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Hepatotherapy activity of breadfruit leaves extract (*Arthocarpus altilis* Fosberg.) in rat induced acetaminophen

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Hepatotherapy Activity of Breadfruit Leaves Extract (*Arthocarpus altilis* Fosberg.) in Rat Induced Acetaminophen

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Abstract. Acetaminophen is kinds of drug for antipyretic, analgetic and anti-inflammatory. Acetaminophen is more affordable, effective and safety. However, consume the drug frequently in a high doses affects to the liver. The side effect of acetaminophen can be treated by antioxidants from organic plants such as Breadfruit (*Arthocarpus altilis* Fosberg.). Breadfruit leaves has flavonoid as secondary metabolite which act as antioxidant. This research aimed to study the hepatotherapy activity from ethanol extract of Breadfruit (*Arthocarpus altilis* Fosberg.) leaves in rat induced acetaminophen. The research used 15 male rats divided into 5 groups. Group 1 (1% Na-CMC); Group 2 (Curliv® 15,595 mg/KgBW); Group 3, 4, and 5 were given ethanol extract of breadfruit leave doses of 600 mg/KgBW, 900 mg/KgBW and 1200 mg/KgBW. The parameter were measure from the serum level of SGPT. As the result, the ethanol extract of Breadfruit leaves dose 1200 mg/KgBW had a greater percentage reduction in SGPT levels than the other groups, it was 42,29 % ($p<0,05$). Ethanol extract of Breadfruit leaves dose 1200 mg/KgBW has the better activity in treating hepatotoxicity induced by acetaminophen in rats.

INTRODUCTION

Acetaminophen is a drug that widely used for antipyretic, anti-inflammatory and analgesic which produces acute liver damage if overdoses are consumed [1]. The analgesic effect of acetaminophen causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. Acetaminophen was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. It was shown that repletion of GSH prevented the toxicity. The reactive metabolite was subsequently identified to be N-acetyl-p-benzoquinone imine (NAPQI). Although covalent bonding has been shown to be an excellent correlate of toxicity, several events have been shown to occur and are likely important in the initiation and repair of toxicity [2].

The liver is the gateway to all materials that enter the body through the digestive tract, so it is very susceptible to metabolic, toxic and microbial disorders [3]. The liver is the organ most frequently damaged by paracetamol because it is involved in metabolism, making it prone to pathological conditions. The liver of rats exposed to acetaminophen will experience hepatocellular changes, dilated and congested sinusoids accompanied by bleeding. The toxic effects of acetaminophen in various organs can be neutralized by antioxidants. Several studies have shown that various antioxidants found in medicinal plant extracts can be used to suppress oxidative stress in experimental animals. Extracts of medicinal plants mainly act as free radical scavengers, form complexes with metal (metal chelation), and stabilize the membrane system [4].

One of the plants that contain high antioxidants is breadfruit leaves (*Arthocarpus altilis*). Breadfruit leaves contain alkaloids, tannins, polyphenol, steroids, saponins, carbohydrates, glycosides [5,6]. Polyphenol compounds function as antioxidants [7]. This research aimed to study the hepatotherapy activity from ethanol extract of Breadfruit (*Arthocarpus altilis* Fosberg.) leaves in rat induced acetaminophen.

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RESEARCH METHOD

Plant Material

This research was conducted at the Pharmacology Laboratory at the Faculty of Pharmacy, Muslim University of Indonesia. Leaves of Breadfruit were collected in Antang, Makassar City.

Preparation of Plant Extract

The extract of breadfruit leaves was prepared by maceration with ethanol for 3 days. All extracts were collected and evaporated by rotavapor.

Experimental Design

The rats were divided into 5 groups, consisting of group 1 as control and receiving Na. CMC suspension. Group 2 as a comparison group were given Curliv® at a dose of 15.595 mg. Group 3, 4 and 5 was extract group were that was given the ethanol extract of breadfruit leaves (EEBL) at a doses 600 mg/kgBW, 900 mg/kgBW and 1200 mg/kgBW, respectively. The animals were adapted for 7 days and fasted for 8 hours before treatment, then 15 rats were taken with initial blood to measure the initial SGPT level (as normal SGPT levels). Then all animals were induced with 2 mL of 2.5 g/kgBW of Acetaminophen (p.o) for 1 day. On 3^{th} day, SGPT levels were measured as SGPT levels after induced. All test groups were given breadfruit leaf ethanol extract orally once a day for 7 days. Then on the 11^{th} day, blood was taken to measure the level of SGPT after therapy.

Data analysis

Data were analyzed with one-way analysis of variance (ANOVA), continued with LSD procedure for multiple comparison tests. A value of $p<0.05$ was considered to be significant.

