0.001) among all groups. A significant difference in ALT (p=0.041) was found between group receiving combination therapy and morphine. There were also significant differences in AST and ALT levels between group receiving combination therapy and control (p=0.045, p=0.032, respectively).
Conclusion: Special consideration should be taken in administering the combination of paracetamol and morphine as it might increase AST and ALT levels in comparison to control group.
Keywords: Paracetamol, morphine, combination, AST levels, ALT levels, pain

INTRODUCTION

Task force on taxonomy of the International Association for the Study of Pain (IASP) stated that pain is 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 classified based on the duration, that is acute pain and chronic pain. Acute pain is the physiological response due to short-term tissue damage and will disappear as it heals, while chronic pain is a continuation of acute pain and lasts more than 3 months[3].

Pain has high morbidity rates in the world. It was estimated that 20% of adults suffered from overall pain and 10% of them were diagnosed with chronic pain each year[4]. Other study showed that chronic pain worldwide was around 30.3±11.7% in which the prevalence in European reached 51.3% of the total population, while in Asian ranged from 7.1– 61%[5]. whereas among the Asian geriatric population, the prevalence was even higher and ranged from 42% to 90.8%[5-7]. For chronic non-cancer pain only, an epidemiology study in Europe showed that the one-month prevalence of moderate-to-severe non-cancer chronic pain was 19%.[8] The overall prevalence of pain has not been studied in Indonesia, but about 12.7 million or 5% of the Indonesian population who had cancer were estimated to have pain[9].

Pain affects the quality of lives of patients and their families[1,2,4]. Chronic pain significantly impacted on patient-perceived health status, affected everyday activities including economic pursuits and personal relationships, and was significantly associated with depressive symptoms.[8] Patients with chronic pain should also take chronic long-term medications so that liver toxicity should be taken into consideration[10].

Analgesics are drugs that relieve pain without causing loss of consciousness. There are two groups of analgesic agents, that are opioid-narcotic analgesics and non-opioid analgesics. Based on The Adaptation of Analgetic Ladder World Health Organization (WHO), there are four stages of pain management based on their intensity. Mild pain can be managed with non-opioid analgesics and/or adjuvants; mild to moderate pain with weak opioids, non-opioids, and/or adjuvants, while moderate to severe pain with strong opioids[11,12]. However, in real world, the majority relied on drugs for pain control and NSAIDs were the most frequent drug choice. Despite pain medications, a large proportion had inadequate pain control.

Analgesic paracetamol is effective in treating acute pain with mild to moderate degree. However, at excessive doses, it may cause side effects in organs involved in the mechanism of action, including liver and kidney[12,13]. Morphine is a strong opioid analgesic drug for severe pain. As a standard for comparison of other opioid groups, morphine undergoes metabolism in the liver and produces 6-glucoronid morphine which adds analgesic effects to morphine[10].

The administration of an analgesic combination is known to increase the effectiveness of the drug in relieving pain and decreasing the likelihood of side effects from each drug[12,14]. The combination of paracetamol and morphine is the third step analgesic in the WHO analgesic ladder. However, side effects of excessive analgesic combination

may be hepatotoxic or result in gastrointestinal complications if both drugs are metabolized in liver[13,15,16].

Liver is the most active tissue in drug metabolism. The role of liver in metabolizing drugs may cause damage to the liver itself.[16] In the United States, approximately 2000 cases of acute liver disease occur annually and more than 50% of them are caused by drugs, which is 37% are due to paracetamol. Early sign in liver abnormalities can be based on the elevation of serum transaminase enzymes such as aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) which indicate a deterioration in the liver cell wall. These enzymes can be used as markers of liver cell integrity for hepatocellular injury[17].

Study in the effects of analgesic combination of paracetamol and morphine was still limited while the use of each drug as monotherapy was widespread. Therefore, this study was to examine the effects of analgesic combination of paracetamol and morphine on serum AST and ALT levels in male wistar rats.

