Effect of Jamblang (*Syzygium cumini*) Seed Extract on ALT and AST Levels in Isoniazid-induced Male Rats

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Keywords: ALT, AST, *Syzygium cumini*, Isoniazide

Abstract: *Syzygium cumini* or known in Indonesia as jamblang fruit which is contain gallic acid, elagic acid, corilagin, ellagitannin, isoquercetin, quercetin and other antioxidant. Elagic acid (EA) has a function as a free radical scavenger that can decrease liver function marking enzymes. Use of isonazide can cause side effects such as an increase in aminotransferase levels that occurs in 10% - 20% of patients several weeks after consumption. This study aims to determine the effect of *Syzygium cumini* seed extract in the ALT and AST values of male rats induced by isoniazid. The research subjects were 30 male white rats strain, weighing 150-200 grams and aged 2-3 months, which were divided into 5 groups. The negative control group was given aquades, while the positive control was given isoniazid as much as 40 mg on the 12th day until the 25th day. The treatment group was given *Syzygium cumini* seed extract with multilevel doses (20 mg / rat, 40 mg / rat, and 80 mg / rat) from day 8 to day 25 and isoniazid 40 mg on the 12th day, on the 12th, on 26thday ALT and AST levels were measured. Data were analyzed using One-Way Anova.The results of the One-Way Anova test in groups with various doses of extract of jamblang fruit extract (20 mg / rat, 40 mg / rat, and 80 mg / rat) showed significant results in reducing ALT and AST levels in isoniazid-induced rats, with p value <0.001 ($\alpha = 0.05$).The result showed that administration of jamblang fruit extract can reduce ALT and AST levels of isoniazid-induced mice.

1 INTRODUCTION

The World Health Organization (WHO) mentions about one third of the world's population or around 2 trillion people with liver disease with a death toll of 1 million. Liver damage can be caused by many things, as evidenced in previous studies by Prasetyo (2010) and Kusuma (2010), not only diseases caused by viruses but can also be from unhealthy lifestyles such as consumption of foods containing excessive cooking oil, and consumption alcohol. In addition, drug consumption can also induce liver damage, for example anti-tuberculosis drugs. Isoniazid (INH) is always given to TB cases, the prevalence of TB in Indonesia is still relatively high, the WHO report in 2010 stated that in 2009 Indonesia's ranking dropped to fifth with the number of TB sufferers at 292,753 people (WHO, 2010). This shows a considerable consumption of INH in Indonesia. The use of INH can cause side effects such as an increase in aminotransferase levels that occurs in 10% - 20% of patients several weeks after consumption but does not cause typical clinical symptoms (Prasetyo, F. A., *et a,* 2010). Nonetheless 0.1% - 2% of patients experience acute liver failure (Maddrey,2013). INH mechanism suspected to cause liver damage can not be proven with certainty, but hypothetically stated that the damage was caused by toxic substances in the form of monoacetylhydrazine (MAH) through the mechanism of free radicals (oxidative stress) (Saukkonen&Jereb, 2012).

Due to its high efficacy, isoniazid (INH) remains the drug of choice for treatment of latent tuberculosis (TB) despite the fact that it can cause liver failure. Although drug-induced liver injury (DILI) caused by different drugs is somewhat different , the clinical characteristics of INH-induced liver injury are fairly typical for idiosyncratic DILI and include malaise, fatigue, nausea and vomiting. The duration of therapy before the manifestation of jaundice can vary between 1–25 weeks with an average of 12 weeks. Fever Affects on average 20%

Hasibuan, A., Bangun, S., Marbun, R., Siregar, S., S., A. and Octora, D.
Effect of Jamblang (Syzygium cumini) Seed Extract on ALT and AST Levels in Isoniazid-induced Male Rats.
DOI: 10.5220/0009974605430550
In *Proceedings of the International Conference on Health Informatics and Medical Application Technology (ICHIMAT 2019)*, pages 543-550
ISBN: 978-989-758-460-2

of the patients and eosinophilia is present in up to 15% of the affected individuals. In most cases, liver injury is asymptomatic and is only detected by measuring markers of hepatocyte injury such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). This is especially true for mild cases of liver injury, which occurs in up to 20% of patients treated with the drug. However, in most patients, liver function returns to normal despite continued treatment with the drug, a phenomenon referred to as' adaptation 'by hepatologists. Severe liver injury is seen in up to 1% of the patients. Elevations in ALT and AST can start as early as 1 week and sometimes as late as 9 months after starting treatment with INH. However, in more than half of the patients an ALT increase occurs between 1–6 months. The abrupt increase in ALT that leads to liver failure is idiosyncratic in nature and is not clearly related to the duration of treatment, the dose of the drug, fever or eosinophil count (Sankhari, *et al*, 2010).

