

## Acute effects of oral administration of roselle extract on renal functions

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- Background** : *It has been reported that long-term drinking of roselle decoction could reduce bacterial infection and resulted in clearer urine in patients with urinary calculus and urethritis. In addition, infusion of roselle extract results in hypotensive effect in rats. However, there has been no study on acute effect of oral administration of roselle extract on renal functions.*
- Objective** : *To investigate the acute effects of the oral administration of roselle crude aqueous extract on renal functions.*
- Research design** : *Experimental design.*
- Materials and Methods** : *The rats were anesthetized and divided into two experimental groups (ROS t<sub>1</sub>, n=7; ROS t<sub>2</sub>; n=10) treated with the crude aqueous extract of roselle at a dose of 450 mg/kg body weight via a gavage tube, and two control groups (CON t<sub>1</sub>, n=6; CON t<sub>2</sub>, n=6) treated with distilled water. Inulin and PAH clearances were studied to evaluate glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. Renal functions including filtration fraction (FF), renal vascular resistance (RVR), urine flow rate (V), urinary excretion of electrolytes (U<sub>e</sub>V), fractional excretions of sodium (FE<sub>Na</sub>),*

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potassium ( $FE_K$ ), chloride ( $FE_{Cl}$ ), calcium ( $FE_{Ca}$ ), osmolarity clearance ( $C_{osm}$ ), free water clearance ( $C_{H_2O}$ ) were determined at the period of 0-30 and 30-60 minutes after administration of distilled water or roselle extract. Moreover, systemic circulation including systolic blood pressure (SP) diastolic blood pressure (DP), mean arterial pressure (MAP), pulse pressure (PP), heart rate (HR) and hematocrit (Hct) were also measured.

**Results** : The results showed that the crude aqueous extract of roselle could decrease ERPF significantly during the period of 0-30 minutes ( $1.18 \pm 0.42$  and  $2.04 \pm 0.67$  ml/min/gm-KW for ROS  $t_1$  and CON  $t_1$ ,  $p < 0.05$ ). The value of FF during the first 30 minutes was significantly increased in ROS  $t_1$  compared to CON  $t_1$  ( $45.13 \pm 10.26$  % and  $32.87 \pm 5.79$  % for ROS  $t_1$  and CON  $t_1$ , respectively).  $FE_{Na}$  was increased during the period of 0-30 minutes ( $1.14 \pm 0.24$  % of ROS  $t_1$  compared to  $0.53 \pm 0.15$  % of CON  $t_1$ ,  $p < 0.05$ ). There were no significant differences between ROS  $t_2$  and CON  $t_2$  on any measuring during the period of 30-60 minutes.

**Conclusion** : This study demonstrates that the crude aqueous extract of roselle has acute effects on renal function by decreasing effective renal plasma flow while the filtration fraction increases without a significant change in systemic circulation. Nevertheless, the increase in fractional excretion of sodium is observed. These effects are shown within 30 minutes after the administration of roselle extract.

