ORIGINAL ARTICLE

Comparison of Diuretic and Electrolyte Excretory Activity between Compounds of Okra Fruit (*Abelmoschus esculentus*) and Furosemide

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ABSTRACT

Introduction: Hypertension takes place of being one of the most concerning health issues, leading to increasing rate of death due to its complications. Pharmacological therapy diuretic become one to intervene on hypertension's pathological sequences through sodium and potassium regulation. Similar diuretic effect can be achieved through fruits and vegetables crop including Okra fruit (*Abelmoschus esculentus*). Objective: to investigate the diuretic variance of different part of Okra fruit's (*Abelmoschus esculentus*). **Methods:** In total of 8 groups of male Rattus norvegicus rats are sorted into control and intervention groups. As control, the C- Group (negative control group) was given Na- CMC 0.5% suspension, and C+ Group (positive control group) was given furosemide. The experimental groups P1, P2, and P3 were given Okra (*Abelmoschus esculentus*) whole fruit ethanolic extract; P4, P5, and P6 Group are were given Okra (*Abelmoschus esculentus*) seedless fruit ethanolic extract within range concentration of 75-300 mg/kg body weight. Urine volume is measured every hour for 6 hours and the 24th hour. Sodium and potassium measurements are executed towards 24-hour urine accumulation. **Results:** Single dose administration of Okra (*Abelmoschus esculentus*) fruit significantly increase 5th hour urine volume as compared to furosemide ($p < 0.001$). Whole fruit of Okra (*Abelmoschus esculentus*) compared to seedless fruit exhibit no significant increment of urine volume and electrolytes ($p > 0.05$). **Conclusion:** Ethanolic extract of Okra (*Abelmoschus esculentus*) fruit produce similar diuretic effect to furosemide. However, seedless Okra (*Abelmoschus esculentus*) fruit exerts a higher kaliuretic effect compared to whole fruit and furosemide.
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Keywords: Diuretic, Okra (*Abelmoschus esculentus*), sodium, potassium

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INTRODUCTION

Hypertension is becoming the world's leading health issue, which is responsible for 10.4 million deaths per year globally due to its renovascular, cardiovascular, and central nervous system complications (1,2). In Indonesia according to Riskesdas (Riset Kesehatan Dasar) 2018 which is a community-based national wide health research by National Institute of Health Research and Development, an increasing hypertension prevalence from 25.8% to 36.32% was detected in individuals aged ≥ 18 conforming to blood pressure measurement (3) Diuretic as pharmacological therapy of hypertension is currently being an intervention agent of primary hypertension along with lifestyle modification through fruits and vegetables diet (1,4). Okra (*Abelmoschus esculentus*) is one of many plants which can grow and cultivate annually in tropical and subtropical climates with warm temperature such as Indonesia (5). Okra (*Abelmoschus esculentus*) fruit has traditionally been utilized as folk medicinal plant due to its high mineral and phytoconstituent content such as polyphenol, quercetin, glycosides, saponin, tannin, catechin, coumarine, phytosterols, and flavonol (5–7). Numerous novel studies had validated Okra (*Abelmoschus esculentus*) as having antidiabetic, antioxidant, immunomodulatory, and microbicidal potential due to its diverse bioactive constiuents mechanism (7–10). Oligomeric proanthocyanidins for example exerts inhibitory action towards α-glucosidase and α-amilase and thus inducing hypoglycemic effect. Polyphenol contained in Okra (*Abelmoschus esculentus*) which increases gluthatione peroxidase acts as antioxidant (10). Various plants with similar phytoconstituents as Okra (*Abelmoschus esculentus*) have also been reported to exert diuretic activity through the increase of urine volume and electrolytes (11–13) yet no further study was made. This phenomenon will eventually lead to the decrease of intravascular volume and cardiac output

(14,15). Maramag reported no significant result in urine volume between Okra (*Abelmoschus esculentus*) fruit and furosemide (16). Thus Okra (*Abelmoschus esculentus*) fruit produce similar diuretic effect to furosemide. The aim of this study is to study diuretic variance of different parts of Okra's fruit (*Abelmoschus esculentus*) which are the whole and seedless part of Okra fruit compared to furosemide by observing urine output, sodium and potassium as a renewal to previous studies.

MATERIALS AND METHODS

Research design

This research was an *in vivo* experimental study with post- test only control group design in which data measurement and collection were done after intervention.

Plant material

Okra (*Abelmoschus esculentus*) whole and seedless fruit which had been processed into coarse powder and authenticated by UPT Materia Medica Batu was used in this research. The powder was then processed into ethanolic extract of Okra (*Abelmoschus esculentus*) which was done in Pharmacology Laboratory, Faculty of Veterinary Medicine, Airlangga University Surabaya.

Preparation of *Abelmoschus esculentus* ethanolic extract Coarse powder received from UPT Materia Medica Batu was repeatedly macerated. Maceration process with 95% ethanol was done at room temperature and 1 atm. The mixture of ethanol and Okra (*Abelmoschus esculentus*) coarse powder was then left for 48 hours. Stirring the mixture periodically was necessary to achieve full extraction. Filtrated mixture after 48 hours was put in water bath at 40°C. Crude ethanol extract was stored at 10-15°C before administration.

