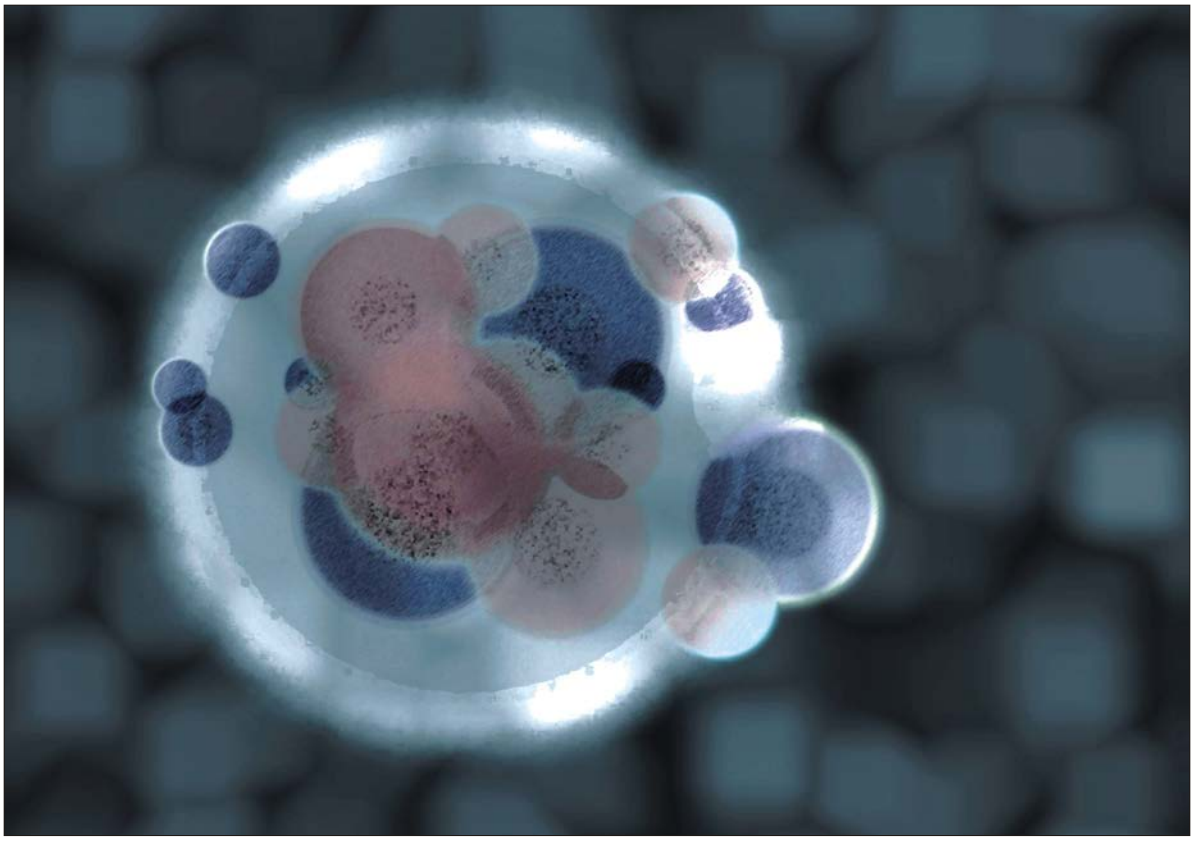

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.388.393

Research Article
Hepatoprotective Activity of Pineapple (*Ananas comosus*) Juice on Isoniazid-induced Rats

[1]Novi Yantih, [2]Yahdiana Harahap, [1]Wahono Sumaryono, [3]Rianto Setiabudy and [1]Lestari Rahayu

[1]Faculty of Pharmacy, University of Pancasila, Jakarta, Indonesia
[2]Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia
[3]Faculty of Medical, Universitas Indonesia, Jakarta, Indonesia

