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Research Article
Ethanol-based Breadfruit Leaf (*Artocarpus altilis*) Extract as Hepatoprotective in Carbon Tetrachloride-induced Liver Injury

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Abstract

Background and Objective: Breadfruit leaf (*Artocarpus altilis*) can be used to cure several diseases such as renal disease, high blood pressure, liver, diabetes, asthma, toothache, skin infection and cancer in Indonesian community. Liver disease including hepatitis and hepatotoxicity require proper management so they may heal faster. This study was aimed to examine the hepatoprotective effect of ethanol extract of Indonesian breadfruit leaf in carbon tetrachloride-induced liver injury Wistar rats by measuring Serum Glutamic Pyruvic Transaminase (SGPT) and malondialdehyde (MDA) levels. **Materials and Methods:** Wistar rats were divided into negative control group, positive control group and three treatment groups. Ethanol-based breadfruit leaf extract were formulated in doses of 125 mg kg^{-1} b.wt., (T1), 250 mg kg^{-1} b.wt., (T2) and 500 mg kg^{-1} b.wt., (T3) applied per oral for 7 days. Positive control and treatment groups were induced by 1 mL kg^{-1} b.wt., dose of carbon tetrachloride intraperitoneal on day 8. Data were statistically analyzed using one-way ANOVA and post hoc test. **Results:** The research revealed that all groups treated with breadfruit leaf have the lower of MDA and SGPT levels ($p<0.05$) when compared to the positive control Ethanol-based breadfruit leaf extraction doses of 250 and 500 mg kg^{-1} b.wt., per oral given for 7 days before induced by 1 mL kg^{-1} b.wt., dose of carbon tetrachloride showed significance in decreased MDA levels ($p = 0.017$) however dose of 500 mg kg^{-1} b.wt., showed the most effective to decreased SGPT levels ($p = 0.000$). **Conclusion:** It is concluded that ethanol-based breadfruit leaf extract had effect on liver protection by decreased levels of MDA and SGPT.

Key words: *Artocarpus altilis*, carbon tetrachloride, drug, hepatoprotective, malondialdehyde

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

INTRODUCTION

Liver function diseases remained a major health problem in both developed and developing countries. The term hepatitis is used for all types of inflammation in the liver cells that can be caused by infection (viruses, bacteria and parasites), drugs, chemicals and alcohol[1]. According to World Health Organization (WHO), the most common cause of hepatitis is due to viruses[1]. In Indonesia, the prevalence of hepatitis in 2013 was 1.2%. Types of hepatitis that infect the population of Indonesia are hepatitis B (21.8%) and hepatitis A (19.3%) indicating that Indonesia is included in the group of countries with moderate to high endemic[2]. In addition to the virus, hepatitis can also be caused by various chemical compounds or drugs that are hepatotoxic[3].

Hepatotoxic drugs are numerous including galactosamine, isoniazid, rifampicin, pyrazinamide and acetaminophen. In addition to drugs, there are highly hepatotoxic compounds namely carbon tetrachloride (CCl_4)[4]. Among these substances, CCl_4 is more commonly used as a liver cell damage inducer so that CCl_4 is often used in testing hepatoprotective activity against experimental animals[5,6].

The CCl_4 compound is a liquid, clear, colorless and volatile compound. In everyday life used as remover stains and carpet cleaners[7]. Giving CCl_4 can cause damage to the liver resulting from the formation of free radicals[8]. The administration of CCl_4 at a dose of 1 mL kg^{-1} b.wt., resulted in a large steatosis and was described with elevated serum glutamic pyruvic transaminase/Serum Glutamic Pyruvic Transaminase (SGPT) levels up to twice as high. CCl_4 compounds damaging liver cells through their reactive toxic metabolites, trichloromethyl ($CCl_{3.}$) through the CCl_4 biotransformation process by the cytochrome P-450 catalyst enzyme in liver cells[9]. The accumulation of free radicals from CCl_4 associated with the lipid peroxidation process will result in oxidative stress which can be determined by measuring one of the parameters of malondialdehyde (MDA). If levels of MDA in the blood increases it induced the risk of hepatocyte cell membrane damage. Increased levels of MDA in the blood can be suppressed with electron-donating compounds (electron donors) called antioxidants. Antioxidants can delay, scavenging and prevent lipid oxidation process[10].

