The Effect of Roselle (Hibiscus sabdariffa Linn) Flower Extract To The SGPT Activity In Male Wistar Rats (Rattus Norvegicus) Induced By High Dose Paracetamol

D Halim¹, E J Sihning, Tehupuring ¹*
¹Faculty of Medicine, Universitas Hang Tuah, Jalan Gadung 1, Surabaya-Indonesia

Abstract. Paracetamol is an analgesic drug that is used widely. The use of high dose paracetamol can cause liver damage. Based on research, Roselle has high antioxidant content. The objective of the research was to find out the effect of Roselle (Hibiscus sabdariffa Linn) flower extract to the SGPT activity of Wistar rats induced by high dose paracetamol.

This study used 24 male Wistar rats as samples and conducted for 10 days. Samples were divided into 3 groups: group of rats fed with standard diet and filtered water for 10 days and received 0.5% CMC-Na orally on the 9th day; group of rats fed with standard diet and Roselle flower extract 200mg/kg body weight for 10 days and received paracetamol 1750mg/kg body weight (in 0.5% CMC-Na) orally on the 9th day. All group were sacrificed on the 10th day. SGPT activity were examined with spectrophotometry method.

The result of Kruskal-Wallis test showed significant difference (p=0.001) of SGPT activity between the group of rats received standard diet (81,44 U/L) and group of rats received high dose paracetamol (240,39 U/L). Furthermore, there is significant difference (p=0.001) of SGPT activity between group of rats received high dose paracetamol (240,39 U/L) and group of rats received high dose paracetamol and Roselle flower extract (72,98 U/L).

It can be concluded that high dose of paracetamol significantly increased SGPT activity, and Roselle flower extract significantly reduced SGOT activity of Wistar rats induced by high dose paracetamol.

Keywords: Hibiscus sabdariffa Linn, Paracetamol, Liver, SGPT

1. Introduction
Paracetamol is a para-aminophenol derivate analgesic-antipyretic drug which is widely used in a single dosage form or in combination with other drugs, can be obtained by doctor’s prescription or sold as free remedies [1]. Under normal conditions, paracetamol is mostly conjugated to sulfate and glucoronate and a small portion will be oxidized by cytochrome P₄₅₀ to N-acetyl-p-benzo-quinone (NAPQI) reactive metabolite which detoxified by liver glutathione into non-toxic conjugates and excreted by kidneys [2]. Paracetamol is metabolized by liver microsome enzymes, 60% of paracetamol is conjugated with glucoronic acid and 30% with sulfuric acid [3] and about 5% is oxidized by cytochrome P₄₅₀ 2E1, 3A4, and 1A2 enzymes in the liver to N-reactive metabolites acetyl-p-benzo-paraquinon-imine (NAPQI) or often referred to as N-acetyl-p-benoquinoine-imine (NAPBOQI) [4][5]. The amount of NAPQI produced at paracetamol therapeutic doses is usually completely detoxified by glutathione to form mercapturi acid which is non-toxic and excreted through urine [1].

In paracetamol overdose, NAPQI production increases beyond glutathione detoxification ability so that NAPQI reacts with liver cells and causes centrolobular necrosis [1]. Damage to liver cell membrane or necrosis, will cause the release of this enzyme into the blood circulation, that makes this enzyme activity increases in blood. Although serum levels of SGPT and SGOT will increase in any disease that affects the integrity of liver cells, SGPT is an enzyme that is more specific in liver.
Elevation of serum SGPT activity is rarely found in conditions other than liver parenchymal disease. In addition, the elevation of SGPT activity lasts longer than the elevation of SGOT activity [6].

Roselle (*Hibiscus sabdariffa* Linn) has been studied as one of natural exogenous antioxidants that has high antioxidants level because it is rich in phenol compounds and flavonoids. Plants that are rich in phenol compounds and bioflavonoids have very high antioxidant activity [7]. Phytochemical studies on Roselle flower calices indicate the presence of various antioxidants such as anthocyanin, ascorbic acid and protocatechuic benefit [8].

This research main objective is to prove that Roselle flower (*Hibiscus sabdariffa* Linn) calices extract can prevent the occurrence of liver cell damage in white rats induced by high dose paracetamol. This study specific aim is to prove that the administration of Roselle flower calices (*Hibiscus sabdariffa* Linn) could reduce oxidative stress in liver cells which refers to the decrease in SGPT activity of white male Wistar rats strain (*Rattus Norvegicus*) induced by high dose paracetamol [9].