METHODS

Sample and Treatment: This study was an experimental research with Post-test Only Control Group Design using 20 male Wistar rats as research objects. Treatments of paracetamol and/or morphine were given for 14 days. The experimental animals were divided into 4 groups (Table 1). Each group was consist of five, healthy and active, male Wistar rats, aged 2-3 months old, weighted 200-250 grams, and without anatomical abnormalities. These groups were control group (group I, no treatment); and treatment groups, consisting of group receiving paracetamol 9 mg only (group II), group receiving morphine 0.18 mg only (group III), and combination of paracetamol 9 mg and morphine 0.18 mg (group III) (Table 1). All samples were given standard foods and drinks. The doses of paracetamol and morphine used in this study were the result of human dose conversion to rat dose. Paracetamol dose calculation was 500 mg x 0.018 =9mg, dose of morphine was 10mg x 0.018=0.18mg, and combination was paracetamol 500 mg x 0.018 = 9 mg and morphine 10 mg x 0.018 = 0.18 mg.

Before being treated, all Wistar rats were acclimatized and fed the same standard food and drink for 1 week *ad libitum*. Each group of wistar rats was then treated according to the previously mentioned for 14 days. Furthermore, wistar rats were drawn blood samples through a retroorbita blood vessel on day 15th to measure the AST and ALT levels in the clinical pathology laboratory. 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 Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels: Blood samples were taken directly from retroorbita blood vessels and examined for the levels of AST and ALT with International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method without Pyridoxal Phosphate 37^{o}C exam. method. The unit used was IU/L.

Data analysis: The data obtained were analyzed by computed statistical program. The normality and homogenity of data were analyzed with Saphiro-Wilk test and Levene test, respectively. The mean differences of AST and ALT levels within group were analyzed using parametric One-Way ANOVA test as the data distribution and variance were normal and homogenous. Post-Hoc test as a further difference test was done to see the difference in each group. Continuous variables were described using mean ± standard deviation. A *p* value of <0.05 was considered as statistically significant.

RESULTS

Serum Aspartate Aminotransferase (AST) Levels: The highest AST levels was in the treatment group administered with the combination of paracetamol and morphine (T3) (88.08±7.49 IU/L), while the lowest AST levels was in the control group (C) (73.84±4.54 IU/L) (Table 2). One-Way ANOVA statistic test revealed a significant difference in AST levels within groups (*p*=0.022) (Table 2). In the Post-Hoc test, there was a significant difference in AST levels between the combination group (T3) and the control group (88.08±7.49 vs 73.84±4.54, *p*=0.045) (Table 3, Figure 1).

No significant differences in AST levels were obtained between the control group with paracetamol only group (T1) (85.15±7.11 vs 73.84±4.54, *p*=0.098) and with morphine only group (T2) (82.69±7.07 vs 73.84±4.54, *p*=0.226). No significant differences were also found between paracetamol only group (T1) with morphine only group (T2) (82.69±7.07 vs 85.15±7.11, *p*=0.992) and with combination group (T3) (88.08±7.49 vs 85.15±7.11, *p*=0.983), and between morphine only group (T2) with combination group T3 (82.69±7.07 vs 88.08±7.49, *p*=0.799) (Table 3, Fig. 1).

Serum Alanine Aminotransferase (ALT) Levels: The highest serum ALT levels was in the group administered with combination of paracetamol and morphine (T3) (216.58±15.62 IU/L), while the lowest was in the control group (C) (144.48±7.09 IU/L) (table 2). One-way ANOVA statistical test revealed a significant difference in serum ALT levels within groups (*p*=0.001) (Table 2). In Post-Hoc test, there were significant differences in ALT levels between the combination group (T3) with the control group (216.58±15.62 vs 144.48±7.09, *p*=0.032) and with the morphine only group (T2) (216.58±15.62 vs 145.50±12.47, *p*=0.041) (Table 4, Figure 2).