Abstract

Background and Objective: Pineapple fruit (*Ananas comosus*) can inhibit the activity of cytochrome 2E1 (CYP2E1). Isoniazid (INH) is one of the most important antituberculosis drugs and it undergoes hydrolysis in the liver via an enzymatic reaction with CYP2E1, resulting in the formation of hepatotoxic compounds. Extracts of ethanol and water from pineapple fruit can decrease alanine transaminase (ALT) and aspartate transaminase (AST) levels in rats, the increased ALT and AST levels are directly proportional to the damage of liver function. The aim of this study was to evaluate the hepatoprotective activity of pineapple juice in INH-induced rats. **Materials and Methods:** Rats were divided into four groups. The normal group (Group 1) was treated with water, the negative group (Group 2) was induced with INH, the positive group (Group 3) was treated with silymarin and the test group (Group 4) was treated with pineapple juice. The treatments for all groups were administered orally for 8 weeks. Rat blood was collected at 0, 2, 4 and 8 weeks after the start of treatment. Levels of ALT and AST were determined using the photometric method and the rat livers were taken for histopathological testing during week 8. Data were analyzed using the one-way analysis of variance test with a 95% confidence interval. **Results:** Based on the data analysis, pineapple juice exhibited hepatoprotective activity, as it decreased the ALT and AST levels in the rats after 4 weeks of treatment. **Conclusion:** Pineapple juice, protected the rats' livers by inhibiting the central venous diameter widening, although the data analysis showed that the liver function in these rats was not as good as that in the positive controls.

Key words: *Ananas comosus*, hepatoprotective, transaminase enzyme, silymarin, cytochrome

Citation: Novi Yantih, Yahdiana Harahap, Wahono Sumaryono, Rianto Setiabudy and Lestari Rahayu, 2017. Hepatoprotective activity of pineapple (*Ananas comosus*) juice on isoniazid-induced rats. J. Biol. Sci., 17: 388-393.

Corresponding Author: Yahdiana Harahap, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia Tel: +62818222192

Copyright: © 2017 Novi Yantih *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver is a regulator of almost all metabolisms in the body. In addition to these functions, this organ also secretes several enzymes, including alanine transaminase (ALT) and aspartate transaminase (AST), into the blood. The secretion of these transaminase enzymes is directly proportional to liver function[1]. The AST is a more sensitive enzyme for detecting muscle and heart damage compared to liver damage, as it is also produced in the muscles and heart. In the liver, the AST enzyme is present in the liver parenchyma cells, the level will increase in the blood if there was damage to the liver cells. In contrast, ALT enzymes were highly sensitive to liver cell damage, as ALT enzymes were mostly produced in the liver. If the liver cells were damaged either by virus or other disorders, this enzyme will move from the liver cells into the blood[1]. One of the drugs that can cause damage to liver cells was isoniazid (INH)[2].

The INH is one of the most important tuberculosis (TB) drugs for the prevention and treatment of TB, both as mono therapy and in combination with other TB medications. Long-term INH administration leads to some unexpected side effects, such as hepatotoxicity[2]. The INH hepatotoxicity is due to its metabolites, approximately 90% of INH undergoes acetylation to acetyl isoniazid, while the rest others undergo direct hydrolysis to hydrazine. Acetyl isoniazid and hydrazine were further metabolised to acetyl hydrazine. Through enzymatic reactions with cytochrome 2E1 (CYP2E1), hydrazine and acetyl hydrazine produce reactive compounds that will bind to proteins in liver cells, thereby causing liver necrosis[3,4]. The damage to liver cells that can be induced by INH is defined as an ALT increase up to 1.5 times above normal limits or at least a two fold increase in 4 weeks of TB treatment. Approximately 10-20% of patients that receive INH therapy have mild liver dysfunction, as indicated by mild and transient elevations of AST, ALT and bilirubin levels[5].