2. Experimental Method
A total of 24 white male Wistar strain rats were divided into 3 groups: group of rats fed with standard diet and filtered water for 10 days and received 0.5% CMC-Na orally on the 9th day; group of rats fed with standard diet filtered water for 10 days and received paracetamol 1750mg/kg body weight (BW) (in 0.5% CMC-Na) orally on the 9th day; and, group of rats fed with standard diet and roselle flower extract 200mg/kg BW for 10 days and received paracetamol 1750mg/kg BW (in 0.5% CMC-Na) orally on the 9th day. All group were sacrificed on the 10th day. SGPT activity were examined with spectrophotometry method in U/L units [10,11,12,13].

Roselle flower extract was obtained from dried calices which is macerated using 70% 6000 mL (1:4) ethanol solvent. After stirring occasionally for approximately 3 hours, then left for 24 hours. Macerate was collected and evaporated with a vacuum rotary evaporator at 60°C and concentrated on a waterbath of 60-70°C to obtain a thick extract [14]. In rats, Roselle extract in a dose of 200 mg / kg BW significantly improved liver function induced by paracetamol [15] So, the dose of roselle in rats with a body weight of 200g = 200 mg / kg BW x 200 g = 40 mg [9].

Rat blood samples were obtained by cardiac puncture after euthanasic anesthesia with 44-60mg / kg BW ketamine hydrochloride intra-muscularly in the quadricep or tricep muscles [16,17]. Then read with spectrophotometer at a wavelength of 340 nm with a factor of 1745. The reading is done in minutes 1, 2, and 3 [10]. Data analysis is done by statistical tests using Kruskal-Wallis followed by Post Hoc Least Significance Difference (LSD). The significance level α used is 5%[13,18].

3. Results and Discussion
The experimental group of animals given only high dose paracetamol without Roselle flower calices extract (240.39 U/L) showed a significant increase in SGPT serum levels (p = 0.001) compared to the experimental animal group that was only given CMC-Na 1% (81.44 U/L). Tabel 1 and 2 showed that there has been significant liver cell damage due to the induction of a single dose of high dose paracetamol orally.

SGPT serum activity in groups of rats receiving high doses of paracetamol without Roselle flower calices extract (240.39 U/L) was significantly different (p = 0.001) to SGPT serum activity in groups of rats receiving high doses of paracetamol and Roselle flower calices 200 mg / kgBW / day (72.98 U/L). This revealed a significant decrease in serum SGPT activity in groups of rats that received high doses of paracetamol and Roselle calices extract 200 mg / kgBW / day. This data shows that Roselle flower calices extract can prevent the occurrence of liver cell damage due to induction of high doses of paracetamol.
Tabel 1. SGPT Activity in Standard Feeding Animal Group, High Dose Paracetamol Animal Group, and High Dose Paracetamol and Roselle Flower Calices Extract Animal Group (*Hibiscus sabdariffa* Linn).

| No. | C (-) (U/L) | C (+) (U/L) | T (U/L) |
|-----|-------------|-------------|---------|
| 1   | 82.7        | 611.7       | 53.6    |
| 2   | 87.1        | 122.3       | 49.2    |
| 3   | 84.7        | 409.4       | 86.3    |
| 4   | 76.8        | 157.4       | 64.4    |
| 5   | 86.1        | 110.5       | 76.1    |
| 6   | 88.3        | 186.2       | 86.8    |
| 7   | 66.9        | 202.8       | 83.5    |
| 8   | 78.9        | 122.8       | 83.9    |

Note: C (-): Control negative group given standard feeding. C (+): Control positive group given high dose of Paracetamol. T: Treatment group given Roselle flower extract and high dose of Paracetamol.

Tabel 2. Mean and Standart Deviation SGPT Activity in Standard Feeding Animal Group, High Dose Paracetamol Animal Group, and High Dose Paracetamol and Roselle Flower Calices Extract Animal Group

| Group | Mean (U/L) | SD (U/L) |
|-------|------------|----------|
| C (-) | 81.44      | 7.0955   |
| C (+) | 240.39     | 178.2119 |
| T     | 72.89      | 15.2257  |

Note: C (-): Control negative group given standard feeding. C (+): Control positive group given high dose of Paracetamol. T: Treatment group given Roselle flower extract and high dose of Paracetamol.

Reactive NAPQI metabolic increases in high dose paracetamol administration [19]. This metabolite is associated with the formation of reactive oxygen species (ROS) which can oxidize biomolecules such as carbohydrates, proteins, and lipids. ROS also has toxic effects on phospholipid membranes and causes wide spectrum cell damage, including lipid peroxidation, enzyme inactivation, abnormalities in intracellular oxidation-reduction function, DNA modification and chromosomal abnormalities [20]. All of these cytotoxic effects will cause centrilobular necrosis with increased hepatic enzyme plasma activity, including SGPT [21]. High serum SGPT is relatively specific for liver cell damage, because SGPT is a cytoplasmic enzyme and is present in high concentrations only in liver cells. This enzyme is released from damaged cells, associated with increased cell membrane permeability or cell necrosis [22].