**Keywords** : Oral administration, Roselle extract, Renal functions.

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- ที่มา** : มีรายงานว่า การให้ผู้ป่วยโรคนี้่วทางเดินปัสสาวะดื่มน้ำสกัดกระเจี๊ยบแดงสามารถลดการติดเชื้อแบคทีเรียในทางเดินปัสสาวะได้ และมีผลให้น้ำปัสสาวะใสขึ้น นอกจากนี้ การศึกษาในหนูทดลองโดยการฉีดสารสกัดกระเจี๊ยบแดงเข้าทางหลอดเลือดพบว่าสามารถลดความดันเลือดแดงได้ อย่างไรก็ตาม รายงานการศึกษาผลเฉียบพลันของน้ำสกัดกระเจี๊ยบแดงที่ให้ทางปากต่อการทำงานของไตยังมีน้อยมาก
- วัตถุประสงค์** : เพื่อศึกษาผลเฉียบพลันของน้ำสกัดกระเจี๊ยบแดงที่ให้ทางปากต่อการทำงานของไตในหนูขาว
- รูปแบบของการวิจัย** : การศึกษาเชิงการทดลอง
- วิธีการทดลอง** : ทำการศึกษาในหนูขาวพันธุ์ Wistar เพศผู้ ที่ทำให้สลบและให้น้ำสกัดกระเจี๊ยบแดงทางปากด้วยขนาด 450 มิลลิกรัมต่อกิโลกรัมของน้ำหนักตัว ทำการศึกษาผลต่อการทำงานของไตและระบบไหลเวียนเลือดหลังจากที่ให้น้ำสกัดกระเจี๊ยบแดงแล้ว 0-30 นาที และ 30-60 นาที ( $ROS t_1$ ,  $n=7$ ;  $ROS t_2$ ,  $n=10$ ) เปรียบเทียบกับกลุ่มที่ให้น้ำกลั่นในปริมาตรเท่ากัน ( $CON t_1$ ,  $n=6$ ;  $CON t_2$ ,  $n=6$  ตามลำดับ)
- ผลการศึกษา** : ผลต่อระบบการทำงานของไต พบว่า น้ำสกัดกระเจี๊ยบแดงทำให้อัตราการไหลของพลาสมาเข้าสู่ไต (มิลลิลิตรต่อนาทีต่อกรัมของน้ำหนักไต) ลดลงอย่างมีนัยสำคัญทางสถิติ ( $p < 0.05$ ) ในช่วงเวลา 0-30 นาที คือ  $1.18 \pm 0.42$  ในกลุ่ม  $ROS t_1$  เมื่อเปรียบเทียบกับ  $2.03 \pm 0.67$  ในกลุ่ม  $CON t_1$  แต่ค่าสัดส่วนการกรองต่ออัตราการไหลของพลาสมาเข้าสู่ไต (%) เพิ่มขึ้นในกลุ่ม  $ROS t_1$  ( $45.13 \pm 10.26$ ) เมื่อเปรียบเทียบกับกลุ่ม  $CON t_1$  ( $32.87 \pm 5.79$ ) ค่าของสัดส่วนการขับถ่ายโซเดียมต่ออัตราการกรอง (%) เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่ม  $ROS t_1$  ( $1.14 \pm 0.24$ ) เปรียบเทียบกับกลุ่ม  $CON t_1$  ( $0.53 \pm 0.15$ ) ค่าที่ศึกษาในช่วงเวลา 30-60 นาทีหลังจากที่ให้น้ำสกัดกระเจี๊ยบแดงแตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม
- สรุป** : น้ำสกัดกระเจี๊ยบแดงที่ให้ทางปากสามารถลดอัตราการไหลของพลาสมาเข้าสู่ไต ขณะที่มีการเพิ่มสัดส่วนการกรองของไตต่ออัตราการไหลของพลาสมาเข้าสู่ไต โดยไม่มีการเปลี่ยนแปลงของระบบไหลเวียนเลือดทั่วร่างกาย อย่างไรก็ตามพบว่าการเพิ่มขึ้นของสัดส่วนการขับถ่ายโซเดียมต่ออัตราการกรอง การเปลี่ยนแปลงนี้พบได้ภายใน 30 นาทีหลังจากให้น้ำสกัดกระเจี๊ยบแดง
- คำสำคัญ** : การให้สารทางปาก, น้ำสกัดกระเจี๊ยบแดง, การทำงานของไต