No significant differences in ALT levels were obtained between the control group with paracetamol only group (T1) (174.11±9.44 vs 144.48±7.09, *p*=0.177) and with morphine only group (T2) (145.50±12.47 vs 144.48±7.09, *p*=1.000). No significant differences were also found between paracetamol only group (T1) with morphine only group (T2) (174.11±9.44 vs 145.50±12.47, *p*=0.425) and with combination group (T3) (174.11±9.44 vs 216.58±15.62, *p*=0.238) (Table 4, Fig. 2).

Table 1. Description of Treatments in All Groups

Group	Treatment	
Control (C)	Rats were administered with standard foods and drinks	No treatment
Treatment 1 (T1)	Rats were administered with standard foods and drinks	paracetamol dose 9 mg, 3 times daily for 14 days
Treatment 2 (T2)	Rats were administered with standard foods and drinks	morphine 0.18 mg, 3 times daily, for 14 days
Treatment 3 (T3)	Rats were administered with standard food and drinks	combination of paracetamol dose 9 mg and morphine 0.18 mg, 3 times daily, for 14 days

Table 2. The Difference of Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels within the Control Group and Treatment Group

Groups	**AST level (IU/L)**	***p* value**	**ALT level (IU/L)**	***p* value**
Control Group (C)	73.84 ± 4.54		144.48 ± 7.09	
Paracetamol Only Group (T1)	85.15 ± 7.11		174.11 ± 9.44	
Morphine Only Group (T2)	82.69 ± 7.07	0.022*	145.50 ± 12.47	0.001*
Combination Group (Paracetamol and Morphine) (T3)	88.08 ± 7.49		216.58 ± 15.62	

*One Way ANOVA test, significant difference if $p<0.05$ mean ± standard deviation (mean ± SD)

Table 3. The Differences of Serum Aspartate Aminotransferase (AST) Levels Among Groups

Groups	**Control Group** (73.84 ± 4.54)	**Paracetamol Only Group** (85.15 ± 7.11)	**Morphine Only Group** (82.69 ± 7.07)
Paracetamol Only Group	0.098		
(85.15 ± 7.11)			
Morphine Only Group	0.226	0.992	
(82.69 ± 7.07)			
Combination Group	0.045*	0.983	0.799
(88.08 ± 7.49)			

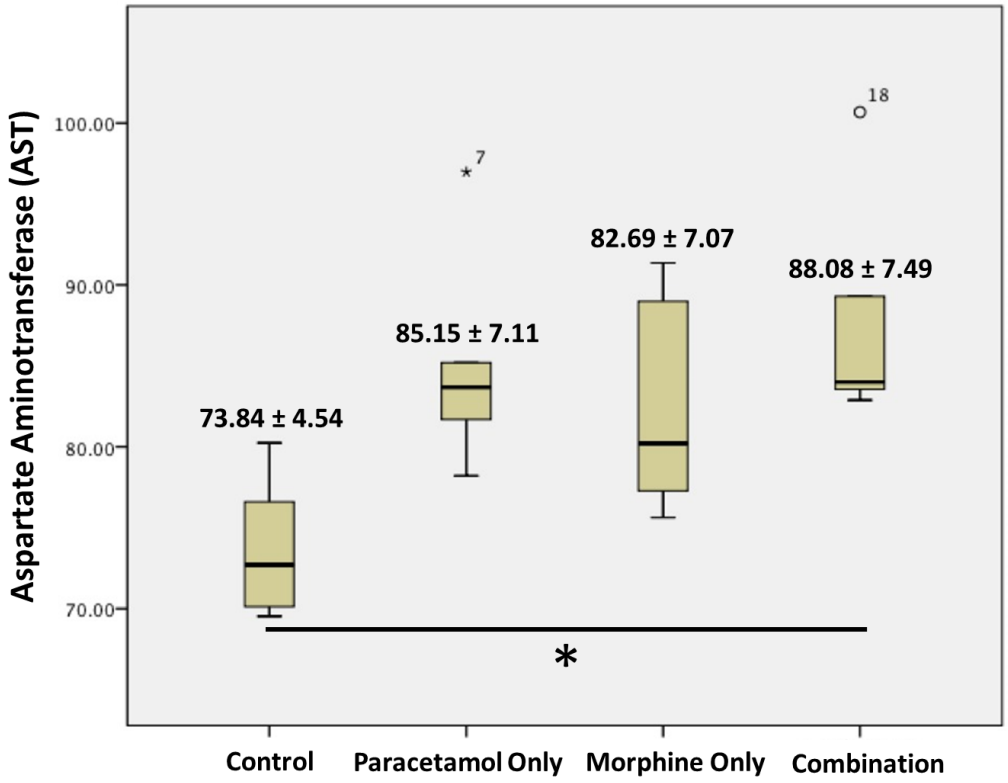
* Post-Hoc Analysis, significant difference if $p<0.05$ Mean ± standard deviation (mean ± SD)

