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## Effect of calyx capsule-ethanol extract *Hibiscus sabdariffa* L. on renal function of healthy volunteers

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**Abstract.** *Hibiscus sabdariffa* contains flavonoid, triterpenoid, anthocyanin which function as immunostimulant. *H. sabdariffa* is considered safe for animal renal; nonetheless, there are known side effects of which need to be further investigated for human renal. This research aims to investigate the effect of calyx capsule-ethanol extract *H. sabdariffa* for renal function of healthy male and female for 30 days period by monitoring Scr and Clcr component in their blood samples. The method of this experimental research was by pre and post-treatment by involving 20 healthy volunteers who have met inclusion and exclusion criteria. The volunteers have completed the informed consent for this experiment. Furthermore, volunteers were divided into two groups (10 male and 10 female). Each group was given orally 500 mg of calyx capsule-ethanol extract *H. sabdariffa* per day for 30 days period. Blood tests were taken on day 0, day 30 after consuming the capsule and day 45 (15 days after the last day of capsule intake) in order to measure the Scr and Clcr concentration in the blood samples by using Jaffe dan Cockcroft-Gault method. The results of each sampling day were further analyzed statistically and compared using Repeated ANOVA dan Friedman test. The results suggest that there was a difference in the renal function on day 0, 30 and 45 samplings. However, there was no significant difference in Scr dan Clcr concentrations on female and male volunteers ( $p>0.05$ ). Specifically, the type of gender affects Scr concentration ( $p<0.05$ ); however, it does not affect Clcr concentration ( $p>0.05$ ). In addition, age and Body Mass Index (BMI) does not affect Scr and Clcr concentrations ( $p>0.05$ ). The side effects discovered through the monitoring increased in mixturation and bloatedness. Calyx capsule-ethanol extract *H. sabdariffa* does not affect on renal function of healthy volunteers.

**Keywords:** *H. sabdariffa*, healthy renal function, Scr, Clcr

### 1. Introduction

*H. sabdariffa* is a natural exogenous antioxidant. It is popular among the well educated women, specifically among the upper middle class who have a great sense of health awareness [1]. *H. sabdariffa* is greatly used to increase stamina since its antioxidant characteristic has immunostimulant effect [2]. The main content of *H. sabdariffa* is vitamin C, flavonoid, polyphenol, beta carotene dan antosianin [3] as well as alkaloid, saponin and tannin [4].



The main component of *H. sabdariffa* i.e. anthocyanin, flavonoid dan chlorogenic acid possess potassium-sparing diuretics, natriuretic in addition to its antioxidant feature [5]. Diuretic is known to maintain the balance of water and electrolyte in the renal system [6].

Renal has a crucial function in the reduction/secretion of medicine/drugs/chemicals in the body [7]. Its function is highly correlated with homeostasis involving urinal, electrolyte, external objects such as toxins and urea secretion. It works through combination of glomerulus filtration (GFR), tubular secretion and body reabsorption [8]. All nutrition and chemicals/drugs will be filtered by GFR hence the GFR capacity is an indicator for renal performance [9].

Glomerulus filtration rate function can be observed from creatinine serum (Scr). Their correlation is not a linear correlation, nevertheless the decrease of GFR is observed when serum creatinine increases, which indicates the reduction in renal cleaning capacity by the reduction of clearance creatinine (Clcr). There is a difference in Scr dan Clcr level of male and female due to difference in Body Mass Index (BMI) [10]. In addition, the difference in eating habits and muscle mass of male and female determine the creatinine production [11].

Drugs effects on renal function are highly correlated with toxic effect which demonstrates the safety of the drugs. Calyx capsul-ethanol extract *H. sabdariffa* is safe for rats with an impact of an increase in Scr depending on the dosage [12]. The impact is found to be toxic for rats once they are given 10,000-7,000 mg/kg body weight of *H. sabdariffa* capsule regularly for a long period [13] also discovers that of *H. sabdariffa* capsule with a dosage of 50,100 dan 200 mg/kg body weight is safe and effective to reduce free radical on rats. In addition, suggests that the LD<sub>50</sub> (acute toxicity on rats occurs) is 850.90 mg/kg body weight [14]. It is also confirmed that toxicity of *H. sabdariffa* extract is deemed low, as low as LD<sub>50</sub> 2,000 up to 5,000 mg/kg/day on rats [15].