Roselle is known as an antioxidant because it contains flavonoids. Other substances contained are vitamin C with 9 times levels higher than oranges [15]. Anthocyanin is a type of flavonoid compound. As an antioxidant, anthocyanin can reduce oxidative damage to DNA, increase glutathione reserves, and increase the expression of glutathione S-transferase P1 protein (hGSTP1) in leukocytes. hGSTP1 is displayed to prevent DNA damage and mutagenesis [23].

Vitamin C in Roselle acts as an antioxidant which functions to bind oxygen so that not support oxidation reactions or as oxygen scavenger [24]. Vitamin C can also bind to variety of reactive oxygen such as super oxides, hydroperoxyl radicals, singlet oxygen and nitric oxide radicals, therefore effectively protecting other substances from oxidative damage. Giving a certain amount of vitamin C can also prevent the glycogenolysis process during the oxidative phase [25]. Vitamin C also works with glutathione peroxidase (the main enzyme to fight free radicals) to re-strengthen vitamin E a fat-soluble antioxidant [26].

Figure 1 showed that there was a decrease in SGPT activity after administration of Roselle flower (*Hibiscus sabdariffa* Linn) calices extract in rats induced by high dose paracetamol. Roselle
flower calices extract administration can increase glutathione reserves so that the toxic metabolites of paracetamol will bind to glutathione to produce cysteine and mercapturic conjugates which excreted in the urine. In addition, reducing oxidant reactions, reduce oxidative stress of the liver. Therefore reduce the level of liver cell damage showed by decreasing SGPT activity [9].

Based on the discussion, it can be concluded that Roselle flower calices (Hibiscus sabdariffa Linn) administration in a dose of 200 mg/kg rat BW was able to significantly reduce the activity of SGPT in rats induced by high dose paracetamol receiving Roselle flower (Hibiscus sabdariffa Linn) calices extract compared to groups of rats induced by high doses of paracetamol without receiving Roselle flower (Hibiscus sabdariffa Linn) calices extract.

**Figure 1.** Mean of SGPT Activity in Standard Feeding Animal Group, High Dose Paracetamol Animal Group, and High Dose Paracetamol and Roselle Flower (Hibiscus sabdariffa Linn) Calices Extract Animal Group. Informations: C (-): Control negative group given standard feeding. C (+): Control positive group given high dose of Paracetamol. T: Treatment group given Roselle flower extract and high dose of Paracetamol.

4. Conclusion
The study of the effect of Roselle (Hibiscus sabdariffa Linn) Flower Calices Extract on SGPT Activity of white male Wistar Rats Strain (Rattus norvegicus) induced by high dose Paracetamol, can concluded as follows:
1. Single dose Paracetamol of 1750 mg / kg body weight can significantly increase SGPT activity in Wistar male white rats strain
2. Roselle flower calices extract (Hibiscus sabdariffa Linn) at a dose of 200 mg / kgBW can significantly reduce SGPT activity in Wistar male white rats strain receiving high dose paracetamol.

References
[1] Darsono, L. 2002, Diagnosis dan Terapi Intoksikasi Salisilat dan Parasetamol, Pharmacology Department Fakultas Kedokteran Universitas Kristen Maranatha, Bandung.
[2] Katzung, BG. 2011, Farmakologi Dasar dan Klinik, 10th edition, Jakarta, EGC Medical Book Publisher.
[3] Walubo, A, Barr, S, Abraham, AM, Coetsee C. 2004, The Role of Cytochrome-P450 Inhibitors in the Prevention of Hepatotoxicity after Paracetamol Overdose in Rats. Human and Experimental Toxicology, 23th edition.http://het.sagepub.com/content/23/1/49.abstract
[4] Kozer, E, Koren, G. 2001, Managementof Paracetamol Overdose. Drug Safety.
[5] Riordan, SM, Williams, R. 2002, Alkohol Exposure and Paracetamol-Induced Hepatotoxicity. Addiction Biology: 7th Ed; pp 191-206.
[6] Tietz, NW, Burtis, CA, Ashwood, ER. 2001, Tietz Fundamental of Clinical Chemistry, 5th Edition, Philadelphia, Toronto, London, W.B. Saunders Company.
[7] Essa, MM, Subramanian, P, Suthakar, G, Manivasagam, T, Dakshyayani, KB, Sivaperumal, P, Subash, S, Vinothini, G. 2006, Influence Of Hibiscus Sabdariffa Of The Levels Of Circulatory Lipid Peroxidation Products And Liver Maker Enzymes In Experimental Hyperammonemia, Journal app. Bio, 4th edition.