Roselle (*Hibiscus sabdariffa* Linn) is a kind of Thai traditional medicine, used as a diuretic agent.<sup>(1-4)</sup> Chronic effect of drinking roselle tea on diuresis has been reported.<sup>(5)</sup> The crude aqueous extract of the petal of roselle is used in patients with lower part of urethritis compared to patients who are treated with antibacterial drug. The results show that the extract of roselle could significantly reduce bacteria infection such as *E. Coli*.<sup>(6)</sup> Other studies have reported that the patients with calculus and urethritis who daily drank roselle tea had clearer urine and decreased urinary pH.<sup>(4,5,7,8)</sup> They suggested that the roselle extract might be used together with modern clinical treatment in order to prevent bacterial infection of urinary system and recurrence of calculi. In normal male human roselle extract decreases urinary salt excretion.<sup>(9)</sup> In addition, decoction of roselle could lower blood pressure.<sup>(10,11)</sup> An *in vitro* study has shown that roselle extract inhibits the tone of rabbit aortic strip.<sup>(12)</sup> Infusion of roselle calyx extract was found to lower significantly both systolic and diastolic pressure in spontaneously hypertensive and normotensive Wistar-Kyoto rats.<sup>(13)</sup> The urine output of the treated spontaneously hypertensive rats was significantly higher than normotensive ones. The hypotensive effect of roselle extract is not mediated through inhibition of the sympathetic nervous system but it could be mediated through acetylcholine-like and histamine-like mechanisms.<sup>(14)</sup>

However, the effects of an oral administration of roselle extract on renal functions and general circulation have not been reported. Therefore, this present study was designed to investigate the acute effects of the oral administration of roselle crude aqueous extract on renal functions and hemodynamics

including glomerular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF), renal vascular resistance (RVR), urine flow rate (V), urinary excretion of electrolytes ( $U_e V$ ), fractional excretions of sodium ( $FE_{Na}$ ), potassium ( $FE_K$ ), chloride ( $FE_{Cl}$ ) calcium ( $FE_{Ca}$ ), osmolarity clearance ( $C_{osm}$ ) and free water clearance ( $C_{H_2O}$ ) in rats. In addition, its effects on general circulation including systolic blood pressure (SP) diastolic blood pressure (DP), mean arterial pressure (MAP), pulse pressure (PP), heart rate (HR) and hematocrit (Hct) were also measured.

## Materials and Methods

### Preparation of a crude aqueous extract of roselle

Dried roselle was purchased from the Marketing Organization for Farmers in Bangkok. An amount of 10 gram of dried roselle was washed with tap water, rinsed with distilled water, and then heated in 500 milliliters of distilled water with the temperature between 80°- 90° C for 15 minutes. After that the dreg of roselle was separated, and the aqueous extract was heated at 65°- 70° C until the total volume was 100 milliliters to yield the final concentration of 0.1 g/ml. The chemical properties of the roselle extract were analyzed for the concentrations of  $Na^+$ ,  $K^+$ ,  $Ca^+$ ,  $Cl^-$ . Osmolarity, pH, and the specific gravity of the extract were also determined. The extract was divided in aliquots and kept at -20° C. Each aliquot was thawed at room temperature when needed.

### Animal preparation

The experiment was carried out in 29 male Wistar rats weighing 280-350 gm from The National Laboratory Animal Center, Mahidol University, randomly divided in two control groups and two

experimental groups. The control and experimental groups were divided into two periods of 0-30 minutes (CON  $t_1$  and ROS  $t_1$ ) and 30-60 minutes (CON  $t_2$  and ROS  $t_2$ ) after the feeding of distilled water or the crude aqueous extract of roselle. Each rat was fasted overnight preceding an operation, but given water *ad libitum*.

### General procedure

Each rat was anesthetized by intraperitoneal injection of 45 mg per kg body weight of pentobarbital sodium (Nembutal Abbott, FE Zuellig Bangkok Ltd.). A tracheostomy was done to facilitate respiration and removal of excess secretion. The animals were allowed to ventilate spontaneously in room air. The left femoral vein was cannulated for infusion of solutions. The right common carotid artery was cannulated for continuous recording of arterial blood pressure and blood sample collection.

A midline abdominal incision was done. The urinary bladder was exposed and inserted with polyethylene tube for collection of urine. Normal saline solution was infused at the rate of 10 ml/kg body weight/hour through the left femoral vein during the surgery for normal hydration. After completion of the operation, normal saline solution containing 1gm/dl of inulin and 0.2 gm/dl of PAH was administered intravenously via the left femoral vein at the same rate.<sup>(15)</sup> A period of 45 minutes was allowed to stabilize plasma inulin and PAH concentration at approximately 20-40 mg/dl and 2-4 mg/dl, respectively.

