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THE EFFECT OF SUBCHRONIC ADMINISTRATION OF ROSELLA (*HIBISCUS SABDARIFFA* L.) EXTRACT ON LEVELS OF UREUM, CREATININE, AND RENAL HISTOPATHOLOGY

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ABSTRACT: Rosella (*Hibiscus sabdariffa* L.) calyx traditionally used to treat various diseases. There is limited evidence based could be found including the efficacy and safety. The present study was aimed to investigate the effects of subchronic administration of *Rosella calyx* extract on the renal function of female Sprague Dawley (SD) rats. The rosella calyx was extracted by 70% ethanol and evaporated with a rotary evaporator to get concentrated extract. The Sprague Dawley rats were randomly divided into five groups including control group which was given CMC Na 0.5% and treated groups were administered orally with the extract of 50, 100, 200 mg/kg bw for 35 days. The satellite group was conducted for observing the reversibility, the observation in this group was conducted 14 days after 35 days of treatment. The biochemical function including ureum and creatinine was analyzed from a blood sample using enzymatic UV-test, and creatinine level were observed by the Jaffe method. The histopathology profile of kidney was observed with Hematoxylin and Eosin staining and followed by microscopic observation. The results showed that ureum levels in treated groups (50, 100, 200, and satellite groups was significantly difference ($p < 0.05$) compare to the control group. The creatinine levels also were found to show significant differences ($p < 0.05$) between the control group with treated groups (50, 100, 200 and satellite group). But the increasing creatinine levels are still within normal range. However, the histopathological profile of animals did not reveal any morphological damage.

INTRODUCTION: The use of medicinal plants for treating various diseases is an increase in line with trend back to nature. Rosella (*Hibiscus sabdariffa*) was used by the community to treat various symptoms of diseases. The previous research has reported the effect of this medicinal plants including anti-hypertensive¹, hepatoprotective²⁻⁴, antihyperlipidemic⁵, anticancer^{6, 7} and antioxidant^{8, 9}. The use of the medicinal plant for clinical application needs to consider the side effects.

The use in a small dose for long duration possibly cause toxicity to organs in the body. The kidney is an important organ in detoxification of many toxicants. Some damage in the kidney could be in the form of necrosis, cell proliferation, inflammatory cell infiltration, and hemorrhage. Various studies have been conducted to evaluate the safety of roselle as a traditional medicine¹⁰⁻¹⁴. It was reported the subchronic treatment of a high dose of water extract of roselle in albino rats could affect the kidney function with parameters of urea, creatinine and uric acid¹³. High dose treatment of rosella extract showed a significant decrease in serum potassium, sodium, chloride, and bicarbonate levels. The renal histopathology also showed the necrotic damage¹⁵. The treatment of a high dose of rosella extract could cause renal damage.

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Therefore, the present research will explore the toxicity effect of rosella extract in an effective dose, with subchronic duration on renal function.

MATERIALS AND METHODS:

Plant Collection and Determination: The rosella calyx was obtained from Kediri (East Java) Indonesia. The plant was identified at the Laboratory of Biology, the University of Ahmad Dahlan with the identification number of B/B.12/1030/VII/2014. The rosella calyx was dried on 50 °C.

Extraction: Rosella calyx was extracted using maceration method with 70% ethanol as solvent and followed by evaporation using a rotary evaporator to get concentrated extract.

Determination of Total Phenolic Content:

Determination of total phenolic content was carried out using gallic acid as standard¹⁶. A total of 10 mg of ethanol extract of roselle calyx was diluted to a volume of 10 ml of a mixture of ethanol: distilled water (1: 1). The 300 ml solution is pipetted and added with 1.5 ml of Folin-Ciocalteu reagent. After settling for 3 min, add 1.2 ml of 7.5% Na₂CO₃ solution and allowed to stand on a range of operating time at room temperature. The absorbance of the extract solution was measured by UV-Vis spectrophotometer at 765 nm.

