

Influence Dried Flower of *Hibiscus sabdariffa* Linn Infusion on Serum Glutamate Pyruvate Transaminase (SGPT) Level against Paracetamol Induced Liver Injury in Rats

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Abstract

*The level of Serum Glutamate Pyruvate Transaminase (SGPT) could be elevated higher than normal due to hepatocellular necrosis caused by virus or hepatotoxin such as paracetamol given in toxic dose. The aim of this research was to determine the ability of dried flower of *Hibiscus sabdariffa* Linn infusion to decrease the level of SGPT in male rats which were induced by toxic dose of paracetamol. This study used 25 Wistar male rats of 3-4 month old, which were randomly divided into 5 groups. Group I (normal control) and Group II (negative control) were given aquadest for 7 consecutive days, groups III until V were given the infusion of *Hibiscus sabdariffa* Linn with concentration of 10%, 20%, and 40% b/v; 2.5 ml/kg BW respectively for 7 consecutive days. Twenty four hours after the last treatment, group II until V were induced liver injury by toxic dose of paracetamol 2.5 g/kg BW and after 36 hours, the blood was taken from the tail vein lateralis and the SGPT activity were measured by GPT ALAT method. The result showed that the administered infusion of *Hibiscus sabdariffa* Linn 20 and 40 % could decrease SGPT significantly to become 388.8 ± 18.79 (67.74%) and 172.2 ± 87.48 U/L (85.55%), whereas the negative control was 1190 ± 443 U/L. Infusion of *Hibiscus sabdariffa* Linn concentration 20 and 40% have hepatoprotective effect against toxicity of paracetamol.*

Key words : *Paracetamol, *Hibiscus sabdariffa* Linn, SGPT, infusion, liver injury*

INTRODUCTION

Liver is an organ involved in the synthesis, storage and metabolism of many endogenous and exogenous compounds, including drugs and other toxins. Liver damage can be caused by drugs, various chemicals, and viruses. Paracetamol is analgesic-antipiretic drug that can cause damage to the liver when used in large dose and long term and can lead to liver necrosis. Mechanism of liver cell damage by paracetamol involve paracetamol metabolic activity via the cytochrome P450 system that generates reactive electrophilic metabolite called N-acetyl-p-benzoquinone-imine (NAPQI), which can conjugate with glutathione (GSH) and

GSH is secreted out of cells. After GSH running out, NAPQI can bind covalently to cell proteins. The consequence of the binding of NAPQI to protein is the occurrence of mitochondrial dysfunction by inhibition of respiration, decrease in liver ATP, spending mitochondrial cytochrome. Glutathione transferase deficiency due to paracetamol exposure causes oxidative stress which can end up to liver necrosis (Dart, 2004). If there is necrosis of the liver, transaminase enzymes such as alanine aminotransferase (ALT) or Glutamate Pyruvate transaminase (GPT) in the liver will come out to the blood, so it will increase SGPT level (parameters of liver function).

One of the medicinal plants that can be used as hepatoprotector is Rosella (*Hibiscus sabdariffa*) flower. Rosella flower contain flavonoid, gossypetine, hibiscetine, sabdaretine, also contain alkaloid, β -sitosterol, anthocyanin, citric acid, 3-rutinoso cyanidine, galaktose, pectin, quersetin, and stearic acid (Vilasinee, et.al, 2005). The antioxidants presence in Rosella, such as gossipetin, anthocyanin, and flavonoid, provide protection against degenerative diseases, such as coronary heart disease (CHD), cancer, hiper-cholesterol, and are efficacious as antioxidants (Mahadevan, et.al, 2009). Flavonoid compound contained in the Rosella flower is suspected have hepatoprotective effect.

In our community, Rosella flower are generally used in a form like tea and served in warm water. In pharmaceutical term, this presentation is known as infusion. Previous research proved that *Hibiscus sabdariffa* calyx extract have hepato-protective effect on CCl_4 induced liver damage (Dahiru, et al, 2003). Based on this background, this research aimed at determining the effect of Rosella flower infusion in the level of Serum Glutamate Pyruvate transaminase (SGPT) in rats induced by toxic dose of paracetamol.

MATERIAL AND METHODS

Equipment: Animal weighing scale (Ohaus), rats holder, oral gauge no 18, injection syringe, ependorf, centrifuged (Kokusan H-1 100 BC), UV spectrophotometer UV-Vis (Stardust), micro-pipette, cuvette, stopwatch, analytical balance (Sartorius), glasses (Pyrex), pan infusion, cabinet dryer, blender, and mesh sieve size 16.

Materials: Rosella flower (*Hibiscus sabdariffa*) was obtained from Tawang-mangu, aquadest, paracetamol (p.a) as a model hepatotoxin, 1% CMC Na as suspending agent, reagent kit GPT-ALAT (Diasys, Germany).

