

Hepatoprotective Activities Of Polar And Non Polar Extract Kembang Sepatu Flower (*Hibiscus rosasinensis* L.)

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Abstract

This study aims to obtain the dominant phytochemical group in polar and non-polar extracts of hibiscus flowers and obtain data on hepatoprotective activity of polar and non-polar extracts of hibiscus flowers on male white rats induced by high doses of paracetamol. This study consists of the stages of extraction, stages phytochemical analysis and test phase of hepatoprotective activity using rat test animals. Treat done is negative control (K -). This group was only given solvents (CMC Na 0.5%) 1ml / head / day for 28 days and was not given heat stress treatment. H0. This group was given solvents (CMC Na 0.5%) 1ml / head / day for 28 days, after which they were given heat stress treatment for eight days. H1 This group was given a polar extract of *Hibiscus rosasinensis* red flowers at a dose of 0.075g mg / 200gramBB / day in a 0.5% CMC suspension of 1 ml for 14 days, and after that was given heat stress treatment for eight days. H2. This group was given a non-polar extract of *Hibiscus rosasinensis* red flowers at a dose of 0.075mg / 200gramBB / day in a 1% CMC suspension of 1 ml for 14 days, and after that was given heat stress treatment for eight days. The measured parameters were the content of SGPT and SGOT . The results showed that the polar extract and non-polar extract *Hibiscus rosasinensis* L. red variety were able to maintain levels of the SGOT and SGPT enzymes in white rats (*Rattus norvegicus*) induced by high doses of paracetamol, as in the negative control group (K-).

Key words: hepatoprotective, extract, flower, *Hibiscus rosasinensis* L

INTRODUCTION

Liver disease is a disease that is often found in the community. This disease can be caused by two factors, namely microorganisms and drugs (Underwood, 1999). One drug that can cause problems in the liver is paracetamol (acetaminophen) (Aslam, et al., 2003). Paracetamol is widely used in Indonesia as a safe antipyretic analgesic. However, its use must still be cautious, because a dose of 6-12 grams has been able to fatally damage the liver (Tjay and Rahardja, 1978). Paracetamol can cause damage to the liver, because in the liver the paracetamol P-450 enzyme will be converted into an active metabolite that is toxic, namely NAPKI (Nasetil-p-benzokuinonimina).

Until now there is no drug that specifically addresses hepatitis (Gestanovia and Hendra, 2004), therefore research is needed to get natural medicines that can be used as hepatoprotectors.

The prevalence of liver disease continues to increase due to people's dependence on synthetic drugs. The drug as a xenobiotic compound will be biotransformed and detoxified in the liver so that liver function becomes very vital. Damage to liver cells can cause tumor formation and develop liver cancer.

As a mega-diversity country, Indonesia has a lot of local wisdom in the use of plant parts as medicine. Based on a preliminary study conducted by Repi and Mocosuli et al (2009), hibiscus flower extract contains phytochemical groups terpenoids, flavonoids and saponins are high. The phytochemical group has the potential as a hepatoprotective component.

In the previous year's study by researchers, it was known the hepatoprotective potential of crude extracts of hibiscus flowers. In this study, a polar extract and non-polar extract activity test from hibiscus flowers will be tested. This study aims to obtain the dominant phytochemical group in polar and non-polar extracts of hibiscus flowers and obtain data on the hepatoprotective activity of polar and non-polar extracts of sepati flower on male white rats induced with high doses of paracetamol.

MATERIALS AND METHODS

2.1. Time and Place of Research

This research was conducted at the Laboratory of Biology Department, FMIPA UNIMA for 4 (four months).

2.2. Tools and Materials

Test animals used were Wistar strain male white rats, uniform weight (\pm 150-180 g), 6-8 weeks old. The material for the hepatotoxin model is in the form of paracetamol obtained from the Pharmacy Farma Manado Pharmacy with a 1% CMC carrier. GPT Kit A tool to measure the activity of GPT-serum, paraffin, 10% formalin, xylol, alcohol, printed wax, hematoxylin-eosin dyes (E. Merck, Darmstadt, Germany). Vitalab Micro (E. Merck, Darmstadt, Germany), electric balance (Mettler Toledo, Model AB 204 made in Switzerland), microscope (Olympus, type BH.2, made in Japan),

camera (Olympus, made in Japan), needle tuberculin, injection syringe, set of surgical instruments, glassware and infusion equipment (complete list of tools / materials in the appendix).