Hepatoprotective agents are compounds that have mechanisms to repair and protect liver function. Pineapple fruit (*Ananas comosus*) contains phenol, vitamins and some proteases, such as bromelain[6]. Pineapple is known to have the ability to inhibit the activity of the CYP2E1 in rat livers and this effect was larger than that of other fruits, like mangosteen, guava, mango, papaya and banana[7]. Thus, pineapple has the potential for development as a hepatoprotective agent.

At a dose of 0.08 and 2mL, pineapple vinegar can reduce the activity of the P450 enzyme on paracetamol-induced mice[8]. Extracts of ethanol and water from pineapple fruit with doses of 200 and 400 mg kg^{-1} b.wt., can decrease ALT and AST levels in rats, based on their antioxidant mechanisms[9]. Pineapple water extract given orally to mice with a dose of 400 mg kg^{-1} b.wt., was more effective than ethanol extract at the same dose. Acute toxicity test results on water and ethanol extracts at a dose of 2000 mg kg^{-1} b.wt., did not show deaths in any rats over 14 days of observation. The hepatoprotective activity of water extract was higher than ethanol extract from pineapple[9], but there has been no report on the hepatoprotective activity of pineapple juice.

In this study, the hepatoprotective activity of pineapple juice was tested in INH-induced rats. This was evaluated from the ALT and AST levels in rat blood, as well as from histopathology test results from 8 weeks of INH induction in the rat liver.

MATERIALS and METHODS

Materials: Pineapple (*Ananas comosus*), smooth cayenne type, was obtained from Subang, West Java, Indonesia. The fruit was determined in November, 2015 (2155/IPH.101/If.07/XI/2015) at the "Herbarium Bogoriense" Botanical Field of the Biological Research Central, Indonesian Institute of Sciences, Cibinong-Bogor, Indonesia. The experimental animals used were white male rats (*Rattus norvegicus*) of the Sprague-Dawley strain that were developed in the Non Ruminansia and Animal Hope Laboratory, Faculty of Animal Science, Bogor Agricultural University. As the hepatotoxic agent, analytical-grade INH was obtained from Zhejiang Jiangbei Pharmaceutical, Zhejiang, China. Silymarin (Legalon, Soho Global Health, Jakarta, Indonesia) was used as a positive control. The ALT-AST reagents and standards (EliTech Clinical Systems, Paris, France) were of pro-analysis grade. Sodium chloride, ether, alcohol (Merck, Darmstadt, Germany) and Bouin solution (Sigma Aldrich, Missouri, America) were of pro-analysis grade for histopathology tests.

Equipment: The main equipment components used in this research were a juicer (Kuche, Jakarta, Indonesia), eppendorf tubes (Eppendorf, Hamburg, Germany) and photometer (Microlab 300 LX, ELiTech Group, Paris, France).

Preparation of pineapple fruit juice: Pineapple was removed of its crown, then peeled and cut into pieces. Pieces of pineapple fruit were inserted into the juicer tube. The juicer was set at high speed for about 5-10 min, until the pineapple juice was obtained.

Preparation and treatment of animals: Rats aged 2-3 months and weighing of 150-200 g were acclimatized in the laboratory environment for 7 days. The animals were divided into four groups, each consisting of 6 rats. Group 1, the normal group, was given distilled water. Group 2 was a negative control group, receiving INH at 27 mg kg^{-1} b.wt., orally. Group 3, the positive control group, was given INH at 27 mg kg^{-1} b.wt. and silymarin at 25 mg kg^{-1} b.wt., orally. Group 4 was the test group, thus rats in this group received INH at 27 mg kg^{-1} b.wt. and pineapple fruit juice at 2 mL orally. All groups were treated for 8 weeks. Silymarin and pineapple fruit juice were given 1 h after administration of INH.