Animal testing: Healthy Wistar male rats, aged 2-3 months, weighing 150-200

gram obtained from the Laboratory of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Muhammadiyah University of Surakarta.

Experimental Procedure Infundasion

Rosella infusion is made by infundasion method. Its reference concentration (in infusion) = 10% (w/v) as the lowest concentration. 10 grams of Rosella powder was added with extra water (2 times of the weight of material) and 100 ml aquadest was put into infusion pan and then heated in a water bath for 15 minutes calculated from the time when the temperature reached 90°C in the pan, while stirring occasionally. Infusion is filtered while hot through a flannel cloth, then add enough hot water to obtain a volume of 100 ml infusion. After that the infusion was divided into three levels of concentration, i.e. 10%, 20%, and 40%. Infusion was given orally to rats with a volume of 2.5 ml/200 g BW.

Determination Dose of Paracetamol Induced Liver Damage in Rats

Determination of of paracetamol dose was based on the hepatotoxic dose in rats, i.e. 2.5 g/kg BW (Donatus, 1983 cit Rosnalini, 1995). Paracetamol was given to the rats orally, using 1% CMC Na as suspending agent.

Preliminary Test

Orientation trial to determine the occurrence of hepatotoxicity in the rats. At this orientation three rats received toxic doses of paracetamol (2.5 g/kg BW). Before treatment, rats were fasted for 18 hours and allowed to drink water *ad libitum*. The blood was taken from vein tail lateralis, at: 0, 12, 24, 36, 48 and 60 hour after administration of paracetamol. Hepato-toxic timing based on the highest level of SGPT.

Orientation trial to determine the duration of treatment with rosella flower infusion. This orientation used two

groups of test animals, each group consisted of three Wistar rats.

Group I: Rosella flowers infusion were given for 1 X 1 day (single dose).

Group II : Rosella flower infusion given 1 X 7 consecutive days (repeated doses) then at the 24th hour after the last administration of infusion, the rats were induced by paracetamol (p.o) 2.5 g/kg BW. Thirty six hours after induction of paracetamol, blood was taken from tail vein lateralis and SGPT level was measured by spectrophotometer UV λ 340 nm.

Hepatoprotective Test of Rosella Flowers

Study were carried out using male Wistar albino rats. Twenty five rats were randomized and divided into five groups, each group consisted of 5 rats. Rats were maintained under standard laboratory condition, and treated as follows:

Group I : Normal control, were given aquadest (p.o)

Group II : Negative control, were given aquadest for 1 X 7 consecutive days (p.o), then at the 24th hour after last administration of distilled water, the rats were induced by paracetamol 2.5 g/kg BW.

Group III: were treated with Rosella flower infusion 10% for 1 X 7 consecutive days (p.o), then at the 24th hour after the last administration infusion, the rats were induced by paracetamol 2.5 g/kg BW.

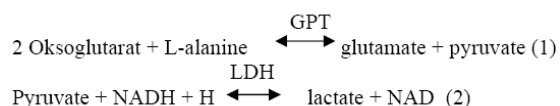
Group IV: were treated with Rosella flower infusion 20% for 1 X 7 consecutive days (p.o), then at the 24th hour after the last administration infusion, the rats were induced by paracetamol 2.5 g/kg BW.

Group V: were treated with Rosella flower infusion 40% for 1 X 7 consecutive days (p.o), then at the 24th hour after the last administration infusion, the rats were induced by paracetamol 2.5 g/kg BW.

At the 36th hour after administration of paracetamol, blood was taken from tail vein lateralis. SGPT level was measured by spectrophotometer UV λ 340 nm with reagen kit GPT-ALAT.

SGPT Assay Using GPL-ALAT Method

SGPT level was determined by photometry using UV spectrophotometer. One hundred milliliters of serum was poured into the test tube, added with 1000 mL monoreagent, and mixed with vortex for 1 minute. It was left standing for 3 minutes to complete the reaction (operating time). Distilled water was used as blank. The absorbance was read at 340 nm wavelength, at 37°C. SGPT level is expressed in units of U/L. SGPT level was determined using GPT-ALAT (Glutamate Pyruvate Transaminase-Alanine Amino transaminase) kinetic method. Serum was analyzed by reaction with 2-L-alanine oksoglutarat and in buffer solution. GPT enzyme present in serum would catalyze the transfer of the amino group of L-alanine to 2-oksoglutarat (1). Pyruvate formed in the presence of NADH and lactate dehydrogenase is converted to lactate enzymatically (2).



NADH have absorption at wavelength of 334, 340, 365 nm. In this examination spectrophotometer will measure the residual unreacted NADH. The reduced absorbance indicates the increased of NADH level (Campbell et al., 2005).