2.3. Research Procedures

a. Phytochomic Analysis (Harborne Method, 1996)

b. Test hepatoprotective activity

Preparation of Experimental Animals Experimental animals were taken randomly and divided into five treatment groups (K-, H0, H1, H2 and H3) each using 6 replications. After that they were given food and drink in an ad libitum and allowed to adapt for 7 days, this is so that the mice can adapt to the new environment. The treatment procedure is as follows:

1. Negative control (K -). This group was only given solvents (CMC Na 0.5%) 1ml / head / day for 28 days and was not given heat stress treatment.
2. H0. This group was given solvents (CMC Na 0.5%) 1ml / head / day for 28 days, after which they were given heat stress treatment for eight days.
3. H1. This group was given a polar extract of Hibiscus rosasinensis red flowers at a dose of 0.075g mg / 200gramBB / day in a 0.5% CMC suspension of 1 ml for 14 days, and after that was given heat stress treatment for eight days.
4. H2. This group was given a non-polar extract of Hibiscus rosasinensis red flowers at a dose of 0.075mg / 200gramBB / day in a 1% CMC suspension of 1 ml for 14 days, and after that was given heat stress treatment for eight days.

2.4. SGOT and SGPT examination

In the examination of SGOT and SGPT blood samples from experimental animals are needed. Blood samples were taken intracardially \pm 3 ml. The experimental animals first dieuthanasia using diethyl ether then the blood is collected in a venoject without being given anticoagulant and covered with a rubber cover. Venoject is placed on its side in order to get a lot of serum (Bijanti et al., 2010).

Blood samples were taken centrifuged at a speed of 3000 rpm for 15 minutes. Serum obtained from the centrifuge was used as a sample for the examination of SGOT and SGPT enzyme levels. Examination of SGOT and SGPT enzyme levels using reagents. The experimental design used in this study was a Completely Randomized Design (CRD). Data analysis using

ANAVA Test (Analysis of Variance). If there are significant differences from these treatments, then followed by Duncan's Multiple Range Test (Kusriningrum, 2009).

2.5. Research Data Analysis

Data on GPT-serum activity were statistically analyzed using a one-way analysis of variance, followed by a Scheffe test of 95% confidence level. Histopathological examination data were analyzed qualitatively.

RESULTS AND DISCUSSION

Phytochemical Extraction and Analysis

Hibiscus (*Hibiscus rosasinensis* L.) used red flower varieties. Simplistic bases are used after being washed thoroughly in running water. Comparison of simplicia and solvent 1: 4 where 500 g of simplicia is macerated with 2000 ml of solvent. Maceration is done for 3 x 24 hours at room temperature. The same comparison is done on 2 types of solvents, namely ethanol (polar solvent) and n-hexane (non-polar solvent). After filtering with Whatman paper No. 1, polar extract and non-polar extract are obtained. For polar filtrate solvents are blackish red with a sharp odor. For non-polar filtrate solvents are dark red with a characteristic odor.

Table 2. Extraction Results

No	Simplisia	Solvent	Extract weight
1	Red Hibiscus Flower	Etanol pa	6,55 gr
2	Red Hibiscus Flower	n-heksan	7,35 gr

Phytochemical Analysis

The extract was then analyzed for phytochemical content using 2 methods, namely the Harborne method (1996) and the Chromatography method. From the results of phytochemical analysis the dominant phytochemical group is flavonoids (Table 3).

Table 3. Phytochemical Content of Aquades Extract

No	Simplisia plants	Exact	Phytochemical Compounds Group	Method
1	Red Hibiscus Flower	Etanol	Flavonoid +++ Alkaloid +++ Tanin ++ Saponin +++ Steroid +	Metode Harborne (1996)
2	Red Hibiscus Flower	n-heksan	Flavonoid +++ Saponin +++ Tannin +++ Steroid +++ Triterpenoid +++	Metode Harborne (1996)

Hepatorprotective Activity

After being given the treatment, the blood of Wistar strain rats was taken by intravenous technique and then analyzed the SGOT content. The data from the SGOT enzyme examination results from five treatments (K-, H0, H1, H2, H3) can be seen from the following table:

Table 5. Mean and standard deviations of SGOT enzyme levels in white rats

Treatment	SGOT Level (U / I) Average \pm SD
K-	42,75 ^c \pm 3,25
HO	47,35 ^a \pm 4,12
H1	23,23 ^b \pm 3,27
H2	20,23 ^{bc} \pm 3,74

Note: The different notations in the tables (a, b, c) indicate a real difference between treatments.

Table 6. Mean and standard deviations of SGPT enzyme levels in white rats

Treatment	SGPT Level (U / I) Average \pm SD
K-	22,45 ^{ab} \pm 2,25
HO	28,25 ^a \pm 3,25
H1	20,23 ^b \pm 4,25
H2	19,24 ^{bc} \pm 4,20

Note: The different notations in the tables (a, b, c) indicate a real difference between treatments.

