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Research Article

THE PROTECTIVE EFFECT OF PANDANUS AMARYLLIFOLIUS LEAVES EXTRACT ON PARACETAMOL-INDUCED HEPATOTOXICITY

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ABSTRACT

Objective: This current study was carried out to investigate the hepatoprotective activity of *Pandanus amaryllifolius* on paracetamol-induced male rats. **Methods:** Thehepatoprotective activity of ethanol extract of *P. amaryllifolius* leaves was evaluated in paracetamol-induced male rats. The extract was administered orally at doses of 50, 100, 200 mg/kg bw for 7 days. On day 7, liver damage was induced by high dose of paracetamol (1.35g/kg bw). The levels of alanin aminotransferase (ALT) and aspartataminotranferase (AST) were determined to evaluate the liver function. Microscopic assessment of hepatic tissue section was also performed.The hepatoprotective effect of extract was compared to normal, positive and negative control. **Results:** All doses ofethanol extract of P. amaryllifolius leaves prevented increase in serum level of ALT and AST as compared to negative control ($P<0.05$). However, only dose of 200 mg/kg bw displayed comparable hepatoprotective activity to normal and positive control ($P>0.05$). The result was supported by histological assessment which showed no liver damage at highest dose of *P. amaryllifolius* extract. **Conclusion:** The result emphasize that ethanol extract of *P. amaryllifolius* leaves possesses hepatoprotective effect.

Keywords: *P. amaryllifolius*, ALT, AST, histopathology.

INTRODUCTION

The liver is the largest digest gland. It produces bilirubin and served as nutrien storage[1]. It receives the blood supply mainly by the portal vein, which drains the spleen and intestines[2].The liver is the most susceptible organ to noxious agents. The number of drugs associated with adverse reactions involving the liver is extensive. Liver necrosis is a predictable reaction secondary to drugs and often a dose-related,such as acetaminophen (paracetamol) [3]. Therefore, the liver must be protected from harmful effect of many substances as liver's function affects almost every other organ system in the body [4].

Pandanus amaryllifolius is a plant belonging Pandanaceae Family. It is widely distibuted in South-east Asia [5]. The unique pleasant of *P. amaryllifolius* is due to the presence of 2-acetyl-1-pyrroline. Others aroma components have been found such as linalool, hexanal, nonanal, 2-penten-1-ol, benzaldehyde and 3-methyl pyridine [6]. Quercetin, carotenoids, tocopherols, tocotrienols also present in this plant [7].

The plant is commonly used in cooking and for treatment of some diseases such as as anti-diabetic, diuretic and to treat common cold with fever. The anti-diabetic effect of *P. amaryllifolius* has been studied previously which reported the ability of *P. amaryllifolius* leaves to inhibit α-glucosidase enzyme, induce insulin productionand increase insulin sensitivity [8-9]. Previous study has also reported the anti-cancer effect of *P. amaryllifolius*[5]. The plant possesses anti-oxidant activity as reported by previous study [10]. In addition, extract of *P. amaryllifolius* prolongs sleeping time and reduces locomotor activity [11]. However, the hepatoprotective activity of *P. amaryllifolius* was rarely reported.

This current study was conducted to investigate the hepatoprotective activity of ethanol extract *P. amaryllifolius*leaves on paracetamol-induced male rats.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used in this study were ethanol (SmartLab, Indonesia) natrium carboxyl methlcellulose (Na CMC) (Sigma, USA), Curcuma® (Soho, Indonesia). A light microscope (Boeco, Germany) and rotary evaporator (Heidolph, Germany) were also used in this study.

Plant materials

The leaves of *P. Amaryllifolius* were collected from Medan, Indonesia. The plant identification was confirmed by Herbarium Medanese (MEDA) Universitas Sumatera Utara, Indonesia.