When liver injury is identified, the first line of treatment is to stop the drug and monitor the patient for recovery. In most cases patients recover. However, the challenge of patients with more severe liver injury can result in a rapid onset of symptoms (within hours) and is contraindicated. Histological characteristics of severe INH-induced liver injury including hepatocellular injury with multilobular necrosis and a mononuclear cell infiltrate, which is generally indistinguishable from viral hepatitis. Steatosis is unusual in INH-induced liver injury. However, during active TB treatment, when INH is given in combination with other agents such as ethambutol, pyrazinamide and rifampicin (RMP), there have been reported cases of steatosis and cholestatic liver injury 7-9. Prolonged treatment with INH can also lead to a lupus-like autoimmune reaction with the presence of antinuclear antibodies hich occurs in up to 20% of patients (Sankhari, *et al*, 2010).

INH-induced liver injury remains a significant clinical problem. Previous studies suggested that bioactivation of AcHz was involved in the injury, but more recent studies point to direct oxidation of INH as the pathway leading to liver injury. Previous studies had also suggested that the injury, especially mild injury, was not immune mediated. However, recent evidence suggests that INH-induced liver injury is indeed immune mediated, but most cases are mild and resolve with immune tolerance. Severe injury may include an autoimmune component, which makes it difficult for patients to recover even if the drug is stopped, often resulting in liver transplantation or death. Understanding the mechanism of INH-induced liver injury may make it possible to prevent progression of the injury after the drug has been stopped. If the injury is immune mediated, in particular mediated by lymphocytes as the histology suggests, treatment with agents such as anti-thymocyte globulin may be effective (Metushi & Phillips, 2016)

However, preventive measures remain a major concern before the occurrence of severity, including the use of natural substances hepatoprotectorwith less side effects. The hepatoprotector substance is expected to prevent liver damage while reducing the impact of damage that has already occurred. Of the various types of medicinal plants that are known to contain antioxidants, one of the ones that attracts attention is Syzygium cumini fruit seeds or known in Indonesia as jamblang fruit. Chemical content of Syzygium cumini fruit's seed are gallic acid, elagic acid, corilagin, ellagitannin, isoquercetin, quercetin, caffeine acid, ferulic acid, guaiacol, resorcinaldimethyl ether, lignaglucoside, veratrole, β-sitosterol, palmitic acid, etc (Sisodia, 2009).

The plant is well known in many countries due to its medicinal properties and fruit value. Traditionally, various parts of the plant were utilized to treat different ailments of humans and animals (Thomson, 2000). The bark has been used for the treatment of sore throat,bronchitis, asthma, thirst, biliousness, dysentery and ulcers (Ayyanar&Subash-Babu, 2012). Leaf juice, alone or in combination with herbs, goat milk and honey is reported to be effective in treating diarrhea, diabetes and stomach-ache (Nikhat et al, 2008). The literature review indicated a number of reports regarding antioxidant activity of extracts, mainly; fruit, peel, leaves and bark of the plant, using various models(Veigas, *et al*, 2008). The antioxidant activityof all parts of the plant was attributed to diverse types ofphytochemical constituents reported previously (Banerjee & Narendhirakannan, 2011).

In previous studies, the seeds of this plant can be used as a drug for diabetes, metrorrhagia, anti-inflammatory, strengthen teeth and gums and as a treatment for non-inflamed type Tinea capitis in the form of a sedion lotion. Elagic acid (EA) has a function as a free radical decomposition. Elagic acid has been reported to reduce liver function marker enzymes on the induction of toxicity with carbon tetrachloride (CCl4) (Dalimartha, 2003).

