Analysis of Degeneration Hepatosit Cell on Female Mice(*Mus musculus*) Induced by Ruthab Dates (*Phoenix dactylifera*) Extract and Paracetamol

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Abstract: *Paracetamol is a drug used as an analgesic antipyretic. One of the fruits used as a hepatoprotection agent is Dates (Phoenix dactylifera) ruthab. This study aims to determine the hepatoprotective activity of various doses of ruthab date fruit extract against liver degeneration of female mice induced by paracetamol. This study used a group pre-experiment with the control group and group with dosing dates of 1, 3, and 7. While the experimental group was the group with 41.4 grams of paracetamol and the group given paracetamol and ruthab dose dates 7. The results of this study showed that there were no significant differences in various doses of ruthab dates extract in the pre-experimental group, although there was a decrease in the mean degeneration score. While the results of the experimental group, namely the group with the administration of ruthab and paracetamol dates, showed significant differences, indicating that there was a hepatoprotective activity of rutab dates extract. The existence of these differences was thought to be due to the flavonoid content in ruthab dates extract which acts as a hydrophilic and lipophilic antioxidant.*

1 INTRODUCTION

Paracetamol (PCT) is a drug commonly used to reduce body temperature during fever (antipyretic), and reduce pain (analgesics). Although paracetamol is declared safe at therapeutic doses, high doses of paracetamol can cause liver failure. The hepatoxic effects of paracetamol are known since around 1960 (Oktiari et al, 2010).

In France, there is a study that shows the population of DILI (Drug Induce Liver Injury) around 13.9 cases / 100,000, two people (5.9%) died and 4 of 34 (11.8%) patients were hospitalized. In Singapore, liver transplants are even 14%. 96% of patients with liver dysfunction are still many who are given liver disease inducing drugs including ranitidine, cefriaxone, and paracetamol in 2012 at Tasikmalaya hospital (Sa'roni, 2012). Paracetamol is widely researched to cause liver toxicity.

Liver is an organ that plays a role in the body's detoxification and metabolic system. Liver damage can be caused by infection or chemical intoxication. The toxicity of the liver can be reduced by the presence of antioxidant compounds. (Hayati et al, 2014). One of the fruits contained in the antioxidant is ruthab dates (*Phoenix dactylifera*).

Ruthab dates (*Phoenix dactylifera*) extract can be used as hepatoprotection because it contains antioxidant compounds. The workings of antioxidant compounds are bound to reactive free radicals to form relatively unreactive free radicals.

Arem *et al* (2014) stated that the effect of hepatoprotection occurred in rats (*Rattus novergicus*) given dichloroacetic acid (DCA) with hepatoprotector of dates extract (*Phoenix dactylifera*) as much as 0.5 and 2 g / l. Other studies it was also found that ruthab dates (*Phoenix dactylifera*) contain fiber which has a good effect on health.

Therefore, it is important to conducted the study in order to determine the effect of various doses comparisons on hepatopotective activity of ruthab palm extract (Phoenix dactylifera) and

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hepatoprotection activity of ruthab date fruit (*Phoenix dactylifera*) on liver histology of female mice (*Mus musculus*) induced by paracetamol.

2 METHOD

2.1 Making Ruthab Dates (*Phoenix dactylifera*) Fruit Extract

Making ruthab dates (*Phoenix dactylifera*) fruit extract with the following steps, ruthab dates *(Phoenix dactylifera)* with 250 grams of wet weight cut into small pieces in an oven for 3 days at 60 ° C (Nurdiana, 2009). Then a dry weight of 150 grams was produced. Macerated with 2 times 300 ml of methanol for 6 days and every 3 days the extract was filtered (Simanjuntak et al, 2014). After that, it is evaporated using a rotary evaporator with a temperature of 50° C for approximately 30 minutes (Qorriaina, 2015). Then extract is put into a vial bottle.

2.2 Making Paracetamol Dosage

The making of paracetamol dosage conducted through several steps as follow:

a. Toxic dosage of paracetamol in human are 10-15 grams (put of median point is 13 grams)
b. Convention factor from human to mice (*Mus musculus*) are 0,0026
c. Weight of mice (Mus muculus) are 25-30 grams (put of median point is 28 grams)
d. Dosage of mice 20 grams
   grams x 0,0026 = 0,0338.
e. Dosage of mice (*Mus msculus*)
   $\frac{28}{20 \times 0{,}0338}$ = 0,04732 gr

2.3 Research Design

This research is a laboratory experimental research with the research design used is a completely randomized design (CRD) with 6 treatment groups divided into 2 large groups and 5 repetitions namely:

a. Experimental group
   K (-): Injection with 0.2 ml Aquades.
   P1: Injected with ruthab dates (*Phoenix dactylifera*) extract with a dose of 1 ml as much as 0.2 ml.
   P2: Injected with ruthab dates (*Phoenix dactylifera*) extract with a dose of 3 dates as much as 0.2 ml.
   P3: Injected with ruthab dates (*Phoenix dactylifera*) extract with a dose of 7 dates as much as 0.2 ml.
b. Experiment group
   K (+): Injected with paracetamol with a dose of 41.4 as much as 0.2 ml.
   P4: Injected with paracetamol with a dose of 41.4 grams as much as 0.2 ml and ruthab dates extract with a dose (best of the preexperiment group) of 0.2 ml.

2.4 Surgery

Surgery in this study was carried out on the 16th day after treatment. Surgery is performed on paraffin blocks and using surgical instruments. After that the liver organ that has been taken is made histological incision

2.5 Presentation of Data

The presentation of the data in this study using the scoring method of the degree of liver damage on this examination was carried out according to the modified Knodell (2000) and Klopfleisch (2013) method, where the degree of damage of each sample was determined by summing all scores of histopathologic lesions as in Table 1.