Based on the findings above, there is a need to further study the effect of calyx capsule-ethanol extract *H. sabdariffa* on renal function of healthy male and female.

## 2. Materials and Methods

This research was conducted using pre and post treatment to determine the effects of *H. sabdariffa* on renal function of 20 healthy volunteers that divided into two groups (10 male and 10 female).

Inclusion criteria in this research were age > 18-45 years, not smoking, IBM 18-30 kg/m<sup>2</sup>, not taking supplements within 7 days before and at the time of treatment who have a health certificate from a registered doctor. While exclusion criteria were pregnant and breastfeeding, using hormonal contraceptives, autoimmune disease, and history of renal impairment.

### 2.1. Variable

The dependent variables in this research were measure the Scr and Clcr concentration in the blood samples on day 0, day 30 and day 45. The independent variables in this research were 500 mg calyx capsule-ethanol extract *H. sabdariffa* orally per day for 30 days period for all volunteers. The confounding variables were body habitus and diet control.

### 2.2. Place and time research

This research was conducted in Hidayatullah Hospital of Yogyakarta for 45 days (April 3<sup>rd</sup> to May 17<sup>th</sup> 2017).

### 2.3. Instruments

Serum creatinine measured by applying Jaffe method using spectrophotometer (486 nm wave length) and picric acid as a reactor. In addition, the measurement of Clcr is performed by Cockcroft-Gault equation.

### 2.4. Health check and preparation

The potential volunteers who have filled the informed consent are physically examined for their hematology, liver and renal by registered doctors. The results are recorded in physical examination and laboratories reports.

The 20 volunteers who have been approved by the Ethical Committee of Universitas Muhammadiyah Yogyakarta (Approval Number No. 255/EP-FKIK-UMY/IV/2017). The volunteers

were given orally of 500 mg calyx capsule-ethanol extract *Hibiscus sabdariffa* (product code/batch number: 5055C/RH162703) per day after meals for 30 days period.

Blood samples are taken on median cubital vein or brachial vein using 5 cc syringe for 4 cc blood then put into microcentrifugation tube containing EDTA as anticoagulant. Plasma separation carried out by centrifugation with a speed of 3500 rpm for 2 hours resulting a clear yellow liquid (serum). If the serum not used immediately, it is stored in the freezer with a temperature of -20<sup>0</sup>C. 0.25 cc to 0.5 cc serum is taken for Scr measurement. Serum that reacts with picric acid placed inside auto-analyzer photometric to measure Scr by applying Jaffe method. Once Scr concentration obtained, Clcr estimated by Cockcroft-Gault equation as follows:

$$\text{Male : Clcr} = (140 - \text{age}) \text{ ABW} / (\text{Scr} \times 72)$$

$$\text{Female: Clcr} \times 0.85$$

2.5. Data analysis

The collected data were tabulated. Continuous variables were presented as mean±SD. We used SPSS 23.0 for data analysis. Scr measurement and Clcr estimation are analyzed using Repeated ANOVA and Friedman test with 95% confidence interval.

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3. Results and Discussion

3.1. Scr and Clcr Measurements

The results of Scr measurement and Clcr estimation from the volunteers on day 0, 30 and 45 (15 days after the last day of capsule intake) is shown in Table 1 dan 2.

**Table 1.** Average male (n=10) renal function on day 0, 30 and 45 (15 days after the last day of capsule intake

Parameter	(Mean±SD)			P value	Normal Value
	Day 0	Day 30	Day 45		
Scr (mg/dL)	1.07±0.31	1.14±0.12	1.22±0.16	0.050	0.5-1.2
Clcr (mL/min )	93.05±29.45	82.99±18.94	77.93±18.64	0.193	80–130

**Table 2.** Average Female (n=10) Renal Function on day 0, 30 and 45 (15 days after the last day of capsule intake

Parameter	(Mean±SD)			P value	Normal Value
	Day 0	Day 30	Day 45		
Scr (mg/dL)	0.84±0.22	0.96±0.11	0.96±0.14	0.132	0.4-1.1
Clcr (mL/min )	85.08±18.19	71.70±8.02	72.93±15.20	0.181	75-120

Notes:

day 0: before *hibiscus sabdariffa* capsule intake

day 30: after *hibiscus sabdariffa* capsule intake (last day)

day 45: 15 days after the last day of capsule intake

Table 1 shows that there is no difference in male renal function on day 0, day 30 and day 45 based on Scr and Clcr content (p>0,05). Likewise, Table 2 demonstrate the same result for female renal function.