Indonesian breadfruit (*Artocarpus altilis*), known locally as sukun, is a tropical plant that can be found in many region of Indonesia. Graham and De Bravo[11] have identified many active components within the breadfruit including steroids, saponins, polyphenols, flavonoids, tannins, riboflavin and champerol. Some of these compounds such as flavonoids have proven benefits as radicals-scavenging antioxidants. Breadfruit plants are rich in flavonoids that act as secondary antioxidants, but the highest flavonoid compounds are found in the leaves[12,13]. Agustin *et al.*[14] have reported that hepatoprotective activity of breadfruit leaf performed by CCl_4 induction method in the infused form with a dose of 13.5 g kg^{-1} b.wt., 27 and 54 g kg^{-1} b.wt., using alanine transferase activity and lipid peroxide content. Breadfruit ethanol extract at doses of 125, 250 and 500 mg kg^{-1} b.wt., can decrease SGPT and SGOT enzyme parameters levels[14].

This study aimed to know the effect of ethanol based breadfruit leaf extract at doses of 125, 250 and 500 mg kg^{-1} b.wt., to protect Wistar rats liver function induced by carbon tetrachloride. The hope is to develop a more effective dose with low toxicity and has protective effect on the liver damage.

MATERIALS AND METHODS

Research was carried out between January and April 2017 at the Animal and Biochemistry Laboratories of the Medical Faculty, Universitas Jenderal Ahmad Yani, Cimahi Indonesia. Thirty Wistar rats were used after ethical approval was obtained from Research Ethics Committee Hasan Sadikin Hospital (RSHS) Bandung with No: 104/UN6.C1.3.2/KEPK/PN/2017. The breadfruit leaves used were harvested in Cibeber-Cilegon iplantations in West Java, Indonesia according to GAMP and were identified in Institut Teknologi Bandung. The leaves were processed to adhere with the herbal medicine standard from BRCC Institut Teknologi Bandung Indonesia.

Chemical materials: Ethanol 96%, CCl_4, liquid paraffin, SGPT reagent, standard solution, butylated hydroxytoluene (BHT) solution, SDS solution, EDTA solution (1.488 g EDTA in 50 mL H_2O always made new), acetic acid solution and thiobarbituric acid (TBA) 0.8% were used.

Ethanol-based breadfruit leaf extract: The breadfruit leaves were macerated to obtain ethanol based breadfruit leaf extract. Ethanol extracts of breadfruit leaf was made bay desiccating 4 kg of shredded leaves that was then placed into an oven at 50°C for 2 days. The remaining 1 kg of semisolid leaves were refined into powder and made into ethanol based extract, using 96% ethanol following the desiccation process. The powder was then placed into a macerator for 24 h until all the powder had become liquid extracts. A rotary evaporator was subsequently used to evaporate the excess liquid to thicken the extract, which resulted in a thick and dark green substance.

Experimental animals: Animals used in this study were white male rats' Wistar strains, with weight ranged from 200-300 g and aged 6-8 weeks. This rat was obtained from Biofarma Bandung. The subjects were acclimation for 7 days, weighing was done at the first taking and before treatment given to determine the doses of ethanol based breadfruit leaf extract and the administration of carbon tetrachloride. Rats were divided into negative control groups, positive control groups and three treatment groups.

Ethanol-based breadfruit leaf extract with a predetermined dose was inserted into the stomach of a mouse using an oral sonde. The doses given adjusted to the weight of the rats. Negative control and positive control were not provided with ethanol based breadfruit leaf extract, whereas three treatment groups were given ethanol based breadfruit leaf extract for a week with different doses [125 mg kg^{-1} b.wt., (T1), 250 mg kg^{-1} b.wt., (T2) and 500 mg kg^{-1} b.wt., (T3)].

All groups except negative control were induced with 1 mL kg^{-1} b.wt., of carbon tetrachloride intra-peritoneal on day 8, after 7 days of ethanol based breadfruit leaf extract oral administration with single dosage. Blood were taken from the retro-orbital vein for examination of MDA and SGPT levels at day 8.

MDA assay: The productions of free radicals were determined by MDA tissues level with TBARs routine procedure perform absorbance readings at wavelength of 532 nm.

SGPT assay: The productions of SGPT were determined by spectrophotometer UV test with International Federation of Clinical Chemistry (IFCC) procedure perform absorbance readings at wavelength of 334-360 nm.

Data analysis: Data were statistically analyzed by one way ANOVA and Tukey's test, after affirming the normality and homogeneity of variances assumptions of the data sets. p values < 0.05 were considered to be significantly different[15].

RESULTS

Hepatoprotective effect of ethanol-based breadfruit leaf extract on MDA plasma levels: Examination of MDA level was done after 7 days of experimental animal giving ethanol based breadfruit leaf extract peroral T1, T2 and T3, then at day 8 rats induced with CCl_4 1 mL kg^{-1} b.wt., for each group except Negative Control (NC).

All subjects in the ethanol based breadfruit leaf extract treatment groups showed better MDA plasma level than the positive control groups when examined on days 8. In the positive control group that was induced with CCl_4 and given no ethanol based breadfruit leaf extract, the average MDA level was 1.555 mg dL^{-1}, whilst the group that received ethanol based breadfruit leaf extract dose 500 mg kg^{-1} b.wt., had a mean MDA level of 0.922 mg dL^{-1} (Table 1).