Based on the background the authors wanted to conduct research to determine the effect of jamblang seed extract as a hepatoprotector by looking at decreasing levels of AST and ALT in rats induced by INH.

2 EXPERIMENTAL METHODS

This research used an experimental with post test only one control groups design.

2.1 Research Sites

Organic chemistry laboratory of the Medical Institute of Lubuk Pakam Medistra is used for the screening process of chemical compounds, the Pharmaceutical Pharmacology Laboratory of the Medical Institute of Medistra Lubuk Pakam is used for rat blood collection and the Regional Health Laboratory for examination of ALT and AST.

2.2 Tools and Materials

The tools used consist of animal balance (GW-1500), electric balance (Mettler Toledo), laboratory glassware, mortar and stamfer, surgical tools (Wells spencer), slide glass, cover glass, parchment paper, millimeter paper block, watch glass, oral sonde, dropper and 1 ml syringe (Terumo), microtube, centrifuge (Velocity 18-R) and microscope (Olympus). The materials used in this study include plant, animal and chemical ingredients. Plant material used is jamblang fruit seeds. The chemicals used are Na-CMC (sodium carboxy methyl cellulose), distilled water, sodium chloride 0.9% (Merck) and EDTA tube. The chemicals used unless otherwise stated are of technical quality, namely ethanol (distillation), acetic acid anhydride, concentrated sulfuric acid, toluene, choral hydrate, and concentrated hydrochloric acid.

2.3 Ethanol Extract of Jamblang Fruit Seeds (*Syzygium cumini*)

The jamblang used should be ripe, with a blackish purple color on the outside. The seeds are taken and then dried in the sun. The extract was obtained by jamblang seeds which were dried, mashed, and then extracted with ethanol liquid. Extraction was carried out by the percolation method. The extraction result is then dissolved with aquadest plus 0.5% carboxymethyl cellulose (CMC) and put in a glass bottle stored in the refrigerator.

2.4 Preparation of Experimental Animals

This research were 30 male white rats (Rattus norvegicus) strain of Wistar male with a weight of 150-200 grams. Before being approved, the animal experiment is conditioned for 2 weeks in a good enclosure to adjust the environment and uniform food. Large samples of each group are determined using the Federer formula.

2.5 Sampling Technique

Purposive sampling technique is sampling of the population is done intentionally according to the required sample requirements. In purposive sampling, the characteristics and the number of samples taken are determined or determined in advance. Sampling was done by purposive sampling, with the criteria for selecting subjects based on characteristics that have been known previously. Experimental animals were divided into 5 groups, each group consisting of 6 mice which were randomly selected. Group 1 as a negative control group (K-), group 2 as a positive control group (K +), group 3 as a control treatment for dose 1 (P1), group 4 as a treatment group for dose 2 (P2), and group 5 as a treatment group for dose 3 (P3).

2.6 Dose of Jamblang Fruit Seed Extract

Jamblang seed extract is made by percolation method. Previously, jamblang seeds were dried, mashed, and then extracted with 70% ethanol liquid. The extract is obtained in the form of a solid paste. Suspension of jamblang fruit seed extract is done by inserting pasta into the glazing bekker then weighing, after that it is diluted with distilled water and added with a suspension agent (CMC 0.5%). The solution is then homogenized with a manual stirrer without heating until a suspension is formed. The weight of the rat used is + 200g (150g - 220g), then the dose of jamblang seed extract that will be given to mice is:

a. 100mg / kg body weight / day
   = (100mg × 200g) / 1000g / day
   = 20 mg / rat / day
b. 200mg / kg body weight / day
   = (200mg × 200g) / 1000g / day
   = 40 mg / rat / day
c. 400mg / kg body weight / day
   = (400mg × 200g) / 1000g / day
   = 80 mg / rat / day

Jamblang fruit extract was administered orally once a day at a dose according to Sisodia and Bhatnagar (2009) research, 20 mg / rat for the P1 group, 40 mg / rat for the P2 group, and 80 mg / rat for the P3 group of mice every day starting from the day 8th to 25th day.