Statistical Analysis

Data of SGPT level were analyzed by one way ANOVA statistics (analysis of variant), followed by LSD (Least Significant difference) test with 95% confidence level.

RESULTS AND DISCUSSION

These studies aimed at determining the effects of Rosella flower infusion to reduced level of SGPT, a marker enzyme in damaged liver. To achieve these objective, the research was conducted in male rats induced by toxic dose of paracetamol (2.5 g/kg BW) using three concentration ranks of Rosella flower infusion 10% (1.25 g/kg BW), 20% (2.5 g/kg BW) and 40% (5 g/kg BW).

Table 1 shows that paracetamol 2.5 g/kg BW could increase the activity of the enzyme glutamate pyruvate trans-aminase (SGPT), which was used as parameter of liver damage. Normal range SGPT level in rats is 21-52 U/L (Paget, 1970). At the 36th hour after administration of paracetamol 2.5 g/kg BW showed the highest SGPT level approximately 1233.33 ± 596.51 U/L.

Liver cell damage is caused by toxic metabolites of paracetamol NAPQI, which is formed in excessive amounts. As a result the amount of glutathione available is not sufficient to neutralize the toxic compound, most NAPQI binds to liver cell macromolecules causing cell necrosis of the liver. If liver cells undergo necrosis, the specific transaminase enzymes in the liver will exit and enter the blood circulation, and the biochemical examination of SGPT will increase.

Rosella 40% infusion which was administered repeated doses (1 x 7 consecutive days) could reduce level of SGPT 7 times compared with single dose given (table 2).

Groups III, IV, and V were treated by Rosella infusion with different concentration, i.e. 10, 20 and 40 % respectively during 1 X 7 consecutive days, and continued to be given paracetamol for 24 hour after the last administration of infusion. Table 3 shows that in group IV and V, which were treated by Rosella infusion concentration 20% and 40% showed a decrease in SGPT level

Table 1. Enzym activity SGPT on rats induced hepatotoxicity by paracetamol toxic dose (2,5 g/kg BW) (n=3)

Treatment	Hour	SGPT level (U/L) in rats			Mean ± SD (U/L)
		1	2	3	
Paracetamol 2,5 g/kg BW (hepatotoxin)	0	31	42	43	38.66 ± 6.65
	12	84	185	101	123.33 ± 54.07
	24	350	1250	450	683.33 ± 493.28
	36	750	1900	1050	1233.33 ± 596.51
	48	550	950	700	733.33 ± 202.07
	60	350	500	550	466.66 ± 104.08

Table 2. Optimalization time of administration Rosella 40% infusion after induced by paracetamol toxic dose 2.5 g/kg BW (n=3)

Length of treatment	Rats	SGPT level (U/L)	Mean ± SD (U/L)
1 X 1 day	1	1500	1400 ± 132.28
	2	1450	
	3	1250	
1 X 7 consecutive days	1	320	205.33 ± 105.62
	2	184	
	3	112	

significantly when compared with negative control. In group III (given infusion 10%) have not been able to lower SGPT compared to negative control ($p > 0.05$), this is probably due to lack of the active substance that is responsible for the hepatoprotective effect.

Negative control group have a significant difference ($p < 0.05$) from normal control group. This was a prove that the administration of paracetamol 2.5 g/kg BW in male Wistar albino rats were able to raise the level of SGPT (Table 3). Administration of Rosella infusion 20% (group IV) and 40% (group V) had a significant difference ($p < 0.05$) against the negative control (group II). This means that infusion of Rosella concentration 20% and 40% had hepatoprotective effects on paracetamol-induced hepatotoxicity. Hepa-toprotective effect of Rosella infusion concentration 40% was greater

than the concentration of 20%. Rosella flower infusion groups (groups IV and V) when compared with normal control aquadest (group I) showed a significant difference ($p < 0.05$), it meant that the decreased level of SGPT due to the administration of Rosella infusion did not reach the normal range (21-52 U/L) (Paget, 1970).

Based on this study, it is necessary to continue testing using infusion of Rosella which were given in longer term (several weeks) and with larger doses. It is based on preliminary test results, group which was given infusion of Rosella with a single dose (1x1 day) cannot decrease SGPT level, meanwhile Rosella infusion that was given repeated dose (1X7 consecutive days) showed a significant decrease in SGPT level, this is probably due to the chemical constituents contained in infusion of Rosella can increase the synthesis of the Glutathione enzyme that has role in neutralizing the toxic metabolites NAPQI excess, so that SGPT level decreased.