Determination of levels of ALT and AST in rat blood: In weeks 0, 2, 4 and 8, blood samples were collected from each group through the orbital vein in the eyes using a capillary pipe and placed in an eppendorf tube. The samples were allowed to stand for about 20 min, they were then centrifuged at 3000 rpm for 15 min. The blood serum was transferred into the eppendorf tube using a syringe. The ALT and AST levels were measured using a calibrated photometer.

Histopathology testing: In week 8, each rat was sedated with ether and then the chest and abdomen were smeared with 70% alcohol and dissected. The heart was removed. The rat liver organ was rinsed with a 0.9% sodium chloride solution to remove the blood attached to the liver tissue; it was then preserved in a container containing bouin solution for histopathological preparation.

Statistical analysis: Data were reported as mean±standard deviation of 6 measurements. One-way analysis of variance test was used to compare all groups and Fisher's least significant difference (LSD) test was performed for determining the different groups. Differences at $p<0.05$ (95% confidence level) were considered to be significant.

RESULTS

Preparation of pineapple fruit juice: The pineapple used in this study was the smooth cayenne type from a plantation in Subang, West Java, Indonesia. Pineapple from Subang has a high water content and little fibre. Pineapple juice was made from ripe pineapple at 7 months. The brix value and acidity of pineapple juice were 1.3497 and 3.52, respectively.

Determination of ALT and AST levels in rat blood: This study received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia and Cipto Mangun kusumo Hospital to use rats as experimental animals in the study. Rats' blood samples of rats were analysed to determine the AST and ALT levels using a calibrated photometer. The ALT and AST standard solutions value were 40.5 and 48.3 U L^{-1}, respectively.

Table 1: Results of the least significant difference (LSD) tests for alanine transaminase (ALT) and aspartate transaminase (AST) levels

	Mean ALT level (U L^{-1})		Mean AST level (U L^{-1})	
Group	Week 4	Week 8	Week 4	Week 8
Normal	25.4±5.9^{a}	25.9±5.8^{a}	50.6±10.5^{a}	52.2±11.2^{b}
Negative	36.8±11.0^{b}	40.2±10.7^{b}	62.8±12.7^{b}	70.6±11.6^{c}
Positive	26.6±9.4^{a}	25.1±9.7^{a}	42.5±7.8^{a}	39.4±7.8^{a}
Test	23.4±5.9^{a}	22.4±5.4^{a}	41.7±8.1^{a}	38.5±6.9^{a}

Values followed by different letters in each column are significantly different at $p<0.05$ and the values are expressed as mean±standard deviation (n = 6)

The measurements of ALT and AST levels by photometry at weeks 0, 2, 4 and 8 are presented in Fig. 1. Data on ALT and AST measurements in each treatment were compared statistically and the results are shown in Table 1.

The ALT and AST levels in all groups showed the same profiles (Fig. 1). The mean ALT and AST levels in Group 1 (normal) exhibited an insignificant increase from the initial week to week 8. Group 2 (negative) showed a continuous increase in ALT levels from the weeks 0-8 of the study. This suggests that INH had a hepatotoxic effect. ALT and AST levels in Group 3 (positive) and Group 4 (test) showed continuous decreases from the initial week to week 8 of the study. The main findings in Table 1 show that pineapple juice exhibited hepatoprotective activity in INH-induced rat after 4 weeks of administration.

The results of statistical tests on all groups found that at weeks 0 and 2, the p-value was more than 0.05. This means that there was no significant difference in ALT and AST levels between groups, illustrating that pineapple fruit juice had not had a protective effect after 2 weeks. The results of statistical analysis in weeks 4 and 8 showed p-values of less than 0.05. This means that there were significant differences between ALT and AST levels among the groups. Statistical analysis with Fisher's LSD test was carried out to assess the significant differences among all groups at the 5% probability level. The LSD test results are presented in Table 1. Based on this table, it can be observed that the negative group had a significant difference from the other three groups, showing that the negative group exhibited a hepatotoxic effect, whereas the test group exhibited a hepatoprotective effect. The LSD test results demonstrated that all groups showed significant differences from group 2 (negative group). This suggests that pineapple juice has the potential to be hepatoprotective and its potential is not significantly different from that of silymarin.