2.7 Preparation of Isoniazid Suspension

Isoniazid (INH) were given in a 300 mg tablet form. The isoniazid drug tablet were crushed with mortar, after that it was diluted with distilled water, homogenized until an isoniazid solution is obtained. Toxic dose of INH in humans is 30 mg / kg BW. The conversion factor for humans weighing 70 kg in mice weighing 200 g is 0.018.

a. Doses in humans weighing 70 kg 30 mg x 70 kg = 2100 mg / human
b. Conversion in mice weighing 200 g 2100 mg x 0.018 = 37.8 mg / rat. Rounding (40 mg / rat)

2.8 Experimental Procedures

The experimental animals consisted of 30 male white rats which were divided into 5 groups:
a. Negative control (K-): aquades 1ml / oral rat
b. Treatment 1 (P1): EEBJ 20mg / kg bw
c. Treatment 2 (P2): EEBJ 40mg / kg bw.
d. Treatment 3 (P3): EEBJ 80 mg / kg bw.
e. Positive control (K +): given INH on the 12th day until the 25th day.

After weighing and determining the dose is completed then on the eighth day treatment of experimental animals began. The negative control group was given aquabides 1 ml orally per rat, treatment group 1 was given jamblang seed extract 20 mg / rat on the 8th day to the 25th day. Also given INH on the 12th day until the 25th day. So that starting on day 12 in one day the rats get jamblang seed extract jamblang fruit seed extract is given 1 hour before INH. Treatment group 2 was given extract of jamblang seeds 40 mg / rat on the 8th day until the 25th day. Also given INH on the 12th day until the 25th day. Starting the 12th day, jamblang fruit seed extract was given 1 hour before INH. Treatment group 3 was given 80 mg jamblang seed extract / rat on the 8th day until the 25th day. Also given INH on the 12th day until the 25th day. Starting the 12th day, jamblang seed extract was given 1 hour before INH. The positive control group was given INH on the 12th day to the 25th day. Outside the treatment schedule the rats were given food pellets and distilled water ad libitum
The Ethanolic Extract of Jamblang Seeds (EEJS) was given on the 8th day until the 25th day. INH is given on the 12th day until the 25th day, giving jamblang seed extract is done 1 hour before INH. On the 26th day rat blood was taken to measure ALT and AST levels.

2.9 Measurement of ALT and AST levels

ALT and AST levels were examined using a spectrophotomester conducted at the North Sumatra Provincial Health Laboratory. Blood is drawn from the heart and arteries as much as 0.5 ml of blood is inserted into the microtube, allowed to stand at room temperature for 5 minutes, centrifuged for 10 minutes at a speed of 3000 rpm to produce a clear serum. Serum was separated and AST and ALT levels were measured.

2.10 Data Analysis

The data were analysis for normality using the Shapiro-Wilk test because the sample size was ≤ 50. Then the variance test was also performed using the Levene's test. Hypotheses were tested using the One-Way Anova (Analysis of Variance) test to find out the existence of mean differences in the five treatment groups.

3 DISCUSSION

3.1 The Results of Phytochemical Screening

The results of phytochemical screening of Jamblang seed ethanol extract showed the content of alkaloids, flavonoids, quinones, polyphenols, tannins, and steroids / triterpenoids groups that could be seen in table 1.

Table 1: Phytochemical Screening Results of Ethanolic Extract of Jamblang Seed

Compound Group	Result
Alkaloids	+
Flavonoids	+
Saponin	-
Quinon	+
Polyphenols	+
Tanins	+
Steroids/ Triterpenoids	+

Notes:
+ :Contains the class of examination compounds
- : Did not contain the class of examination compounds

The seeds are reported to contain jamboline, traces of pale yellow essential oil, chlorophyll, fat,

resin, albumen, tannins, phenolic compounds such as ellagic acid, gallic acid, caffeic and ferulic acids and their derivativesand flavonoids like rutin and quercetin (Charles River Laboratories, 2008). Based on such constituents, seed extracts are expected to possess excellent astringent and antioxidant potential, which may be beneficial in relieving gastroenteritis and liver inflammation.