The results of the study and calculation of SGOT and SGPT levels using Variation Analysis (Anava) showed that the calculated F value is greater than the F table of 5%. In Duncan's multiple range test it was found that the H0 group (CMC Na 0.5%) gave the highest increase in SGOT enzyme levels and was significantly different from the K- (control), H1, H2 ($P < 0.05$) treatments. This proves that there is a significant increase in SGOT levels in a group of white mice suffering from heat stress without being given extracts of *Andrographis paniculata* and *Echinacea purpurea* before. Subsequent results revealed that there were significant differences between the H0 groups with the H1, H2 groups ($P < 0.05$). Where from these results the average SGOT levels of H1, H2, and H3 are smaller than those of SGOT H0. In the K-group, there was no significant difference with the H2 group.

In the analysis of the results of SGPT levels, it is known that the H0 group gave the highest increase in SGPT enzyme levels, it showed that there was an increase in the highest SGPT levels in white mice suffering from heat stress. Furthermore, there were significant differences between the H0 and H1, H2 groups ($P < 0.05$). This means that there are significant differences in SGPT levels in the H0 group with the H1, H2 white mouse group; where there was a decrease in SGPT levels in groups given a combination of the two extracts. In the analysis of SGPT data it is known that the K-group was not significantly different from the H0, H1, and H2 groups ($P > 0.05$); but significantly different from the H3 group ($P < 0.05$).

An increase in SGOT and SGPT enzymes in body fluids (plasma or serum) can provide clues to cell membrane changes or cell damage so that intracellular molecules can escape or penetrate cell membranes (Bijanti et al., 2010). One of the main problems that occur in heat stress is the formation of several enzymes that produce ROS (Reactive Oxygen Species), known as free radicals as byproducts. ROS produced as a byproduct of these enzymes can flood cells so that intracellular free radicals accumulate and suppress endogenous antioxidants or what is commonly called oxidative stress. ROS can cause structural damage to the liver cell membrane consisting of lipid bilayers (cell membranes with unsaturated fats), through the mechanism of lipid peroxidation. Phospholipids, which are the main constituents in the plasma membrane and cell membrane, are often the subject of lipid peroxidation. An important consequence of lipid peroxidation is an increase in membrane permeability and disrupt the distribution of ions that cause cell and organelle keratin (Devlin, 2002; Mudipali, 2007; Yin et al., 1995). Mild damage from liver cells, cytoplasmic enzymes (one of which is SGPT) will leak out of the cell into

the bloodstream. While the increased release of the SGOT enzyme into the bloodstream is caused by damage to the mitochondria, due to oxidative stress in the liver induced by heat stress (Rogers, 2009).

Consumption of foods rich in antioxidants can reduce the risk of diseases caused by oxidative stress and inflammation. Antioxidants play an important role in inhibiting and neutralizing free radicals (Raj Kapoor et al., 2008). As is well known that both *Hibiscus rosasinensis* L. and *Andrographis paniculata* plant extracts have several antioxidant properties that play an important role for health.

Paracetamol oxidation by the liver produces toxic metabolites, N-acetyl-p-benzoquinone (NAPO) which can cause glutathione emptying and lipid peroxidation. This oxidation of paracetamol also produces free radicals which can cause lipid peroxidation and is also a precursor of NAPO. Both of these oxidation results paracetamol if in large quantities (on the use of a single dose 10-15gr) can cause liver cell damage resulting in liver intra-cell enzymes, including GPT entering the blood vessels so that the level of GPT enzymes in the serum will increase. In addition to hibiscus flowers, Sambiloto is reported to also be able to increase the activity of antioxidant enzymes, decrease the activity of the enzyme lipidperoxidase and increase glutathione filling. By increasing the activity of antioxidant enzymes while preventing the formation of free radicals that occur due to the oxidation process of paracetamol by cytochrome P450. Increased glutathione filling allows reactive metabolites formed due to the oxidation process of paracetamol to be conjugated by glutathione so as to prevent covalent binding of reactive metabolites with macromolecular components of hepatic cells. Whereas by decreasing the activity of the bitter peroxidase enzyme, it can reduce the lipid peroxidation process in the liver cell membrane. Sambiloto can also reduce depleting glutathione.

CONCLUSION

Based on the results of this study it can be stated that the polar extract and non-polar extract of *Hibiscus rosasinensis* L. red variety are able to maintain levels of the SGOT and SGPT enzymes in white rats (*Rattus norvegicus*) induced by high doses of paracetamol, as in the negative control group (K-).

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