Extraction procedure

The leaves materials were washed, dried and ground. Then 500 g of sample was macerated in ethanol. The extraction was repeated twice on the residue.The filtrates were combined and the solvent was removed using rotary evaporator to obtain extract of *P. amaryllifolius.*

Experimental Procedure

The hepatoprotective activity evaluation was performed by a modified method by Olaleye et al. (2014) [12].The white rats were divided into several groups, which include normal, negative control, positive control and treatment groups. The animals in treatment group were treated with ethanol extract of *P. amaryllifolius* leaves at doses of 50, 100, 200 mg/kg bw for 7 days. Meanwhile, the negative control and normal group received Na CMC 0.5% as vehicle. Curcuma® was used as positive control at dose of 54 mg/kg bw. On day 7, except normal group, all rats received the treatment of 1,35g/kg bw of paracetamol one hour after extract administration. Then, after 24 h of paracetamol induction, all rats were sacrified and blood were collected for biochemical parameters determination. Meanwhile the livers were collected for histopathological study. The use of rast was approved by the Animal Research Ethics Committees of Universitas Sumatera Utara (approval number 674/KEPH-FMIPA/2017).

Biochemical Parameters Evaluation

The blood were inserted into microcentrifuge tubes and immediately centrifuged for 20 min at 3000 rpm. The serum was separated to determine the levels of alanin aminotransferase (ALT) and aspartataminotranferase (AST).

Microscopic examination

The liver was immersed in a 10% formalin buffer solution and then embedded in paraffin. Slicing was done using a microtome and

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stained with hematoxylin and eosin and observed using a light microscope.

Statistical analysis

The data were analysed using Statistical Package for Social Sciences (SPSS). The data presented as mean ± standard error of the mean (SEM) and analysed using a one-way analysis of variance (ANOVA) and followed by Tukey post hoc test. P<0.05was considered to be different significantly.

RESULTS AND DISCUSSION

Biochemical Parameters

The treatment with large dose of paracetamol in rats induced a liver damage which indicated by the increasing value of ALT and AST. Paracetamol is an analgesic and antipyretic drug which considered safe at therapeutic doses. High dose of paracetamol may induce centrilobular necrosis due its toxic metabolite. Paracetamol is bioactivated mainly in the liver by the enzymes cytochrome P450 (CYP2E1) [13]. Bioactivation of acetaminophen result in a reactive intermediate metabolite known as N-acetyl-p-benzoquinone imine (NAPQI). At normal paracetamol dosing, NAPQI is detoxified to Glutathione S-transferase pi 1(GSTP1) to non toxic metabolite. However, when taken in overdose, the liver's glutathione stores are depleted and NAPQI detoxification may decrease. It begins to react directly to hepatocyte [3].

Table 1 shows that ethanol extract of *P. amaryllifolius* leaves decreased the alanin aminotransferase (ALT) and aspartataminotranferase (AST) levels of male rats after induced by high single dose of paracetamol, indicating the hepatoprotective activity of *P. amaryllifolius* leaves.The liver function was commonly monitor by measuring serum level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [14]. Most hepatic cells have high concentrations of AST and ALT. They present in high concentrations and was released from the hepatocyte cytoplasm in liver damage. Hence, AST and ALT are sensitive parameter of liver function [15]. The levels of ALT and AST increased after treatment paracetamol, however it can be prevented by the treatment with ethanol extract of *P. amaryllifolius*leaves (Table 1).

The ethanol extract of *P. amaryllifolius* prevent the elevation of ALT and AST levels in a dose dependent manner as compared to negative control (P<0.05). The *P. amaryllifolius* extract at dose of 200 mg/kg bw displayed comparable hepatoprotective effect with normal and positive control (P>0.05).