RESULT AND DISCUSSION

Effect of the ethanol extract of breadfruit leaves (EEBL) by acetaminophen induced liver injury in rats with SGPT level serum are given in Table 1, Table 2 and Table 3.

TABLE 1. The level of serum SGPT in rat (mg/dl) before induced acetaminophen.

No	Group 1 (Sodium CMC)	Group 2 (Curliv®)	Group 3 EEBL dose 600 mg/kgBB	Group 4 EEBL dose 900 mg/kgBB	Group 5 EEBL dose 1200 mg/kg BB
1.	33,8	31,4	33	37,4	33,7
2.	32,3	36,5	31,3	36,8	36,7
3.	32,7	38,8	32,4	36,8	38,2
Mean	32,93	35,56	32,23	37	36,2

In Table 1 represents the level of serum SGPT on normal rat before induced acetaminophen. The values range from 32 to 37 mg/dl. After that, all animals were induced with acetaminophen orally with dose of 2.5 g/kg BW. According to Gad, normal levels of SGPT in white rats were 17.5-30.2 U/L [8]. The analysis differences between normal rats SGOT and SGPT activities in this research compared to predetermined values, was probably due to several stress factors that could occur through increased in this research and difference in the weight of the rats.

TABLE 2. The level of serum SGPT in rat (mg/dl) after induced acetaminophen.

No	Group 1 (Sodium CMC)	Group 2 (Curliv®)	Group 3 EEBL dose 600 mg/kgBB	Group 4 EEBL dose 900 mg/kgBB	Group 5 EEBL dose 1200 mg/kg BB
1	168,5	103,5	80,7	84,5	125,7
2	86,5	93,6	104,3	96,5	106,2
3	78,4	102,1	77,7	66,2	70,3
Rata-rata	111,13	102,6	87,6	82,4	100,7

In Table 2 represents the level of serum SGPT on rat after induced acetaminophen. The increase of SGPT serum level was 2 until 3 times rather than normal value. This is indicated that there was liver injury occurred.

According to B Hemabarathy et al, when taken in over dose, acetaminophen (N-acetyl-p-aminophenol; APAP) will produce a reactive metabolite called N-Acetyl-p-benzoquinoneimine (NAPQI). NAPQI is an intermediate product of acetaminophen produced in the presence of cytochrome-p450 that causes hepatic damage and tubular necrosis in the kidney in both humans and experimental animals [9]. Hepatotoxicity effect from acetaminophen can be assessed by monitoring liver function, such as Serum Glutamic Pyruvic Transaminase (SGPT).

Serum Glutamic Pyruvic Transaminase or SGPT is an enzyme in the human body. This enzyme is mostly found in the liver. However, ALT is also present in several other organs, but in small amounts. In a normal or healthy body, SGPT is usually found in the liver cells. However, when these organs are damaged, this enzyme will leave the cells and then enter the blood vessels. This condition causes an increase in SGPT in the body [10]. After that, group 3, 4 and 5 were given the ethanol extract of breadfruit leaves (EEBL) at a doses 600 mg/kgBW, 900 mg/kgBW and 1200 mg/kgBW, respectively until 7 days. Group 1 was given only Sodium CMC suspension and Group 2 was given Curliv® at a dose of 15.595 mg.

TABLE 3. The level of serum SGPT in rat (mg/dl) after therapy.

No	Group 1 (Sodium CMC)	Group 2 (Curliv®)	Group 3 EEBL dose 600 mg/kgBB	Group 4 EEBL dose 900 mg/kgBB	Group 5 EEBL dose 1200 mg/kg BB
1	145,6	50,7	58,3	54,3	52,4
2	75	50	65,2	52,4	54,3
3	77,6	49,8	34,9	36,9	46,5
Mean	99,4	50,1	52,8	47,8	51,06

The Table 3 represents the level of serum SGPT on rat after therapy. There is reducion of SGPT serum level was occurred. This is indicated that there was liver injury occurred. The average data on the table 3 then calculated become the percent reduction to see the dose of breadfruit leaf ethanol extract which is more effective in repairing liver damage. It can be seen in Table 4 below:

TABLE 4. Percent Reduction of serum SGPT level.