### Experimental procedure

The rats were divided in 4 groups. After the stabilization period, the control rats (CON  $t_1$ , n=6;

CON  $t_2$ , n=6) were administrated with distilled water at the amount of 4.5 ml/kg body weight via a gavage tube while the experimental rats (ROS  $t_1$ , n=7; ROS  $t_2$ , n=10) were treated with the crude aqueous extract of roselle at the dose of 450 mg/kg body weight. Inulin and PAH clearances of all groups were studied to evaluate GFR and ERPF, respectively. Plasma and urine samples were analyzed for inulin concentration by an anthrone colorimetric technique and for PAH concentration by the method of Bratton and Marshall as modified by Smith, 1962<sup>(16)</sup> using spectrophotometer (model BTS 320, BioSystems). Arterial blood pressure was recorded using a blood pressure transducer (model MLT 1050, MacLab System, ADInstruments) and a polygraph (ML 740 MacLab/4s, MacLab System, ADInstruments). Hematocrit was determined by microhematocrit centrifuge (model Z230H, BHG HERMLE) and measured by micro-capillary reader (I.E.C. Cat. No. 2201, DAMON/IEC DIVISION). Other parameters were derived by calculation as follows :

$$\begin{aligned} FF &= GFR/ERPF \\ RVR &= MAP/RBF \\ FE &= U_e V/GFR \\ C_{osm} &= U_{osm} V/P_{osm} \\ C_{H_2O} &= V-C_{osm} \end{aligned}$$

### Statistics

The experimental data were presented as mean  $\pm$  SD. Statistical comparison of the parameters between CON  $t_1$  and ROS  $t_1$ , and between CON  $t_2$  and ROS  $t_2$  were analyzed by using Student's unpaired t-test. The significance level was determined at  $p < 0.05$ .

## Results

The compositions and chemical properties of the crude aqueous extract of roselle.

**Table 1.** The compositions and chemical properties of the crude aqueous extract of roselle.

Compositions and chemical properties	Conantrations and property values
Na+	1.00 mEq/l
K+	32.05 mEq/l
Cl-	11.5 mEq/l
Ca++	3.45 mg/ml
pH	2.68
Osmolarity	210 mOsm/l
Specific gravity	1.0067

Table 1 shows the compositions and selected chemical properties of the crude aqueous extract of roselle, which the concentration of the extract was prepared by dried roselle 10 gram in 100 ml of distilled water as the procedure mentioned in the method section.

## Effect on renal functions

In Table 2. the crude aqueous extract of roselle demonstrates a non-significant decrease of GFR in thirty minutes after the administration ( $0.47 \pm 0.13$  in ROS  $t_1$ ) comparing with the control group ( $0.67 \pm 0.24$  in CON  $t_1$ ). A significant decrease in ERPF is shown in ROS  $t_1$  compared with CON  $t_1$  ( $1.18 \pm 0.42$  vs  $2.04 \pm 0.67$  ml/min/gm-kw;  $p < 0.05$ ) (Figure 1), meanwhile RVR in ROS  $t_1$  was slightly increased ( $47.34 \pm 16.83$  vs  $30.26 \pm 10.88$  mmHg/ml/min/gm-kw) but the change was not statistically significant. Interestingly, filtration fraction was increased in ROS  $t_1$  at  $p \leq 0.05$  (Figure 2). The result shows that there was no significant change of the parameters in ROS  $t_2$  comparing with CON  $t_2$ .