Table 3. Effect of Rosella (*Hibiscus sabdariffa*) infusion on serum enzymes SGPT (U/L) in paracetamol induced liver damage in rats

Animal No	Group I Normal control (aquadest)	Group II Negative control (aquadest 7 days respectively + Paracetamol 2.5 g/kgBW	Group III (Rosella infusion 10% 7 days respectively + Paracetamol 2.5 g/kgBW	Group IV (Rosella infusion 20% 7 days respectively + Paracetamol 2.5 g/kgBW	Group V (Rosella infusion 40% 7 days respectively + Paracetamol 2.5 g/kgBW
1	38	750	1100	380	320
2	35	1900	875	412	184
3	33	1050	700	366	112
4	39	1300	725	404	117
5	37	950	650	382	128
Mean±SD	36,4±2,40	1190±443,56	810±182,52	388,8±18,79	172,2±87,48
% decreasing SGPT	-	-	31,93±15,33	67,33±1,57	85,44±±7,28
P value		<0.05**		<0.05*(**)	<0.05*(**)

*Considered significant when compared with negative control (paracetamol group)

**Considered significant when compared with normal group

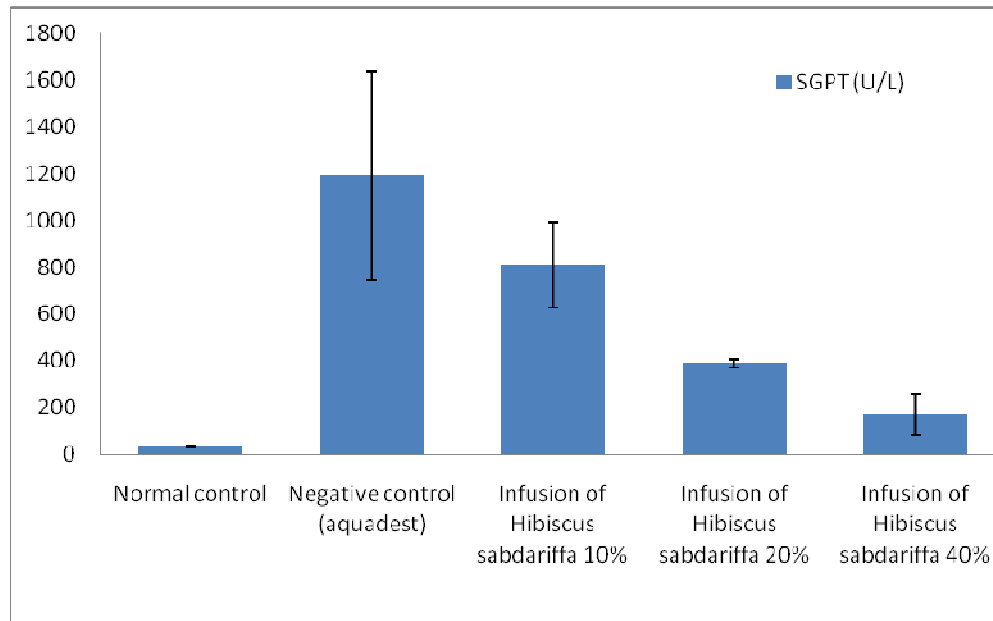


Figure 1. Effect of Rosella (*Hibiscus sabdariffa*) infusion on serum enzymes GPT level in paracetamol-induced liver damage in rats (n=5)

In the previous study (Ali, et al, 2003), *Hibiscus sabdariffa* water extract 200 mg/kg BW given for 4 weeks in rats induced by paracetamol 700 mg/kg BW, was able to improve damaged liver cells returned to normal, while the lower doses were not effective. Liu et.al (2005) showed that *Hibiscus sabdariffa* dried flowers extract had a protective effect against liver damage (fibrosis) induced by CCl₄. Concentrations of dried flower extracts of *Hibiscus sabdariffa* given were 1-5% for 9 weeks, whereas induction of CCl₄ was carried out for 7 weeks. The result showed that this extract could reduce liver damage including steatosis and fibrosis, while also lowering plasma level of aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), and restore glutathione content decreased and inhibit lipid peroxidation product during CCl₄ administration. *Hibiscus sabdariffa* is also relatively non-toxic with LD₅₀ > 5000 mg/kg BW (Ali et al, 2005). Hepatoprotective effects of rosella flower is probably caused by the antioxidant content in *Hibiscus sabdariffa*.

CONCLUSION

The results showed that infusion of rosella flower (*Hibiscus sabdariffa*) concentration of 20% (2.5 g/kg BW) and 40% (5 g/kg BW) given daily seven days respectively (p.o) could reduce level of SGPT in male Wistar rats induced by toxic dose of paracetamol (2.5 g/kg BW). Level of SGPT in treatment with rosella infusion 20 and 40 % decrease to be 388.8 ± 18.79 and 172.2 ± 87.48 U/L respectively, whereas the negative control group 1190 ± 443 U/L.

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