Determination of Total Flavonoid Content:

The total flavonoid content was determined using quercetin as standard¹⁷. The extract was weighed of 1 gram and dissolved in 100 ml of 70% ethanol, The 3 ml of extract solution was added with ethanol until 10.0 ml. The solution of 0.5 ml was taken and diluted with 1.5 ml of ethanol, and added with 0.1 ml 10% AlCl₃; 0.1 ml Na acetate; and 2.8 ml of distilled water, the solution was mixed until homogeneous and measured by UV-Vis spectrophotometer at 430 nm.

Preparation of Sample: The rosella extract was diluted in 5% of CMC Na.

Animal Treatment: The animal handling in the present research had the ethical approval from Research Ethics Committee Universitas Ahmad Dahlan with ref. number 011505043.

A total of 30 female mice strain Sprague Dawley were divided into 5 groups, each consisting of 6

rats and treated for 35 days. The CMC Na 0.5% gave normal group during the study. We also provided the control group for a reversible group which maintains until 14 days after treatment. The treated group was treated with 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW of extract for 35 days.

Blood Serum Preparation: Blood sampling was carried out on day 36 except for the reversible group was done on day 50. The blood was taken from sinus orbital of an eye and then collected in the Eppendorf tube. Following collection, the blood was then centrifuged for 10 minutes at 8000 rpm. Serum was collected from supernatant at the top of the sample.

Determination of Ureum Level:

Determination of ureum level was carried out using an enzymatic method using Urea FS* (Diasys). The principle of this method was urease catalyzes the hydrolysis of urea into ammonia and carbonate. This ammonia then is reduced along with oxidation of NADH to NAD⁺ in the process catalyzed by enzymes GLDH. NADH oxidation causes a decrease in absorbance at 340 nm, the decrease rate of absorbance is directly proportional to the concentration of ureum. The absorbance at 30 seconds was recorded as (A1), and then the absorbance at the 90 sec was recorded as (A2). Urea levels in the serum can be calculated by comparing with a standard.

Determination of Creatinine Level:

Blood creatinine levels determined by the Jaffe method using Creatinine FS* (Diasys). In this method, creatinine forms an orange-red colored complex in alkaline picrate solution. Creatinine forms a complex reaction of creatinine-picric using reagents Creatinine FS. The absorbance was observed using spectrophotometry at a 492 nm. The creatinine level was counted by comparing with a standard.

Histopathological Observation:

Animals were anesthetized using CO₂ gas for a few minutes. Mice then sacrificed by cervical dislocation. The organs were separated immediately and put in 10% formalin solution after washing with normal saline. The renal organ was made histopathological preparations and stain with hematoxylin and eosin (HE).

RESULTS AND DISCUSSION:

Total Flavonoid and Total Phenolic Content of *H. sabdariffa* L. Extract: Anthocyanin, one of the flavonoids group members, is the major compound of *H. sabdariffa*. The anthocyanin level found abundant in various color fruits, vegetables, and flowers. The previous research reported the high level of flavonoids and phenolics compound on *H. sabdariffa* L. extract. The total phenolic content was expressed as gallic acid equivalent. It was found that the total phenolic content of rosella extract was $1.96 \pm 0.05\%$. The previous studies reported the phenolic content of *H. sabdariffa* L. calyx from different place found 1.40-2.20% of gallic acid equivalent¹⁸. The flavonoid contents of the extracts regarding quercetin equivalent was $0.52 \pm 0.02\%$.

The Effect of Ethanol Extract of Rosella on Renal Function: The safety aspect is very important in the utilization of medicinal plant in formal medication. Rosella was reported to have a therapeutic effect as antihyperlipidemic and clinically proved as antihypertension¹⁹. The utilization of rosella for therapeutic was increasing as immunomodulator⁹ for maintaining health status.

Renal is an organ with an important role in detoxication of some toxin. The glomerular filtration rate is important parameter to evaluate renal function in toxicant metabolism. Accurate measurement of glomerular filtration rate is time-consuming and expensive, but some filtered substances may be measured to estimate glomerular filtration rate through the clearance activity including blood urea and serum creatinine²⁰. Ureum level and glomerular filtration rate is the best estimate of a number of functioning nephrons and renal function.

The effect of rosella extract treatment on ureum level was performed on **Table 1**. The subchronic treatment of rosella extract increases the ureum level significantly ($p < 0.05$). The effect showed the dose-dependent. The observation of ureum level also was done at 14 days after treatment was stopped to observe the reversibility of biochemical changes. The result showed that after 14 days, the ureum level still at the high level as shown in **Table 2**.