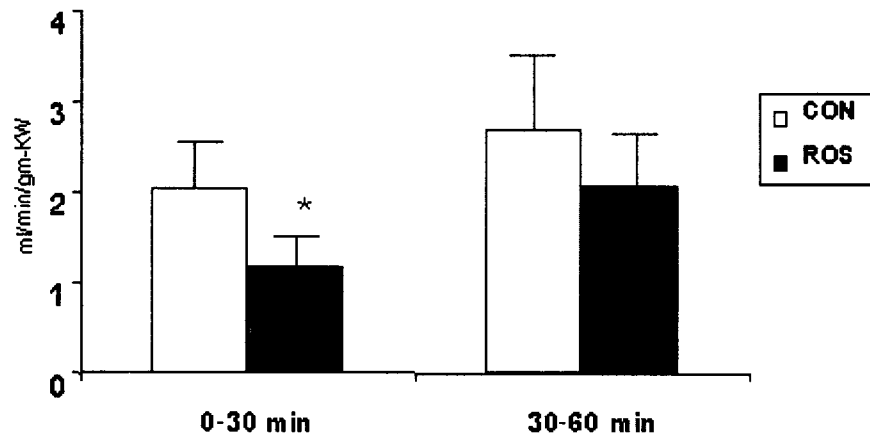
In Table 3. there is no significant alteration of the values of urinary flow rate, urinary excretion rate of the electrolytes, osmolarity clearance and free water clearance in ROS  $t_1$  comparing with CON  $t_1$  and ROS  $t_2$  comparing with CON  $t_2$ .

In Table 4. and Figure 3. the results show that  $FE_{Na}$  was significantly increased in only ROS  $t_1$  compared with CON  $t_1$  ( $1.14 \pm 0.24$  vs  $0.53 \pm 0.15$  %;  $p < 0.05$ ). No significant change in fractional excretion of other electrolytes of both periods was observed.

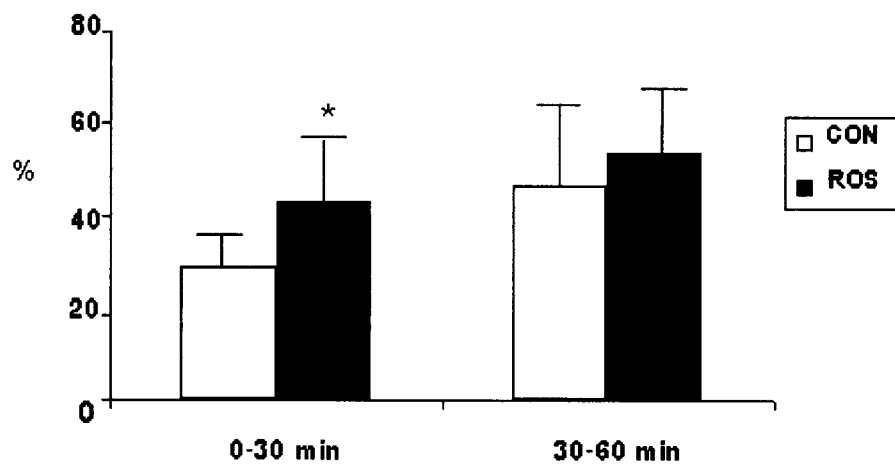
**Table 2.** Renal hemodynamics in rats after feeding crude aqueous extract of roselle 0-30 minutes and 30-60 minutes in experimental groups compared with the control ones.

	CON $t_1$	ROS $t_1$	CON $t_2$	ROS $t_2$
GFR (ml/min/gm-kw)	$0.67 \pm 0.24$	$0.47 \pm 0.13$	$1.29 \pm 0.80$	$1.13 \pm 0.53$
ERPF (ml/min/gm-kw)	$2.04 \pm 0.67$	$1.18 \pm 0.42$ *	$2.70 \pm 1.00$	$2.03 \pm 0.65$
FF (%)	$32.87 \pm 5.79$	$45.13 \pm 10.26$ *	$47.80 \pm 17.30$	$54.93 \pm 11.98$
RVR(mmHg/ml/min/gm-kw)	$30.26 \pm 10.88$	$47.34 \pm 16.83$	$23.67 \pm 6.85$	$28.71 \pm 7.98$

(Mean  $\pm$  SD). \*  $p < 0.05$  glomerular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF), renal vascular resistance (RVR)



**Figure 1.** Mean  $\pm$  SD of effective renal plasma flow in ROS  $t_1$  and ROS  $t_2$  compared with CON  $t_1$  and CON  $t_2$ , respectively. \*  $p < 0.05$



**Figure 2.** Mean  $\pm$  SD of filtration fraction of ROS  $t_1$  and ROS  $t_2$  compared with CON  $t_1$  and CON  $t_2$ , respectively. \*  $p < 0.05$

**Table 3.** Effects of the crude aqueous extract of roselle on urinary flow rate, urinary excretion rate of the electrolytes, osmolarity clearance and free water clearance during 0-30 minutes and 30-60 minutes after the feeding comparing with the control groups.