No.	Groups	Percent Reduction (%)
1.	Group 1	10,55
2.	Group 2	49,78
3.	Group 3 EEBL dose 600 mg/kgBW	39,68
4.	Group 4 EEBL dose 900 mg/kgBW	41,91
5.	Group 5 EEBL dose 1200 mg/kgBW	42,29*

Note " * = $P < 0.05$ by LSD test

In this work, the test was carried out on the effect of giving ethanol extract of breadfruit leaves (*Artocarpus altilis* (Parkins.) Fosberg) (EEBL) on the acetaminophen-induced rat liver. The test parameters carried out in this study were the measurement of SGPT levels in rats. SGPT is an enzyme that is located in the cytoplasm of liver cells and its value increases in the presence of hepatocellular injury. SGPT levels were measured before acetaminophen induction, after acetaminophen induction, and after therapy with breadfruit leaf ethanol extract. This measurement before induction becomes the initial parameter to see the normal serum SGPT level. Measurement after acetaminophen induction becomes a parameter for the occurrence of liver damage. This is indicated by an increase in SGPT levels by 2-3 times rather than normal. Measurement after therapy with EEBL to saw any improvement in the liver after therapy. This is indicated by a decrease in SGPT levels.

The sample used in this study was the ethanol extract of breadfruit leaves. To test the ability of EEBL to repair liver damage, it was tested on rat (*Rattus norvegicus*). A total of 15 rat test animals were prepared and then divided into 5 groups. Group I (control) were given 1% Sodium CMC suspension, group II (comparison) were given Curliv® suspension at a dose of 15.595 mg/KgBW, groups III, IV, and V (treatment) were given EEDS at a dose of 600 mg/kgBW, 900 mg/kgBW, 1200 mg/kgBW. The administration is carried out for 7 days once a day. Before the treatment, acetaminophen was induced to see the damage to liver.

Acetaminophene is one of the most frequently used drugs, which in prolonged use with high dose can cause liver damage. The mechanism of liver damage is the levels of GSH in the liver cells are greatly reduced which results in the susceptibility of liver cells to injury by oxidants and also allows NAPQI to bind covalently to cell macromolecules [11]. An oxidative stress is characterized by excessive production of reactive oxygen radicals (ROS) combined with weakened endogenous antioxidant defense system. However, once the antioxidant defense system fails, it can arise cellular oxidative damage by irreversible. The harmful effects of oxidative stress were reported that oxidative stress initiates or promotes the pathological progress of diverse human diseases. And there is evidence supporting oxidative stress as a detrimental pathway that intensifies the hepatocytes injury caused by acetaminophen [12]. Polyphenols are the compound that can act as a free radical-scavenger,metal ion chelator and/or singlet oxygen-quenchen [13].The results of the measurement of serum SGPT levels in the rats' liver can be seen in tables 1, 2, and 3. The table shows that before the treatment the rats had a normal liver condition. This is indicated by the average measurement of serum SGPT levels ranging from 32 - 37 mg / dl (data can be seen in table 1). The results of measuring the SGPT levels in rats after paracetamol induction increased 2 until 3 times (data can be seen in table 2). In table 2, it can be seen that the SGPT levels of the rats ranged from 82 -111 mg / dl. This increase is a parameter of liver damage which is indicated by an increase in ALT levels. The measurement of ALT levels after therapy can be seen in Table 3. In this table, it can be seen that after being given therapy for 7 days once a day, there was an effect of improvement in the liver except in group 1. This is indicated by a decrease in SGPT levels ranging from 17 - 52 mg/dl.

The data on the average serum SGPT level measurement was calculated for the percentage reduction to estimate the effective dose (data can be seen in table 4). In the Table 4, the EEBL dose of 1200 mg/dl has the greatest percentage reduction, but does not exceed Group 2 (Curliv®). The decrease in SGPT levels after EEBL therapy is due to the presence of chemical component of polyphenol compounds which act as antioxidants. Antioxidants are able to repair liver damage. The phenolic compounds that was found in the plant are protective against oxidative damage and adverse effects by ROS and free radicals act as both competent radical scavengers and metal chelators [14].

CONCLUSIONS

Based on the results and discussion, it can be concluded that the ethanol extract of breadfruit leaves (EEBL) dose of 1200 mg/kgBw has activity in improving liver function which had been damaged by acetaminophen better than the other extract groups by reduced serum SGPT level in rat. A total of 15 rat test animals were prepared and then divided into 5 groups. Group I (control) were given 1% Sodium CMC suspension, group II (comparison) were given Curliv® suspension at a dose of 15.595 mg/KgBW, groups III, IV, and V (treatment) were given EEDS at a dose of 600 mg/kgBW, 900 mg/kgBW, 1200 mg/kgBW. The administration is carried out for 7 days once a day. Before the treatment, acetaminophen was induced to see the damage to liver. The mechanism of liver damage is the levels of GSH in the liver cells are greatly reduced which results in the susceptibility of liver cells to injury by oxidants and also allows NAPQI to bind covalently to cell macromolecules. The harmful effects of oxidative stress were reported that oxidative stress initiates or promotes the pathological progress of diverse human diseases.

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