The present research found that subchronic duration treatment of rosella increases the ureum level significantly. Observation of ureum level at 14 days after subchronic treatment was stopped also found that ureum level is still significantly higher than normal. The high level of ureum could be caused by increasing protein catabolism and renal damage. Renal damage could be followed by increasing of glomerular filtration and ureum level.

TABLE 1: THE EFFECT OF SUBCHRONIC TREATMENT OF ROSELLA EXTRACT ON UREUM LEVEL OF SPRAGUE DAWLEY RAT

Group	Ureum (mg/dl)
Normal	20,77 ± 1,99
50 mg/kg bw	40,71 ± 3,89*
100 mg/kg bw	42,79 ± 4,54*
200 mg/kg bw	62,02 ± 3,72*

The * significant difference with the normal group

TABLE 2: THE UREUM LEVEL OF ROSELLA EXTRACT TREATED RATS FOR SUBCHRONIC TREATMENT ON 14 DAYS AFTER TREATMENT (SATELLITE GROUP)

Groups	Doses (mg/kg bw)	Ureum level ± SD (mg/dl)
Normal	0	44.58 ± 3.23
Observing on day 36	200	62.02 ± 3.72
Observing on day 50	200	66.04 ± 2.39*

The * significant difference with the normal group

The effect of rosella extract on renal function also evaluate on creatinine level. The creatinine level of the rat after subchronic treatment of rosella extract was performed on **Table 3**. The creatinine level was significantly increased after 35 days of treatment. The creatinine level stayed at a high level after 14 days treatment was stopped as shown in **Table 4**. It could be caused by permanent changes after a long duration of rosella treatment. This could be due to the biochemical response, which may initially be adaptive, if the exposure of oxidant was sustained, it would lead to various biochemical changes or pathological disorders²¹.

TABLE 3: THE EFFECT OF SUBCHRONIC TREATMENT OF ROSELLA EXTRACT ON CREATININE LEVEL OF SPRAGUE DAWLEY RAT

Groups	Creatinine ± SD (mg/dl)
Normal	0.11 ± 0.00
50 mg/kg bw	0.31 ± 0.05*
100 mg/kg bw	0.28 ± 0.06*
200 mg/kg bw	0.50 ± 0.06*

The * significant difference with the normal group

TABLE 4: THE CREATININE LEVEL OF ROSELLA EXTRACT TREATED RATS FOR SUBCHRONIC TREATMENT ON 14 DAYS AFTER TREATMENT (SATELLITE GROUP)

Groups	Doses (mg/kg bw)	Creatinin level \pm SD (mg/dl)
Normal	0	0.36 \pm 0.00
Observing on day 36	200	0.50 \pm 0.06
Observing on day 50	200	0.64 \pm 0.10*

The observation of creatinine level also found that treatment of rosella extract for subchronic duration increase the creatinine level significantly. The creatinine level stayed at a high level after 14 days treatment was stopped. It could be caused by permanent changes after a long duration of rosella treatment. This could be due to the biochemical response, which may initially be adaptive, if the exposure of oxidant were sustained, it would lead to various biochemical changes or pathological disorders²¹.

The previous study on water extract of rosella also found that treatment on higher dose increased the ureum and creatinine level significantly^{22, 23, 13, 15}. This finding confirms the data of toxicity of rosella extract on renal function for long duration

treatment. The functional integrity of the renal is for body homeostasis. The kidney plays an important role in the excretion of metabolites including toxic metabolites. Kidney also plays a role in the regulation of intracellular fluid volume, electrolyte composition, and acid-base balance²². Exposure of toxic material to the kidney, therefore, could have a profound effect on total body metabolism²⁴.

Creatinine was the end product of high energy compound, creatine-phosphate. Creatinine has been reported to be a marker of renal function with elevated concentration of creatinine indicate the decreasing of glomerular filtration. The kidney damage contributed to the increases in the increasing level of urea and creatinine in serum

Histopathological Profile of Kidney: The histopathologic observation was carried out to confirm the toxic effect which is caused by rosella extract on biochemical parameters after subchronic duration treatment. The histopathologic profile of renal after HE staining was presented on **Fig. 1**.