3.2 Measurement Results of ALT (Alanin Aminotransferase) and AST (Aspartate Aminotransferase) Levels

AST and ALT both are vital transaminase enzymes and play central role in amino acid metabolism. Both of these are found in the different body's organs such as liver, heart, skeletal muscle, kidneys, brain, and red blood cells(Agrawal, 2013). Serum AST and ALT level, and their ratio (AST/ALT ratio) are frequently measured clinically as biomarkers for liver health. Their increased level has been linked with abnormal liver functions, though these are not very specific to liver disease. Though, the toxin treated animals showed increased levels of these enzymes. Conversely, extract therapies attenuated the increased level of these enzymes in serum. Recovery towards the normalization suggests that these extracts caused parenchymal cell regeneration in liver, thus protecting membrane fragility and thereby decreasing enzyme leakage (Achliya, *et al*, 2004).

The results of measurements of ALT and AST levels could be seen on table 2 and table 3 below.

Table 2. Measurement Results of ALT

Goup	Level of AST(IU/L) ± SD
Negative control	50,90±6,65
EEJS20mg/kg bb	50,80±6,49*
EEJS40 mg/kg bb	48,80±4,14*
EEJS 80 mg/kg bb	45.25±4.78*
Positive control	71,20±10,20

Note:
* = p< 0,05, significant difference with positive control group

The results of statistical analysis byone way ANOVA showed that there was a significant difference (p <0.05) between mice given EEBJ and an isonazide positive control group. This shows that the administration of EEBJ has an effect of decreasing the ALT value on isoniazid-induced test animals. Based on Charles River Laboratories (2008) the normal ALT value for white mice is 14-64 IU / L.

Table 3. Measurement Results of AST

Group	Levels of AST(IU/L) ± SD
Negative control	207,80±4,55
EEJS 20mg/kg bb	246,00±2,55*
EEJS 40 mg/kg bb	216,80±2,28*
EEJS 80 mg/kg bb	211,75±2,98*
Positive control	246,00±8,33*

The results of statistical analysis using one way ANOVA showed that there was a significant difference (p <0.05) between mice given EEBJ and an isonazide positive control group. This shows that the administration of EEBJ has an effect of decreasing AST value on isoniazid-induced test animals.

Based on Charles River Laboratories (2008) the normal AST value for white rats is 64-222 IU / L.

The INH used here, is actually an antibiotic drug but also known to cause oxidative injury particularly in liver cells(Achliya, *et al*, 2004).

INH is a hydrazide that is readily oxidized 11. Three metabolites have been proposed to be responsible for INH-induced liver injury, acetyl hydrazine (AcHz), hydrazine (Hz) and more recently a metabolite resulting from the bioactivation of INH itself.Experiments implicating AcHz and Hz as hepatotoxic species were performed several decades ago, mostly in rats where the acute liver injury correlated with covalent binding of AcHz and with blood levels of Hz 12. At the time, the parent drug (INH) was not thought to contribute to liver injury because its administration did not produce severe liver injury. However, these experiments utilized ring-labelled acetylisoniazid (AcINH) (Meng, 2015).

This conclusion was not warranted because the drug that was administered was not INH. It was AcINH in which the hydrazine is blocked. If hydrolysis led to AcHz and isonicotinic acid, no covalent binding of the pyridine ring would occur. In addition, the characteristics of the liver toxicity in these studies were different from that in humans. In particular, it was an acute rather than a delayed onset idiosyncratic liver injury. Furthermore, the metabolism in humans may be different from that in rats. However, the conclusion that direct bioactivation of INH does not occur has persisted (Metushi, 2011). Recently, a reactive metabolite resulting from bioactivation of INH itself has been shown to form covalent adducts to liver

macromolecules 13-17. Covalent binding of this metabolite is more likely to lead to an immune response than the reactive metabolite of AcHz which would only acetylate proteins (Figure 1).