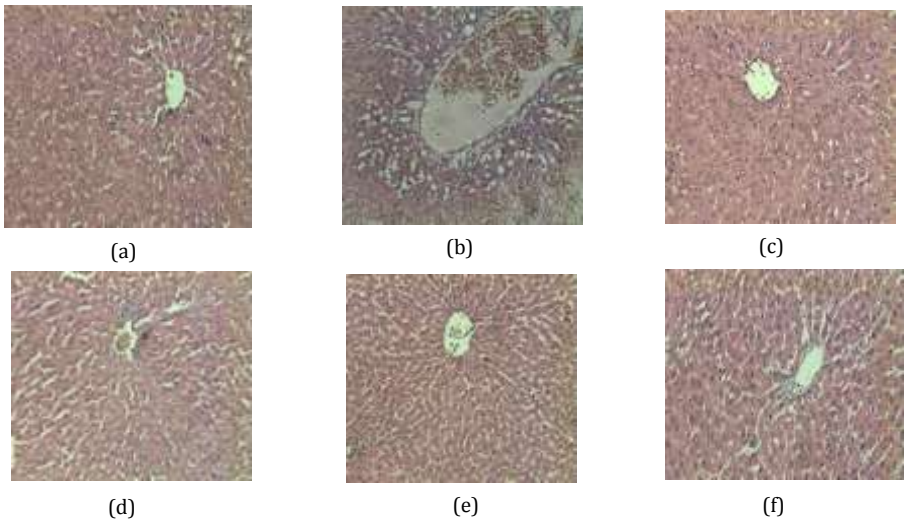
Table 1: Effect of *P. Amaryllifolius* extract on AST and ALT levels

No.	Groups	AST (IU/L)	ALT (IU/L)
1.	Normal	198.58± 3.49	202.60± 2.89
2.	Negative control (Na-CMC 0,5%)	806.14±2.16*	820.80 ± 1.24*
3.	Positive control (Curcuma 54 mg/kg bw)	198.72± 1.27[a]	205.94± 2.58[a]
4.	*P. amaryllifolius* extract 50 mg/kg bw	434.26± 6,27[a,b,*]	452.34± 3.47[a,b,*]
5.	*P. amaryllifolius* extract 100 mg/kg bw	368.94± 3.32[a,b,*]	357.82± 2.79[a,b,*]
6.	*P. amaryllifolius* extract 200 mg/kg bw	209.24± 4.28[a]	207.14± 3.65[a]

Data were analyzed by one-way ANOVA, and followed by Tukey post hoc test. *P< 0.05 compared to the normal control.[a]P< 0.05 compared to the negative control.[b]P< 0.05 compared to the positive control

Microscopic examination

The microscopic evaluation of the liver after treatment with paracetamol showed signficant histopathological changes as compared to normal group. As shown in Fig. 1, haemorrhage, sinusoidal dilatation and necrotic cells were observed in negative control group, indicating liver damage arised. The ethanol extract of *P. amaryllifolius*leaves was able to prevent liver damage, especially at dose of 200 mg/kg bw. Signs of liver damage were still observed at dose of 50 and 100 mg/kg bw. In agreement with the biochemistry examinations, the ethanol extract of *P. amaryllifolius* leaves protected the necrotic lesion within the liver. Of all the doses of extract, dose of 200 mg/kb bw depicted the strongest liver protection and was comparable with normal and positive control groups. The microscopic examination were similar to the normal condition after treatment with ethanol extract of *P. amaryllifolius* at dose of 200 mg/kb bw. The ability of ethanol extract of *P. amaryllifolius*leaves to prevent liver damage may cause by the presence of various bioactive compounds. Previous study by Ghasemzadeh and Jaafar (2013) reported that *P. amaryllifolius* extract contains high flavononids (specially cathechin and kaempferol) and exhibited strong antioxidant activity [16]. The free radical scavenging ability of flavonoids may protect the liver from toxic metabolite of acetaminophen although other constituents may also contribute.



(a) (b) (c)

(d) (e) (f)

Fig.1. Microscopic examination (10 × 10). (a) Liver of normal control rat; (b) liver of negative control rat; (c) Liver of positive control rat; (d) Liver of treatment group at 50mg/kg; (e) Liver of treatment group at 100 mg/kg; (f) Liver of treatment group at 200 mg/kg

CONCLUSION

The ethanol extract of *P. amaryllifolius* leaves showed hepatoprotective effect. However, only at dose of 200 mg/kg bw prevented paracetamol-induced increase in serum ALT and AST as well as necrotic lesion within the liver which was comparable with normal and positive control groups. However, further studies are required to elucidate the hepatoprotective mechanism of *P. amaryllifolius* leaves.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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