	CON t <sub>1</sub>	ROS t <sub>1</sub>	CON t <sub>2</sub>	ROS t <sub>2</sub>
V (μl/min/gm-kw)	5.68 ± 2.79	5.66 ± 2.23	10.11 ± 4.73	8.65 ± 2.92
U <sub>Na</sub> V (μEq/min/gm-kw)	0.47 ± 0.22	0.84 ± 0.29	1.26 ± 0.65	1.55 ± 0.62
U <sub>K</sub> V (μEq/min/gm-kw)	0.89 ± 0.24	0.75 ± 0.15	1.11 ± 0.31	0.96 ± 0.18
U <sub>Cl</sub> V (μEq /min/gm-kw)	1.07 ± 0.90	0.93 ± 0.60	2.08 ± 1.24	1.53 ± 0.82
U <sub>Ca</sub> V (μg/min/gm-kw)	0.81 ± 0.19	0.68 ± 0.20	0.86 ± 0.59	0.96 ± 0.89
C <sub>osm</sub> (μl/min/gm-kw)	20.93 ± 11.37	28.1 ± 9.58	36.45 ± 13.81	34.40 ± 11.03
C <sub>H<sub>2</sub>O</sub> (μl/min/gm-kw)	-15.05 ± 8.22	-21.69 ± 7.21	-26.34 ± 11.22	-23.50 ± 8.67

(Mean ± SD) urine flow rate (V), urinary excretion of electrolytes (U<sub>e</sub> V), osmolarity clearance (C<sub>osm</sub>), free water clearance (C<sub>H<sub>2</sub>O</sub>)

**Table 4.** Effects of the crude aqueous extract of roselle on fractional excretion rate of electrolytes during 0-30 minutes and 30-60 minutes after the feeding compared with the control groups.

	CON t <sub>1</sub>	ROS t <sub>1</sub>	CON t <sub>2</sub>	ROS t <sub>2</sub>
FE <sub>Na</sub> (%)	0.53 ± 0.15	1.14 ± 0.24*	0.68 ± 0.48	1.16 ± 0.54
FE <sub>K</sub> (%)	38.44 ± 2.87	48.53 ± 13.84	20.88 ± 5.79	28.41 ± 13.81
FE <sub>Cl</sub> (%)	1.26.71 ± 0.73	1.45 ± 1.03	1.17 ± 0.96	1.31 ± 0.83
FE <sub>Ca</sub> (%)	2.70 ± 0.66	3.23 ± 0.81	2.32 ± 2.87	1.86 ± 1.80

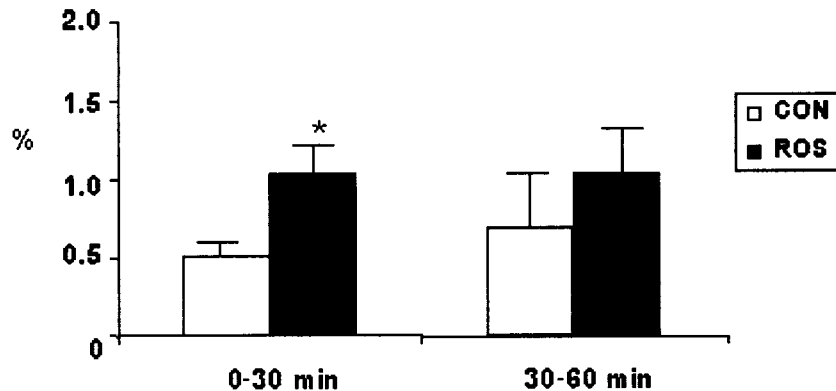
(Mean ± SD). \*p<0.05 fractional excretions of sodium (FE<sub>Na</sub>), potassium (FE<sub>K</sub>), chloride (FE<sub>Cl</sub>) calcium (FE<sub>Ca</sub>)

**Table 5.** Effects of the crude aqueous extract of roselle on systemic circulation after feeding 0-30 minutes and 30-60 minutes compared with the control groups.