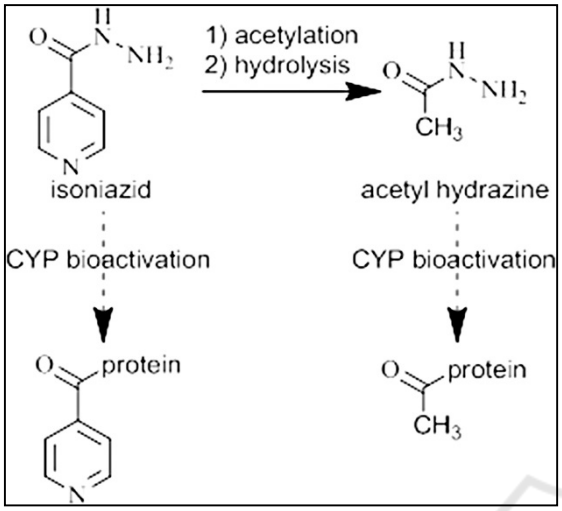

Figure 1. Proposed pathway for an immune mediated reaction to INH in the liver.

Using western blots and mass spectrometry, it has been shown that the reactive metabolite of INH can react with multiple lysine residues on hepatic proteins. Further characterization using mass spectrometry revealed drug adducts on D-dopachrome decarboxylase, prohibitin 2 and macrophage migration inhibitory factor 18. Autooxidation of INH involving free radicals has also been reported. It is unclear whether this is significant *in vivo* where there are many antioxidant systems.Moreover, INH produces hydrazine metabolites (nitrogen free radicals) after metabolism. These reactive free radicals act as stimulator of lipid peroxidation resulting in cell death and hepatic necrosis. In this investigation also, INH treatment showed considerable liver injury. Which was resembles with earlier investigations (Meng, 2015).

The administration of ethanolic extract of seeds of the plant in two doses lowered the level of biochemical markers, which were increased by free radicals of INH. It is probable that the administration of extract for 14 days increased the antioxidant capacity of animal to scavenge the free radicals generated by INH. The free radical scavenging activity of the plant, under investigation, has been attributed to the presence of flavonoids and related compounds. The plant also contained ellagic acid, a polyphenol having lipid peroxidation inhibition activity.

The ethanol extracts of E. jambolana seeds showed hepatoprotective effects in carbon tetrachloridetreated rats. In addition, another study has reported on the hepatoprotective and antioxidant activity of E. jambolana seeds. Hepatoprotective effects are attributed to its antioxidant activity, which restores the activity of superoxide dismutase, catalase, and glutathione peroxidase to normal levels, and increases glutathione content and levels of lipid peroxidation and hydroperoxides in the liver. Seed content includes glycosides, traces of pale yellow essential oil, fat, resin, albumin, chlorophyll, the alkaloid jambosine, gallic acid, ellagic acid, corilagin and related tannins, 3,6-hexahydroxydiphenoyl glucose and its isomer 4,6-hexahydroxydiphenoyl glucose, 1-galloyl glucose, 3-galloyl glucose, quercetin, and elements such as zinc, chromium, vanadium, potassium, and sodium. Unsaponifiable matter of the seed fat contains b-sitoterol. Dried seeds of E. jambolana have been reported, with 11.67% alcohol-soluble extractive fiber, 3.397% inorganic fiber, 40% water-soluble gummy fiber, and 15% water-insoluble neutral detergent fiber. Kumar and colleagues (2009) state that the ethyl acetate and methanol extracts of the seeds of S. cumini show the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins, and triterpenoids. Since the current worldwide morbidity and mortality due to liver disease is increasing every year, with corresponding increases in expenditure for drug treatment, alternative plant therapies may be beneficial.

Presumably, treatment with ethanolic extracts of jamblangcan modulate liver cytochrome P-450 enzymes to enhance scavenging of hepatotoxic free radicals and by increasing antioxidant defense activities.

4 CONCLUSIONS

Based on the results of the study it can be concluded that the induction of isoniazid (INH) can increase levels of ALT and AST, giving EEBJ 20mg, 40mg and 80mg / kg bw in male white rats which induced by isoniazid gave the effect of decreasing ALT and AST levels. A moderate to high dose increase (80 mg / rat / day) did not increased the effect of increasing ALT and AST due to INHinduction. So that the use of EEJS was expected to be developed as an herbal hepatoprotector product.

ACKNOWLEDGEMENT

Thank you to the Medistra Foundation for the laboratory facilities provided for this study.

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