The highest MDA levels were demonstrated by a positive control group induced only CCl_4 1 mL kg^{-1} b.wt., with an average MDA value of 1.555 mg dL^{-1}. The MDA levels in T1, T2 and T3 showed a low value compared with MDA levels of positive control group because it was administered breadfruit ethanol extract.

A Kruskal-Wallis test was performed on the data relating to MDA plasma levels as the data distribution was abnormal. The test results ($p<0.05$) on MDA plasma levels showed significant difference among all groups ($p = 0.017$).

Mann-Whitney post hoc tests showed that the most significant differences ($p<0.05$) were between the positive control group and all the treatment groups who received ethanol based breadfruit leaf extract per oral T1, T2 and T3 (Table 2).

Hepatoprotective effect of ethanol based breadfruit leaf extract on SGPT serum levels: Examination of SGPT level was done after 7 days of experimental animal giving ethanol based breadfruit leaf extract per oral T1, T2 and T3, then at day 8 rats induced with CCl_4 1 mL kg^{-1} b.wt., for each group except Negative Control (NC).

All subjects in the ethanol based breadfruit leaf extract treatment groups showed better SGPT plasma level than the positive control groups when examined on day 8. In the positive control group that was induced with CCl_4 and given no ethanol based breadfruit leaf extract, the average SGPT level was 102.6 U L^{-1}, whilst the group that received ethanol based breadfruit leaf extract dose 500 mg kg^{-1} b.wt., had a mean SGPT serum level of 59 U L^{-1} (Table 3).

Anova test was performed on the data relating to SGPT serum levels as the data distribution was normal. The test results ($p<0.05$) on SGPT serum plasma levels showed significant difference among all groups ($p = 0.000$).

Bonferroni comparative tests showed that the most significant differences ($p<0.05$) were between the positive control group and all the treatment groups who received ethanol based breadfruit leaf extract per oral T1, T2 and T3 (Table 4).

Table 1: Effect of breadfruit leaf ethanol extract in carbon tetrachloride-induced liver injury Wistar rats on malondialdehyde levels

Groups	N	Mean (mg dL^{-1})	SD	Min-Max	p-values
Negative control (NC)	6	0.975	0.292	0.67-1.38	
Positive control (PC)	6	1.555	0.475	1.28-2.40	
125 mg kg^{-1} b.wt., (T1)	6	1.300	0.552	0.79-2.38	0.017*
250 mg kg^{-1} b.wt., (T2)	6	0.970	0.213	0.61-1.20	
500 mg kg^{-1} b.wt., (T3)	6	0.922	0.413	0.70-1.10	

Descriptive: Krusskall wallis test, *Statistically significant (p<0.05), SD: Standard deviation

Table 2: Breadfruit leaf ethanol extract effect on malondialdehyde (MDA) levels value significance of differences between treatment groups

Groups	Comparison group	p-values
	Positive control (PC)	0.028*
Negative control (NC)	125 mg kg^{-1} b.wt., (T1)	0.337
	250 mg kg^{-1} b.wt., (T2)	0.936
	500 mg kg^{-1} b.wt., (T3)	0.936
Positive control (PC)	125 mg kg^{-1} b.wt., (T1)	0.045*
	250 mg kg^{-1} b.wt., (T2)	0.006*
	500 mg kg^{-1} b.wt., (T3)	0.006*

Descriptive: Mann-whitney test, *Statistically significant (p<0.05)

Table 3: Distribution of mean values, standard deviation, min-max and p value effect of breadfruit leaf ethanol extract on SGPT levels

Groups	N	Mean (U L^{-1})	SD	Min-Max	p-values
Negative control (NC)	6	39.5	4.08	35-45	
Positive control (PC)	5	102.6	9.55	93-115	
125 mg kg^{-1} b.wt., (T1)	6	94.0	4.05	90-100	0.000*
250 mg kg^{-1} b.wt., (T2)	6	85.6	5.87	80-95	
500 mg kg^{-1} b.wt., (T3)	6	59.0	6.36	50-67	

Descriptive: ANOVA test, *Statistically significant (p<0.05), SD: Standard deviation

Table 4: Breadfruit leaf ethanol extract effect on SGPT levels value significance of differences between treatment groups

Groups	Comparison group	p-values
	Positive control	0.000*
Negative control	125 mg kg^{-1} b.wt., (T1)	0.000*
	250 mg kg^{-1} b.wt., (T2)	0.000*
	500 mg kg^{-1} b.wt., (T3)	0.000*
Positive control	Negative control	0.000*
	125 mg kg^{-1} b.wt., (T1)	0.297
	250 mg kg^{-1} b.wt., (T2)	0.001*
	500 mg kg^{-1} b.wt., (T3)	0.000*