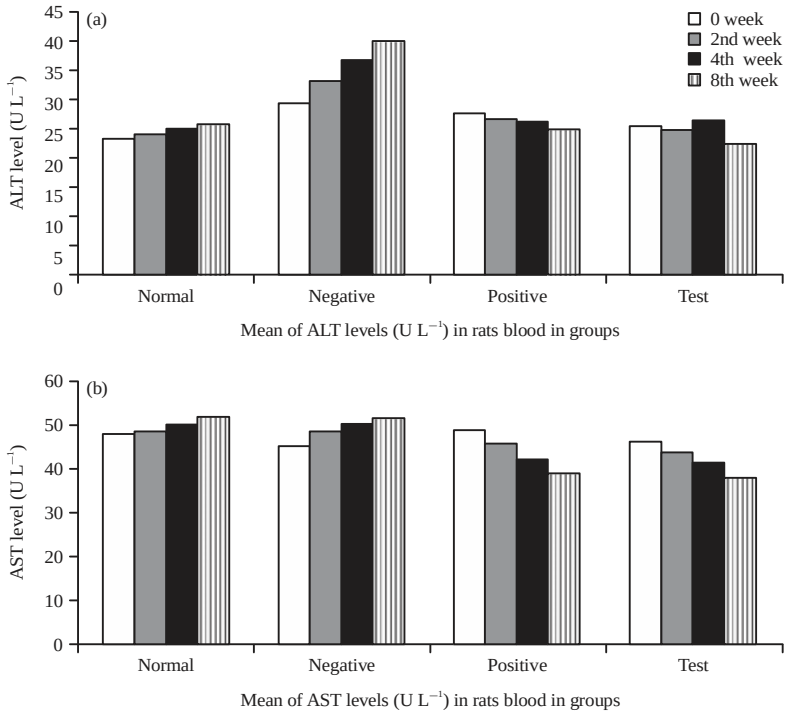

Fig. 1(a-b): (a) Profiles of alanine transaminase (ALT) and (b) Aspartate transaminase (AST) levels in rat blood over 8 weeks