Table 4. The Differences of Serum Alanine Aminotransferase (ALT) Levels among groups

Groups	**Control Group** (144.48 ± 7.09)	**Paracetamol Only Group** (174.11 ± 9.44)	**Morphine Only Group** (145.50 ± 12.47)
Paracetamol Only Group	0.177		
(174.11 ± 9.44)			
Morphine Only Group	1.000	0.425	
(145.50 ± 12.47)			
Combination Group	0.032*	0.238	0.041*
(216.58 ± 15.62)			

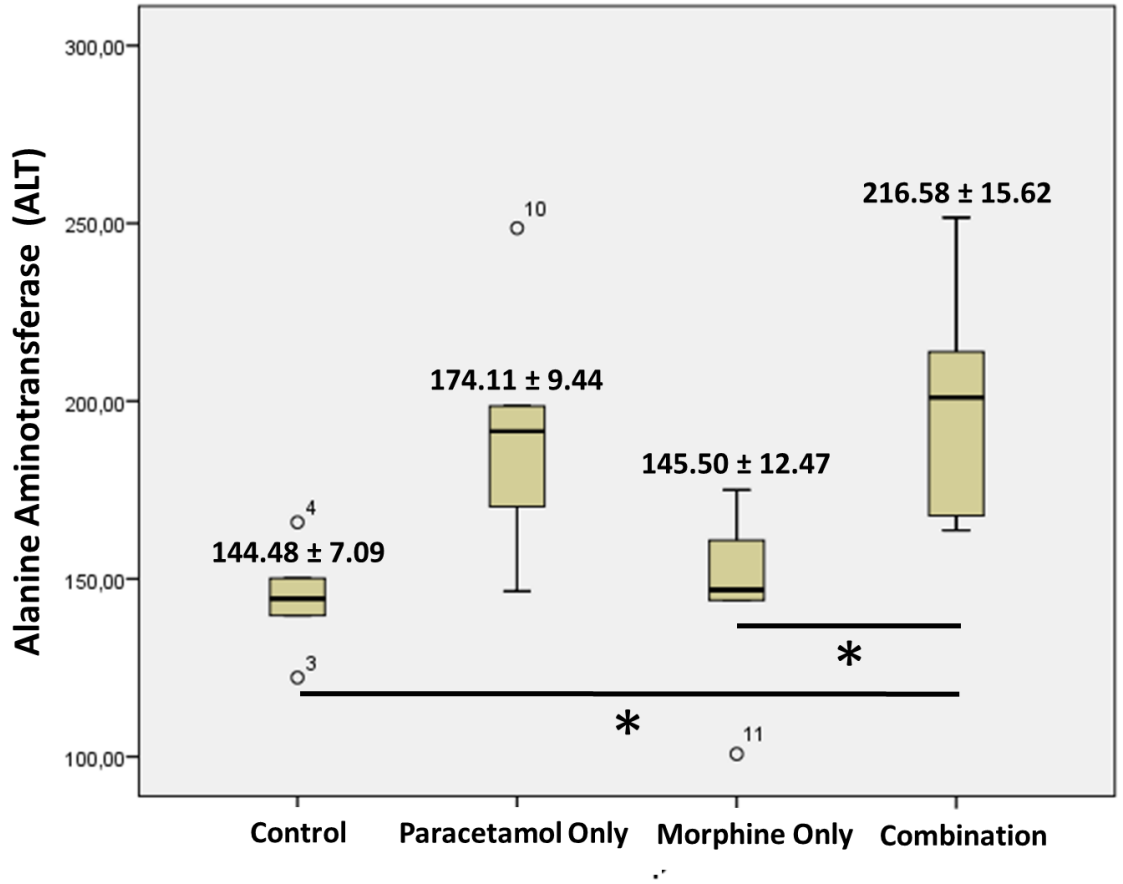
* Post-Hoc Analysis, significant difference if $p<0.05$ Mean ± standard deviation (mean ± SD)