Descriptive: Bonferroni test, *Statistically significant (p<0.05)

DISCUSSION

This study found that liver damage induced by CCl_4 can be protected with the used of ethanol based breadfruit leaf extract medications. Per oral formulas can be applied in vivo which are relatively easy to used offering hepatoprotective effect. This study showed that breadfruit leaf extract had equal strength in reducing the MDA levels of male white rat Wistar which induced by toxic compound. The CCl_4 compound is a liquid, clear, colorless and volatile compound. Previous studies have found that CCl_4 compounds can cause damage to the liver caused by the formation of free radicals. The CCl_4 compounds damage the liver cells through their reactive toxic metabolites, trichloromethyl (CCl_3) through the CCl_4 biotransformation process by cytochrome P-450 catalyst enzyme in hepatic cells. The accumulation of free radicals associated with the lipid peroxidation process will lead to oxidative stress that can be determined by measuring one of the parameters of malondialdehyde (MDA)[8-10].

Many active constituents of breadfruit leaf extract are believed to promote hepatoprotective in liver diseases. One of breadfruit leaf compound flavonoids have antioxidant properties. It can scavengers free radicals induced by CCl_4 induction. Finally, it was proven that the breadfruit leaves were able to prevent the formation of free radicals and prevent the occurrence of hepatocyte lipid cells of male CCR_4 induced Wistar CCR_4 white rat rats in terms of decreased MDA levels in the treatment group treated with breadfruit ethanol extract[12-14].

This study showed that breadfruit leaf extract had equal strength in reducing the SGPT serum levels of male white rat Wistar which induced by CCl_4. This indicates that there is an effect of saponin leaves ethanol extract on significant levels of SGPT among the five groups. Provision of breadfruit ethanol extract at doses of 125 and 250 mg kg^{-1} b.wt., has shown a significant decrease ($p<0.05$) in SGPT levels. However, the largest decrease in SGPT levels at doses of 500 mg kg^{-1} b.wt., was seen from the mean difference. This result is in accordance with previous studies which proved that infusion of breadfruit leaf given for 7 days in a row with the highest doses was able to protect the liver of mice due to CCl_4 exposure because the activity of SGPT fell to close to the value of normal group activity[16]. Saponin has anti-inflammatory effect that may lower the risk of liver infection[17]. Flavonoid found in breadfruit leaf have antioxidant properties, which eliminate free radicals and unpaired oxygen induce by CCl_4 that may cause hepatocyte cell damage. The current results also show that efficacy dose 500 mg kg^{-1} b.wt., of medication directly proportional to decrease MDA levels and SGPT level to its active ingedients[16]. Ethanol based breadfruit leaf extract possibility of a role in preventing liver injury in the early phase, decreasing the levels of MDA and SGPT in the acute hepatotoxic which will give a significantly reduced ($p<0.05$) free radicals and alanine transferase activity produced hepatoprotective effect. However, with the application ethanol based breadfruit leaf extract can stimulate hepatocyte cell healing in hepatitis and liver diseases. Expected when standardized natural ingredients from ethanol based breadfruit leaf extract were applied to liver injured will help the healing process for optimal liver health. The limitations of this study are cellular and humoral mechanism of ethanol based breadfruit leaf extract to hepatocyte cell in liver injury model study not explored.

CONCLUSION AND FUTURE RECOMMENDATION

Based on research conducted to know the hepatoprotective effect of ethanol based breadfruit leaf extract on wistar rats induced carbon tetrachloride it can be concluded that the extract of breadfruit ethanol has an effect on decrease of MDA level and SGPT level of male white wistar rats CCl_4 induced and effective dose of ethanol based breadfruit leaf extract in lowering SGPT level was 500 mg kg^{-1} b.wt. This study demonstrated that breadfruit leaf extract effective in protecting the liver from CCl_4 injury according to MDA and SGPT level measurements. In further studies we will examine inflammatory cell counts in liver injury, perform a histopathology analysis and immunohistochemistry analysis of hepatocyte cell as a possible important indicator of liver injury repair.

SIGNIFICANCE STATEMENTS

This study discovers the possible liver protection and antioxidant effect of Indonesian breadfruit leaf ethanol extract that can be beneficial for hepatoprotective drugs. This study will help the researcher to uncover the critical areas of preventive mechanism of liver injury by herbal medicine that many researchers were not able to explore. Thus a new theory on breadfruit leaf as a natural remedy empirically efficacious as hepatoprotective drugs in hepatitis patient according to free radicals and liver enzyme level measurements.

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