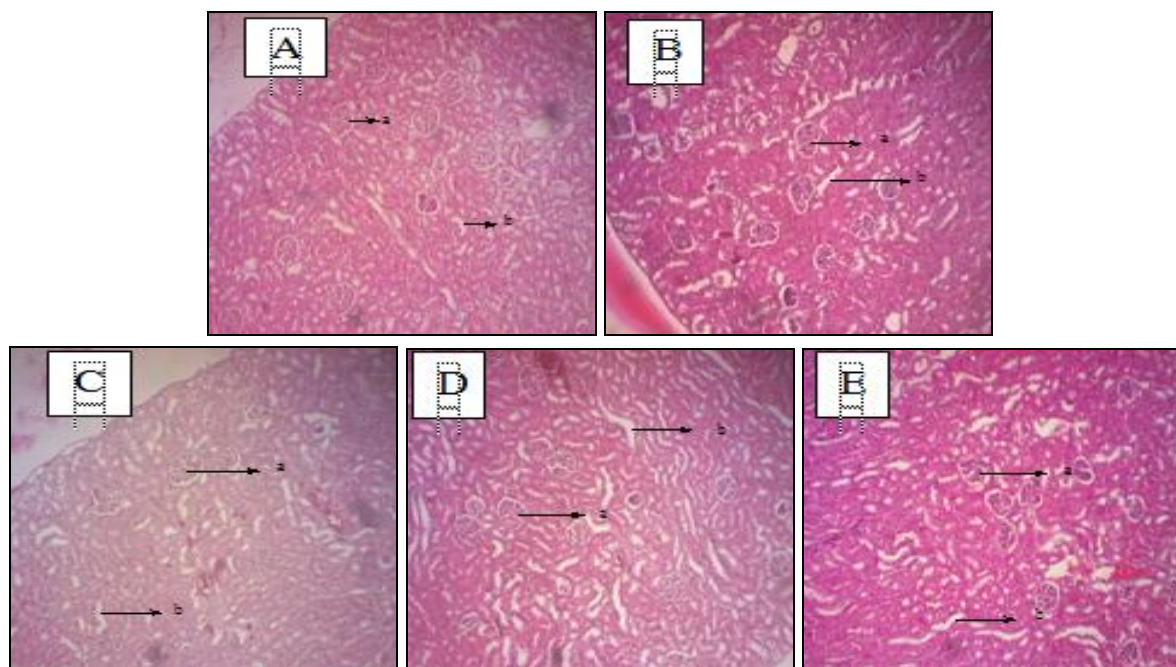


FIG. 1: HISTOPATHOLOGICAL PROFILE OF RAT KIDNEY WHICH SUBCHRONICALLY TREATED BY ROSELLA EXTRACT OF NORMAL GROUP (A) 50 mg/kg bw (B) 100 mg/kg bw (C) AND 200 mg/kg bw (D) AND SATELLITE GROUP OF 200 mg/kg bw (E) WITH HEMATOXYLIN AND EOSIN STAINING (HE) AND 100x MAGNIFICATION

Treatment of rosela extract did not show the significant pathological changes of the kidney as shown in **Fig. 1**. The histopathological of kidney

showed no pathological damage. This result recommended that increasing of ureum and creatinin level was not caused by the kidney

damage. The increasing level of ureum and creatinine could be caused by food intake. High protein intake could cause an increased level of ureum and creatinine. Although, this result showed no significant difference on histological of the kidney, using of rosella for a long duration, need special attention as previous research reported the kidney damage on higher dose²⁵. Treatment higher dose 200, 500, dan 800 mg/kg.bw of rosella extract for 21 days reported to cause the biochemical of renal shown by the increasing of ureum and creatinine and also the histopathological of the kidney.

CONCLUSION: Treatment of *Rosella calyx* ethanol extract with dose 50, 100 and 200 mg/kg BB for 35 days increase the ureum and creatinine level significantly. The histopathological observation of kidney was found normal. This result recommends that using of rosella ineffective dose did not damage the kidney. But higher dose using of rosella extract could cause the kidney damage.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest in this research.

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