Figure 1. Serum Aspartate Aminotransferase (AST) levels among each group.



* Post-Hoc Analysis.
Significant difference $p<0.05$ between combination group and control.
Mean ± standard deviation (mean ± SD).

Figure 2. Serum Alanine Aminotransferase (ALT) levels among each group



* Post-Hoc Analysis.
Significant difference $p<0.05$ between combination group with control group and morphine only group.
Mean ± standard deviation (mean ± SD).

DISCUSSION

Liver is the main organ in human body metabolizing various substances that enter the body, including drugs. Liver cells' injury during drug metabolism may increase the production of hepatic enzymes, including AST and ALT enzyme. AST and ALT can be used as a marker of integrity of liver cells.[16, 17]

In the United States, 2000 cases of acute liver disease occur annually and approximately 37% are due to paracetamol. Paracetamol is the leading cause of drug induced toxic injury in liver. Drugs account for 2- 5% of cases of patients hospitalized with jaundice and approximately 10% of all cases of acute hepatitis. Chronic liver disease and cirrhosis account for some 2% of mean in 17 countries with nearly 40,000 deaths per year.[17]

The primary metabolic pathway for paracetamol is hepatic glucuronidation, most of which are conjugated by glucoronic acid and sulfuric acid that yields a relatively non-toxic metabolite. A small amount of the drug is metabolized via hydroxylation by cytochrome P-450 pathway such as CYP3A4 and CYP2E1, into highly reactive and potentially harmful metabolites NAPQI (N-acetyl-p-benzoquinone imine). NAPQI is an extremely hepatotoxic byproduct produced during the xenobiotic metabolism of the analgesic paracetamol as well as being 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.[18]

When a toxic dose of paracetamol is ingested, the normal glucuronide pathway is saturated and large amounts of NAPQI are produced. Liver reserves of glutathione are depleted by conjugation with this excess NAPQI. The mechanism by which toxicity results is complex, but is believed to involve reaction between unconjugated NAPQI and critical proteins as well as increased susceptibility to oxidative stress caused by the depletion of glutathione. Increased levels of AST in the blood occurs due to the reduced amount of glutathione by NAPQI which causes a decrease in the potency of the mitrocondrial membrane so that the enzyme AST goes out into the bloodstream.[18]

Our study showed that the administration of all agents, including paracetamol, morphine, or combination of both agents in therapeutic doses led to a slightly increase in AST and ALT levels compared to control, but they were not significant. The administration of oral paracetamol in wistar rats showed no significant increase in AST and ALT levels. This was in line with the study from Peacock *et al.*[19]

which showed that a single dose of intravenous paracetamol was as safe and effective as oral paracetamol in reducing endotoxin-induced fever with low side effects. Wininger *et al.*[20] also showed that intravenous paracetamol (1000 mg and 650 mg) revealed good analgesic efficacy compared with placebo and were well tolerated in patients after abdominal laparoscopic surgery.

In line with our study, paracetamol have been reported safe in eldery and children.[21,22] Jahr *et al.*[21] also documented the safety and efficacy paracetamol in the elderly subpopulation which were comparable with the subpopulation younger than 65 years. While a systematic review from Lavonas *et al.*[22] showed that therapeutic paracetamol was not associated with liver injury in children. There was no hepatotoxic effects in children taking paracetamol at doses of <0.75 mg/day.