Both female and male renal function (Scr) is not affected by the *H. sabdariffa* capsule intake from day 0, day 30 as well as day 45 (p>0.05). However, there is a difference in the average Scr level on day 0 and day 30 of which Scr increases on day 30 within the acceptable level for human renal. Toxic effect occurs at 10,000-66,000 mg/kg body weight *H. sabdariffa* intake for rats (regular and long period intake). The effects could be muscular dystrophy leading to renal disturbance [12]. The increase

in Scr level is due to the content of anthocyanin and quersetin found in *H. sabdariffa* extract. These flavonoid substances are effective free radicals scavenger [16]. Anthocyanin found in the extract ethanol is less than one using water [12] hence the increase of Scr is not found to be significant in this research. If muscular dystrophy occurs, the creatinine production will diminish due to muscle formation inefficiency leading to increase in creatinine in the blood [17]. The Muscular dystrophy effect is not discovered from the volunteers since the creatine in the blood is still within permissible 50-100 mg/kg body weight.

Scr measurement on male volunteers shown that there is a surge of Scr on day 45 which shows that there is a potential *H. sabdariffa* residue or other influencing factors such as low water intake or unhealthy lifestyle. In contrast, there is no surge in Scr on day 45 on female volunteers.

There is an insignificant difference in Clcr estimations for male and female volunteer ( $p > 0.05$ ). There is a decrease in Clcr level on day 30 for male and female volunteers; however it is still within permissible limit. The reduction in Clcr is due to the increase in creatinine production being wasted by body [18]. The increase in creatinine by products is due to quersetin content which stimulates the release of Nitric Oxide (NO) on vascular endothelial. This phenomenon is known as vasorelaxation which results in failure in renal function [5].

### 3.2. Volunteers response to Scr and Clcr monitoring

Creatinine level does not only depend on muscle mass but also muscle activities, diet, health conditions, age, gender, and race [19]. Scr and Clcr monitoring based on gender, body mass index (BMI) and age is provided in Table 3.

**Table 3.** Scr and Clcr monitoring based on gender, body mass index (BMI) and age.

Group	Scr (Mean±SD)	p-value	Clcr (Mean±SD)	p-value
<b>Gender</b>				
Male	1.14±0.11	0.001	82.99±18.94	0.207
Female	0.96±0.11		72.94±15.21	
<b>Body Mass Index</b>				
<18.5	1.2±0.00	0.495	64.95±0.00	0.262
18.5-22.9	1.03±0.13		74.78±16.96	
23.0-24.9	1.03±0.21		92.31±16.32	
25.0-29.9	1.2±0.00		95.70±0.00	
Age	1.05±1.39	0.621	77.79±17.49	0.559

Clcr level is not affected by age, BMI and age ( $p > 0.05$ ); nonetheless, Scr is affected gender factor ( $p < 0.05$ ) due to difference is muscle mass. Apart from muscle mass, the difference in Scr is due to the difference in muscle mass and diet in different gender [8].

### 3.3. The side effects

The side effects discovered from intake of calyx capsule-ethanol extract *H. sabdariffa* are increase in micturition and bloatedness. It is found that 3 male experiences micturition increase and 2 male experiences bloatedness. Increase in micturition can still be considered as minor diuretic. This result is aligned with the result from rats [20]. It is found that *H. sabdariffa* can increase renal filtration by 48% since quersetin found in *H. sabdariffa* stimulates the release of Nitric Oxide (NO) on vascular endothelial. This phenomenon is known as vasorelaxation which results in diuretic effect. Diuretic effect can be found in the volunteers who consume the *H. sabdariffa* with dosage of 25, 50, and 100 mg/ body weight [21].

Bloatedness occurs since calyx capsul-ethanol extract *H. sabdariffa* contains ascorbic acid (260-280 mg/100 g) found in the dray leaves of hydroxycitric acid and hibiscus acid [22]. Bloatedness can result in discomfort in the digestion system.

#### 3.4. Research limitation

Research limitation were variables measured only in adult volunteers, without measuring the elderly and children volunteers. As well this research only measured sub chronic toxicity, further research is needed to determine chronic toxicity.

#### 4. Conclusions

It can be concluded that calyx capsule-ethanol extract *H. sabdariffa* does not affect on renal function of healthy volunteers.

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