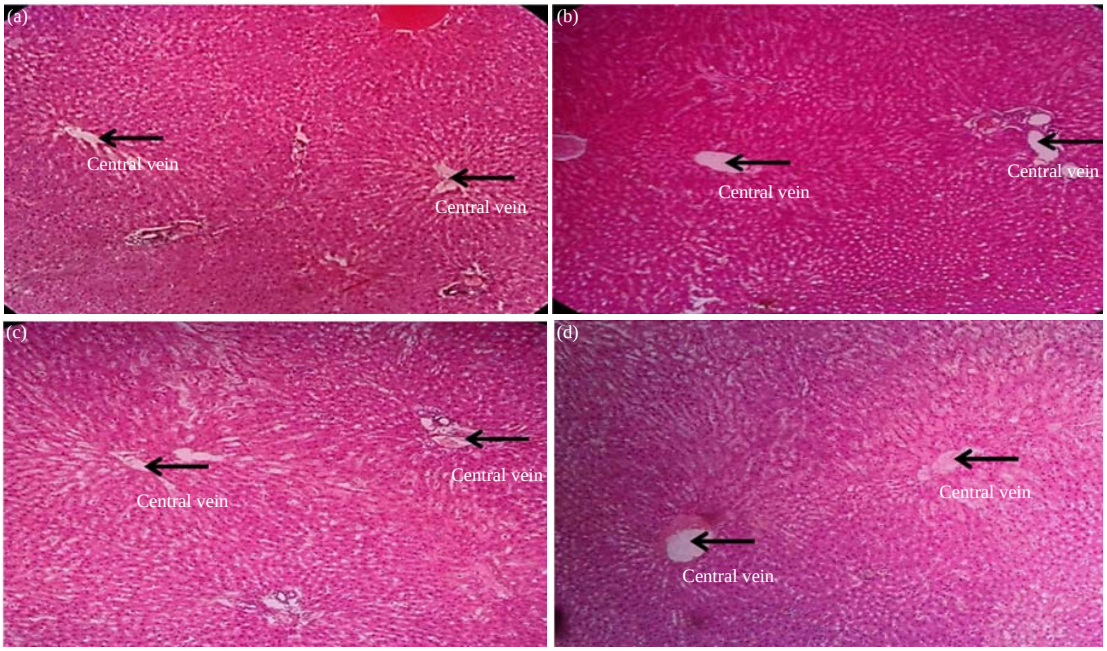

Fig. 2(a-d): Hepatic histopathology of the (a) Normal, (b) Negative, (c) Positive and (d) Test groups

Histopathological testing: The central venous diameters from the four groups are shown in Fig. 2. The central veins in the normal group showed no widening and were arranged normally, in contrast, in the negative control group, the central veins exhibited extensive widening. The positive control and test groups showed less dilation than the negative control group did (Fig. 2, 3). The results are clarified by the measurement of each central venous diameter of each group.

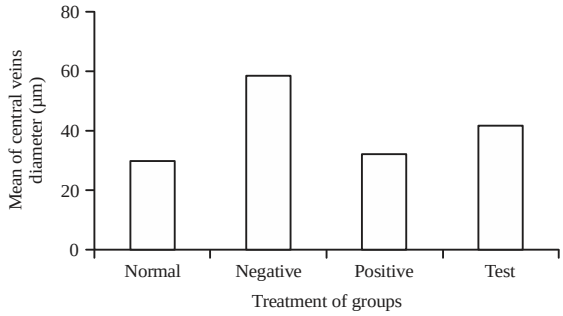

Fig. 3: Mean of central vein diameter (µm) after week 8 in the normal, negative, positive and test groups

Table 2: Least significant difference (LSD) test results for central vein diameter in the four groups

Group	Central vein diameter (µm) at week 8
Normal	29.90±1.31[a]
Negative	58.81±1.78[d]
Positive	32.40±2.39[b]
Test	42.08±1.64[c]

The different letters in each column represent a significant difference at $p<0.05$, values are expressed as mean±standard deviation (n = 6)

The one-way analysis of variance test showed significant differences, which were supported by the LSD test, with a 5% probability level. The results of the statistical analysis in week 4 showed a p-value of less than 0.05. This means that there were significant differences among the groups and the LSD test could be used to observed the significant differences in all groups (Table 2).

The central venous diameter in the negative group exhibited significant differences from the other three groups (Table 2). This suggests that there was a hepatotoxic effect in the negative control group, whereas, there was a hepatoprotective effect in the positive control and test groups. The statistical results also showed that the hepatoprotective activity of pineapple fruit juice was smaller than that of silymarin.

DISCUSSION

The brix value of pineapple fruit juice used in this study met the requirements set by SNI HS (number 2009.41.00, i.e., it was less than 20). The brix value of pineapple juice was relatively low, meaning that the amount of soluble solids dissolved in the water of pineapple fruit was extremely small.

Rats were selected as experimental animals because they have similar enzyme functions and responses to humans[10], as well as because it was easy to breed and obtain rats. Male rats were used to reduce the effects of hormones. The Sprague-Dawley strain was chosen because these rats are calmer and easier to handle.

In the positive control group, silymarin was used because it has hepatoprotective activity. Silymarin at 25 mg kg^{-1} b.wt., was equivalent to 400 mg kg^{-1} b.wt., of pineapple[8]. The INH dose used in this study was a toxic dose, the appropriate INH dose for rats of 37.8 mg/200 g b.wt., was determined by converting the toxic INH dose for humans (30 mg kg^{-1} b.wt.)[11]. Silymarin and pineapple fruit juice were administered at 1 h after the rats were induced with INH. The timing was related to the bioavailability of INH, which decreases with food and the t_{max} of INH, which was 1-3 h[12].