Differ to our study, a study from Watkins *et al.*[23] have shown a potential toxic effect on administration of paracetamol at higher dose of 1200 mg/kgBW, 2400 mg/kgBW and 4800 mg/kgBW in comparison with those without paracetamol. The incidence of maximum ALT of more than 3 times the upper limits of normal was 31% to 44% in the treatment groups receiving paracetamol, including those subjects treated with paracetamol alone. Treatment with paracetamol was associated with a markedly higher median maximum ALT compared with placebo. Taken together, the administration of therapeutic paracetamol within a short duration might be safe and did not increase AST or ALT levels, but there might be elevated AST and ALT levels or even liver damage in higher doses and a relatively longer time.

Our study showed that morphine administration within therapeutic doses in wistar rats did not significantly increase AST and ALT. This was different with the study from Salahshoor *et al.*[24] which revealed that morphine administration increased the liver enzyme level (AST and ALT), mean diameter of the central hepatic vein and hepatocytes in male mice. Similarly, Cui *et al.*[25] also revealed that the serum hepatic enzyme levels of AST and ALT increased after dosing morphine 100 mg/kg and the magnitude of the analgesic effect of morphine depended on dosing time.

Atici *et al*.[26] showed that there were histopathological and biochemical changes due to chronic usage of morphine in rats liver. Serum ALT and AST were significantly higher in morphine group compared to the control group, while light microscopy revealed severe centrolobular congestion, focal necrosis and perivenular necrosis in the morphine group. Their findings pointed out the risk of increased lipid peroxidation, hepatic damage due to long term use of morphine.

Gurantz and Correia[27] demonstrated that acute injections with high doses of morphine caused the loss of cytochrome P-450 resulting in increased AST enzyme activity in the rat liver. This acute loss of cytochrome P-450 was a result of morphine-mediated accelerated turnover (degradation) of its heme moiety and was associated with hepatotoxicity of the drug.

Morphine metabolism is in the liver. It is conjugated with glucoronic acid and hydrolyzed by cytochrome P-450. Continuous use of drug, including morphine will decrease the ability of the liver fraction resulting in decreased liver morphine metabolism.[27-29] Taken together, the discrepancies between our findings with previous studies might depend on the duration and dose of morphine used to the increased serum AST and ALT levels. The use of morphine within therapeutic doses was relatively safe.

Our study showed that the administration of paracetamol and morphine in therapeutic doses as combination resulted in significant increase of AST and ALT levels in comparison with the control group, but not with paracetamol only group. Furthermore, there was significant difference in ALT between the combination group compared to the morphine only group. Different to our findings, Watkins et al.[23] showed that initiation of recurrent daily intake of 4 gram of paracetamol in healthy adults was associated with ALT elevations. However, concomitant treatment with opioids did not increase this effect. The theoretical plausibility was that combination of analgesics was used to decrease the dose of each drug so that it could reduce side effects of each single drug without decreasing the effectiveness of the drugs. The intravenous administration of paracetamol might reduce the need for morphine.[14]

This study did not mean to prevent us to use combination therapy in clinical practice. However, the use of drugs combination of paracetamol and morphine need monitoring due to the potential side effects on the liver. History of paracetamol ingestion must be considered in the differential diagnosis of serum aminotransferase elevations, even in the absence of measurable serum paracetamol concentrations. Other considerations should be taken in administering combination therapy, such as alcohol consumption, chronic liver infection or other hepatotoxic drugs usage.

This study was not calculating the dose-dependent pattern and time-dependent pattern as the limited study time, yet further research is still needed. This study did not control some external factors such as environmental factors and other diseases, as well as internal rat factors such as resistance and stress levels.

CONCLUSIONS

Special attention should be taken in administering the combination of paracetamol and morphine, due it might increase AST and ALT levels and cause side effect on liver in comparison to control group and mono-therapy group.

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