[8] Hirunpanich, V, Utaipat, A, Morales, NP, Bunyapraphatsara, N, Sato, H, Herunsalee, A. 2005, Antioxidant Effects Of Aqueous Extracts From Dried Calyx Of Hibiscus Sabdariffa Linn. (Roselle) In Vitro Using Rat Low-Density Lipoprotein (LDL), Biol Pharm, 28.

[9] Susilowati, A.E. 2009, Pengaruh Pemberian Ekstrak Bunga Roselle (Hibiscus Sabdariffa L.) Terhadap Kerusakan Sel-Sel Hati Mencit (Mus Musculus) Akibat Paparan Parasetamol, Skripsi, Fakultas Kedokteran Universitas Sebelas Maret, Surakarta.

[10] Bregmeyer, HU, Bernt, E. 1971, Methods of Enzymatic Analysis, 2th edition, New York, Academic Press Inc.

[11] Herwiyararasanta, I. 2010, Efek Pemberian Sari Kedelai Hitam Terhadap Kadar LDL(Low Density Lipoprotein) Tikus Putih (Rattus norvegicus) Dengan Diet Tinggi Lemak, Fakultas Kedokteran Hewan Univeristas Airlangga.

[12] Indriania 2013, Populasi, Sampel, Dan Teknik Sampling, Universitas Negeri Semarang.

[13] Steel, RGD, Torrie JH, alih bahasa Sumantri, B 1991, Prinsip dan Prosedur Statistika. Suatu Pendekatan Biometrik, Cetakan kedua, Jakarta, PT Gramedia Pustaka Utama, pp.105-146.

[14] Rahardian, M 2013, Efek Hepatoprotector Ekstrak Etanol Kelopak Bunga Roselle (Hibiscus Sabdariffa L.) Pada Tikus Sprague Dawley Yang Diinduksi 7,12-Dimetilbenz(A)Antrasen: Kajian Aktivitas SGPT, SGOT, ALP, Dan Picturean Histopatologi Hati, Tesis, Fakultas Pascasarjana Farmasi Universitas Ahmad Dahlan.

[15] Maryani, H, Kristiana, L 2005, Khasiat dan Manfaat Roselle, Jakarta, PT Agro Media Pustaka, pp.2-33.

[16] Beeton, Christie, Garcia, Aridna, Chandy, George, 2007, Drawing Blood from Rats through the Saphenous Vein and by Cardiac Puncture. J Vis Exp.10.3791/266.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2565848/

[17] Office of Laboratory Animal Medicine Univeristy of Delaware 2010, Cardiac Puncture Blood Collection (Terminal Procedure).https://www.udel.edu/research/pdf/cardiac-puncture-blood-collection-terminal.pdf.

[18] Sudjana 1984, Metoda Statistika, 3rd Edition, Bandung, Penerbit Pustaka, pp.157-170, 213-261.

[19] Brunton, L, Chabner, B, Knollmann, B. 2011, Analgesic-Antipyretics and Antiinflammatory Agent: Drugs Employed in The Treatment of Rheumatoid Arthritis and Gout, Goodman and Gilman’s The Pharmacological Basic of Therapeutics. 12th edition. USA, Mc. Graw Hill medical.

[20] Sheeja, K, Shihab, PK, Kuttan, G 2006, Antioxidant and Anti-Inflammatory Activities of the Plant Andrographispaniculata Ness, Immunopharmacology and Immunotoxicology.

[21] Mahadevan, SBK, McKiernan, PJ, Davies, P, Kelly, DA 2006, Paracetamol Induced Hepatotoxicity. British Medical Journal.

[22] Sulaiman, Daldiyono, Akbar, Rani 1990, Gastroenterologi Hepatologi, Biokimia Penyakit Hati, CV. Infomedika Jakarta.

[23] Corredo, RG 2007, Medox, Purified Anthocyanins: Antioxidant Power with Many Biological Effects. Scientific Review.http://www.chaipanich.co.th/research/ Purified_Anthocyanins.pdf

[24] Kumalaningsih, S 2007, Antioksidan, Sumber & Manfaatnya.http://antioxidantcentre.com/index.php/Antioksidan/3.-Antioksidan-Sumber-Manfaatnya. html.

[25] Argapay 2008, Daya Hambat Vitamin C terhadap Kerusakan Membran Sel Darah Merah akibat Fotosensitisir Ofloksasin yang Diinduksi Ultraviolet.http://one.indoskripsi.com/judul-skripsi/kedokteran/daya-hambat-vitamin-cterhadap-kerusakan-membran-sel-darah-merah-akibat-fotosensitisirofloksasin-ya

[26] Null, G. 1993. The Antioxidant Vitamin-Vitamin Chttp://www.garynull.com/Documents/ vitaminc.htm.