	CON t <sub>1</sub>	ROS t <sub>1</sub>	CON t <sub>2</sub>	ROS t <sub>2</sub>
SP (mmHg)	134.70 ± 28.23	130.26 ± 15.88	125.06 ± 7.38	123.68 ± 11.62
DP (mmHg)	106.04 ± 17.57	102.62 ± 12.15	94.88 ± 5.84	97.54 ± 12.87
MAP (mmHg)	110.45 ± 19.67	111.75 ± 13.22	103.15 ± 7.33	103.54 ± 13.23
PP (mmHg)	24.98 ± 12.50	27.64 ± 5.95	25.97 ± 9.68	26.73 ± 7.74
HR (beat/min)	395.61 ± 52.01	386.00 ± 19.01	390.41 ± 36.11	352.07 ± 68.45
Hct (%)	43.10 ± 3.13	40.00 ± 2.24	40.89 ± 2.87	40.84 ± 3.46

(Mean ± SD) systolic blood pressure (SP) diastolic blood pressure (DP), mean arterial pressure (MAP), pulse pressure (PP), heart rate (HR) and hematocrit (Hct)





**Figure 3.** Mean  $\pm$  SD of fractional excretion of sodium in ROS  $t_1$  and ROS  $t_2$  compared with CON  $t_1$  and CON  $t_2$ , respectively. \*  $p < 0.01$

### Effects on general circulation

The feeding of the aqueous extract of roselle did not significantly alter SP, DP, MAP, PP, HR, and Hct of ROS-groups comparing with those of CON-groups as shown in Table 5.

### Discussion

In this study, the concentrated crude aqueous extract of roselle 100 mg/ml which was administrated via a gavage tube to the rats at dose 450 mg/kg of BW could significantly decrease ERPF ( $p < 0.01$ ) during 0-30 minutes after the delivery without an alteration of arterial blood pressure (Table 2 and Table 6). The decrease in ERPF is possibly due to the increase in RVR, although it was non-significant. This result seems that roselle has the vasoconstriction effect on renal arterioles, causing the tendency of the decrease in GFR in ROS  $t_1$ <sup>(17)</sup>, however it has no effect on systemic circulation. Hypotensive effect was not observed by the oral administration of the roselle extract in this study, which was different from the

study of intravenous administration of roselle extract to the rats.<sup>(10,11)</sup> Filtration fraction was significantly increased during the period of 0-30 minutes. It showed that the roselle extract was able to increase the glomerular filtration respected to the renal plasma flow. Interestingly, the natriuretic effect, which may simultaneously lead to diuresis, was observed, and might be revealed unless GFR decreased. The results from Table 3 did not show any effects on urinary flow rate. It disagrees with some previous studies reported by Leclerc, 1938 and Muangmun, et al., 1982.<sup>(6)</sup> However, from Table 4, the significant increase in the excretion of sodium respected to GFR ( $FE_{Na}$ ) implies that roselle has the effect on renal tubular function.<sup>(18)</sup> The roselle extract could possibly decrease sodium reabsorption in renal tubule. This effect on the increase in fractional excretion of sodium might be applied by chronic administration of roselle extract on patients with hypertension. However, the mechanism of the effect of roselle extract on tubular functions needs further investigations.

## Conclusion

This study demonstrates that the crude aqueous extract of roselle has an acute effect on renal function by decreasing effective renal plasma flow while the filtration fraction was increased without a significant change in systemic circulation. Nevertheless, the increase in fractional excretion of sodium was observed. These effects were shown within 30 minutes after the administration of roselle extract. However, the mechanisms of the effects have not been elucidated, and therefore need further investigations.

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