Blood samples for determining ALT and AST levels were taken through the eye orbital sinus. It was easy to obtain samples via this route and the method can minimise haemolysis, which usually occurs when blood samples were taken through the lateral veins of the tail. Blood sampling haemolysis should be prevented, as this may result in dilution effects on substances that are present in plasma, but low in erythrocytes. Enzymes like phosphatase acid, lactate dehydrogenase and AST exhibit high content in erythrocytes. AST and serum activity increase by 2% for every 10 mg dL^{-1} increase in haemoglobin serum content[3]. Rats were fasted for 6 h before treatment to minimise biological variation. The serum was stored in a freezer at a temperature of -40°C to minimise enzyme activity during storage. Serum was used as the samples for photometric measurement of AST and ALT values, since the reagents were compatible with serum.

To ensure the quality and performance of the device according to its function, the photometer equipment needed to be calibrated before the sample analysis. Tool calibration used standard solutions of ALT and AST as substitutes for the samples. The calibration results showed the appropriate ALT and AST measurement values. The standard values are 32.6-52.2 U L^{-1} for ALT and 37.3-59.7 U L^{-1} for AST[13].

In general, substances will be metabolized by the liver. Therefore, the liver is an important body organ that needs to be protected from substances that can damage it. Liver damage can be seen in elevated ALT and AST levels, as well as from liver histopathology analysis. One of the examinations of liver histopathology involves measuring the diameter of the central veins. The central veins are venules bounded by the endothelium. This examination was selected because blood was supplied through the portal vein and the hepatic artery, which was the afferent vessel and supplies the central vein, the efferent vessel in the middle of the lobule. Liver damage involves the widening of central venous diameter[14].

Based on the ALT and AST levels in rat serum, as well as the widening of central venous diameter in rat liver, pineapple juice has the potential to generate a hepatoprotective effect after administration of 2 mL kg^{-1} b.wt., for 4 weeks,

but its potential was smaller than that of silymarin (25 mg kg^{-1} b.wt.). The results are shown in Table 1 and 2, Fig. 1-3. Pineapple fruit contains phenol and bromelain[6]. The hepatoprotective activity of pineapple fruit juice was probably due to its bromelain and phenolic acid contents[8,9]. Previous studies have reported that pineapple can inhibit the CYP2E1 enzyme[7]. The inhibition of CYP2E1 enzyme can reduce the hepatotoxic of INH, because of that enzyme plays an important role in metabolising INH into toxic metabolites[7]. Moreover, the phenolic acid contained in the fruit acts as an antioxidant and it has been shown to reduce levels of AST and ALT enzymes induced by paracetamol[8]. Other studies have shown that pineapple extracted into ethanol and water extracts also contain antioxidants, which were thought to have a protective effect on rat-induced liver paracetamol[9]. In terms of the bromelain content of mature pineapple fruit, bromelain has antioxidant and anti-inflammatory properties, thus, this enzyme is also suspected to have hepatoprotective characteristics[6].

This study was intended as a preliminary study of the pineapple juice as a potential adjuvant for patients receiving INH. It was found that 2 mL of pineapple juice could decrease the ALT and AST levels of INH-induced rat after daily treatment orally for 4 weeks. It is recommended that pineapple juice should be used in further research as an adjuvant for patients taking INH. Pineapple juice can reduce the hepatotoxicity of INH because of its activity as an inhibitor of CYP2E1. Further, *in vivo* study should be carried out in patients to obtain evidence-based results on the hepatoprotective activity of pineapple juice in patients receiving with INH.