Table 1. histopathologic scoring

Form of Lesion	Score	Information
DEGENERATION	0	Degeneration does not occur
	1	If degenerative changes < 25% from all fields of view
	2	If degenerative changes 26 – 50% from all fields of view
	3	If degenerative changes 51 - 75% from all fields of view
	4	If degenerative changes >76% from all fields of view

2.6 Data Analysis

Liver cell scores that experienced degeneration in liver histology incision of mice (Mus musculus) in the form of nominal ordinal data scale, comparative test, and will be processed using SPSS 21.0 application. Based on the scale of the data, the test used was Chi-Square test both in the pre-experimental group and the experimental group because the sample in this study consisted of 2 samples or more and were independent (independent), then included in the non-parametric test

3 RESULT AND DISCUSSION

The results of observations of cell nucleus undergoing degeneration in the pre-experimental group can be seen in Figure 1.

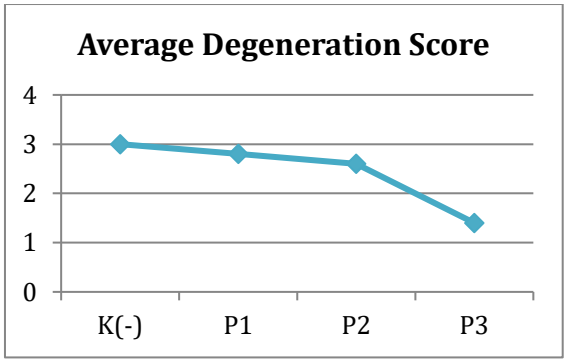

Figure 1. Mean Scores of Liver Degeneration Pre Experimental group

From Figure 1 it was found that there was no difference in scores on the mean score of liver degeneration on the administration of various doses of ruthab dates (Phoenix dactylifera L.). According to the chi-square test, the data in this pre-experimental group had an α value of 0.420 where α> 0.05. In this case it can be concluded that H0 is accepted that there is no difference between the degeneration change score and the ruthab palm extract (Phoenix dactylifera L.) which has been given.

Although according to the test, it showed that there is no effect between necrosis, paracetamol, and palm ruthab (Phoenix dactylifera L.) extract dates, but in Figure 1 there is a decrease in the mean score of degeneration changes. This is due to the presence of flavonoid compounds in the ruthab palm fruit extract (Phoenix dactylifera L.).

This compound is widely known as hepatoprotection and is closely related to the prevention of the emergence of several diseases such as liver disease (Bandy, 2009). According to Midlleton (2007), flavonoids are active compounds included in the type of antioxidant intermediates that act as hydrophilic and lipophilic antioxidants. Flavonoids are compounds that act as antioxidants. The antioxidant mechanism of flavonoids is to capture ROS directly, prevent regeneration of ROS and can indirectly increase the antioxidant antioxidant cellular antioxidant enzymes.

Flavonoids are the most effective compounds as scavanger reactive species, such as super dioxide, peroxyl radicals, and peroxinitrite by transferring H + atoms (Bandy, 2009). The prevention of the formation of ROS by flavonoids is done in various ways, namely inhibiting the action of the enzyme xanthine oxidase and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, and chelating metals (Fe2 + and Cu2 +) so as to prevent redox reactions that can produce free radicals (Bandy, 2009). Whereas for the second 15 days, the experimental group, the results of the observation of necrosis change scores can be seen in Figure 2 below.

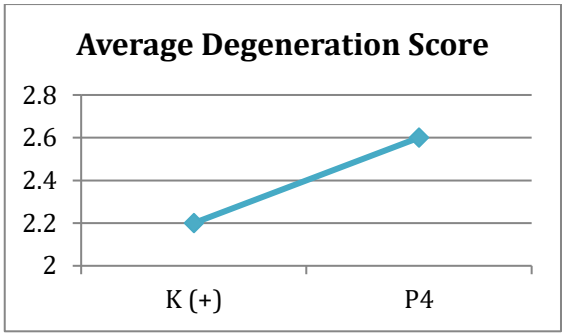

Figure 2. Mean Scores of Liver Degeneration Experimental Group

Chi-square test results show that the value of α in this experimental group is 0.05 where α = 0.05. In this case it can be concluded that H0 is rejected, namely there is a difference in the score of degeneration changes, paracetamol, and ruthab dates extract (Phoenix dactylifera) that have been given.

In Figure 2 shows that there is an increase in the graph from the score 2.2 towards the number 2.6. This is because the p4 group had previously been induced with paracetamol. Therefore, it can increase the average score of degeneration. Cheville (1976) states that fat degeneration occurs because cells experience oxygen deficiency and food substances.

Oxygen deficiency and these food substances can interfere with the process of energy formation, so protein synthesis decreases, which eventually the cell is unable to form proteins.

According to Carlton and Mc Gavin (1995), there are two reasons that cause the liver to be susceptible to toxins. First, the liver receives 89% of the blood supply from the portal vein that drains blood from the gastrointestinal system. The substance of toxic substances including plants, fungi, metals, minerals, and other chemicals absorbed into the portal is transported to the liver. Second, the liver produces enzymes that have the ability to biotransform various kinds of exogenous and endogenous substances that are eliminated by the body.

4 CONCLUSIONS

In this study it can be concluded that in the pre-experimental group there was no difference in the various doses of ruthab dates extract (Phoenix dactylifera), but there was an average reduction in damage scores. Whereas in the experimental group there were differences between the groups given paracetamol and the combination of ruthab dates (Phoenix dactylifera) and paracetamol

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