CONCLUSION

Pineapple juice had hepatoprotective activity in INH-induced rats after oral administration of 2 mL for 4 weeks. Its activity was smaller than that of silymarin at a dose of 25 mg kg^{-1} b.wt.

SIGNIFICANCE STATEMENT

This study assessed the hepatoprotective activity of pineapple juice on INH-induced rats. It was intended as a preliminary study concerning pineapple juice's potential as an adjuvant to INH, administered to patients to minimize the hepatotoxic side effects of drugs like INH.

ACKNOWLEDGMENT

The authors are grateful for a research grant (Grant No. 355/UN2.R3.1/HKP.05.00/2017) from the Directorate of Research and Community Service, Universitas Indonesia.

REFERENCES

1. Seef, L.B. and R.J. Fontana, 2011. Drug-Induced Liver Injury. In: Sherlock's Diseases of the Liver and Biliary System, Dooley, J.S., A. Lok, A.K. Burroughs and J. Heathcote (Eds.). Blackwell Publishing Ltd., UK., pp: 478-506.
2. Rahman, A.O., 2013. The correlation of hydrazine (Isoniazid metabolite) levels with SGPT levels at the end of an intensive phase of treatment of pulmonary tuberculosis patients. Ph.D. Thesis, Gadjah Mada University, Indonesia.
3. Tostmann, A., M.J. Boeree, R.E. Aarnoutse, W.C.M. de Lange, A.J.A.M van der Ven and R. Dekhuijzen, 2008. Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. J. Gastroenterol. Hepatol., 23: 192-202.
4. Teixeira, R.L.D.F., R.G. Morato, P.H. Cabello, L.M.K. Muniz and A.D.S.R. Moreira *et al.*, 2011. Genetic polymorphisms of NAT2, reCYP2E1 and GST enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. Mem. Inst. Oswaldo Cruz, 106: 716-724.
5. Kishore, P.V., S. Palaian, R. Paudel, P. Mishra, M. Prabhu and P.R. Shankar, 2007. Drug induced hepatitis with anti-tubercular chemotherapy: Challenges and difficulties in treatment. Kathmandu Univ. Med. J., 5: 256-260.
6. Murniati, E., 2006. The Sweet Scaly Pineapple on the Tongue. SIC Printing, Surabaya, Indonesia.
7. Chatuphonprasert, W. and K. Jarukamjorn, 2012. Impact of six fruits-banana, guava, mangosteen, pineapple, ripe mango and ripe papaya-on murine hepatic cytochrome P450 activities. J. Applied Toxicol., 32: 994-1001.
8. Mohamad, N.E., S.K. Yeap, K.L. Lim, H.M. Yusof and B.K. Beh *et al.*, 2015. Antioxidant effects of pineapple vinegar in reversing of paracetamol-induced liver damage in mice. Chin. Med., Vol. 10. 10.1186/s13020-015-0030-4.
9. Mallik, D., C. Bhattacharjee and T.S. Gouda, 2014. Pharmacological intervention of the fruit of plant *Ananas comosus* acting as hepatoprotective activity in animal models. Indian J. Res. Pharm. Biotechnol., 2: 1167-1172.
10. Yue, J., R.X. Peng, J. Yang, R. Kong and J. Liu, 2004. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. Acta Pharmacol. Sin., 25: 699-704.
11. Himawan, R., 2008. Effect of green tea leaf extract (*Camellia sinensis*) on levels of SGPT white rats (*Rattus norvegicus*) induced isoniazid. Ph.D. Thesis, Sebelas Maret University, Surakarta.
12. Preziosi, P., 2007. Isoniazid: Metabolic aspects and toxicological correlates. Curr. Drug Metab., 8: 839-851.
13. Dialab, 2006. Liquid reagents of GOT (AST) and GPT (ALT). DIALAB Production von Chemishtechnishen, Austria.
14. Taylor, C.R., 2015. Cirrhosis imaging. Jun 24, 2015. https://emedicine.medscape.com/article/366426-overview.