Experimental animal and housing

Thirty-two male rats (Rattus norvegicus), aged 8-12 weeks, weighing about 150- 250 grams, originated from external farming company were chosen for this study. The rats were allowed to acclimate for 7 days and given free access to 50 grams of standard rodent diet and water ad libitum prior to the day of experiment. Acclimation process was necessary in order to adjust the rats into the new environment in standardized pharmacological laboratory of Faculty of Veterinary Medicine, Airlangga University, Surabaya. Each rats were housed in single standard metabolic cages in the size of 25 cm^2 and maintained under standard room temperature of 25- 26 °C with each 12 hour period of day and night. The rats were kept away from noise to avoid physiological stress which would affect diuresis.

Grouping and intervention

All rats were assigned into 8 groups. Two control groups were given Na- CMC 0.5% (C-) and furosemide (C+) of 10 mg/kg.BW. P1- P3 group were given ethanolic extract of whole Okra fruit (*Abelmoschus esculentus*) whereas P4- P6 group were given ethanolic extract of seedless Okra fruit (*Abelmoschus esculentus*) within a dosage range of 75 to 300 mg/kg.BW.

The intervention towards Rattus norvegicus was begun with rehydration followed by oral administration of Na-CMC 0,5%, furosemide, and ethanolic extract of Okra (*Abelmoschus esculentus*) an hour later. Each rat was placed in one metabolic cage with restricted access to food and water during this period. Observation of urine volume was done every 1st, 2nd, 3rd, 4th, 5th, 6th, and 24th hour after oral administration of ethanolic extract of Okra (*Abelmoschus esculentus*). After the 24th hour urine collection was tested for sodium and potassium level using ion selective electrode (ISE) method (17).

Statistical analysis

Experimental data were presented as mean ± SD. One-way ANOVA and Tukey HSD Post Hoc test was used to compare variables in between groups.

Ethical clearance

This study was approved by Widya Mandala Surabaya's Health Research Ethics Committee Ref no 160/WM12/KEPK/MHSW/T/2021.

RESULTS

Ethanolic extract of compounds of *Abelmoschus esculentus* significantly increased excretion level of 5th hour urine volume ($p < 0.001$) as shown in Table I yet no significant increment were found in and electrolyte excretion level ($p > 0.05$). Tukey HSD analysis showed no significant difference in urine output and electrolytes between furosemide and *Abelmoschus esculentus* ethanolic extract receiving animals, which shows similar diuretic effect. No significant differences were also found in urine volume and electrolytes excretion between whole fruit and seedless *Abelmoschus esculentus*.

As seen in Table I, 24 hour urine volume in ethanolic extract of whole *Abelmoschus esculentus* within a dosage of 150 mg/kg.BW (P2) group exhibited higher result than positive control group (C+). In terms of urine sodium excretion, Table II shows ethanolic extract of seedless *Abelmoschus esculentus* within a dosage of 300 mg/kg.BW (P6) group also showed higher result than positive control group (C+). Most *Abelmoschus esculentus* ethanolic extract of both whole and seedless groups exhibit higher potassium excretion level than furosemide as shown in Table I. Hence, the increase was seen highest in animals that received *Abelmoschus esculentus* ethanolic extract.

Fig. 1 does not show dose- dependent increment of 24- hour urine volume exerted by *Abelmoschus esculentus* ethanolic extract yet Fig.2 show increment

Table I: Effect of *Abelmoschus esculentus* Ethanolic extract on Urine Output and Electrolytes

		C-	C+	P1	P2	P3	P4	P5	P6 *
Urine Volume	1st	3.85 ± 1.33	3.84 ± 1.84	4.63 ± 1.50	4.81 ± 2.92	3.51 ± 1.53	4.40 ± 0	3.22 ± 0.37	2.56 ± 0.44
	2nd	0.98 ± 1.01	1.45 ± 1.59	0.30 ± 0.51	0.58 ± 1.01	0.33 ± 0.49	-	-	-
	3rd	0.21 ± 0.24	0.83 ± 0.96	-	0.54 ± 0.69	-	-	-	-
	4th	-	0.25 ± 0.36	0.23 ± 0.40	-	-	-	-	-
	5th	0.02 ± 0.05*	0.14 ± 0.27*	0.05 ± 0.08*	-	-	1.76 ± 0*	0.20 ± 0.28*	0.16 ± 0.28*
	6th	0.12 ± 0.25	-	-	-	-	-	0.22 ± 0.31	0.16 ± 0.28
	24th	1.23 ± 0.84	0.68 ± 0.74	1.00 ± 0.60	1.48 ± 0.44	1.21 ± 0.88	0.55 ± 0	1.59 ± 1.49	1.28 ± 0.79
	Total	6.43 ± 2.82	6.92 ± 2.89	6.22 ± 1.31	7.47 ± 2.56	5.1 ± 0.87	6.71 ±0	5.24 ± 1.99	4.03 ± 1.52
	Sodium	19.40 ± 10.25	25.79 ± 13.16	24.28 ± 13.55	21.76 ± 8.10	20.44 ± 8.44	1.28 ± 0	20.43 ± 3.83	27.18 ± 19.73
	Potassium	39.77 ± 10.03	38.24 ± 11.97	37.18 ± 14.57	39.87 ± 13.15	50.04 ± 16.21	29.20 ± 0	60.34 ± 14.14	54.28 ± 23.05

Notes: Values represent mean ± standard deviation
* $p < 0.001$ is considered significant compared to control group
* C- = Na- CMC 0.5% ;
C+ = Furosemide ;
P1 = Whole *Abelmoschus esculentus* Fruit 75 mg/kg.BW ;
P2 = Whole *Abelmoschus esculentus* Fruit 150 mg/kg.BW ;
P3 = Whole *Abelmoschus esculentus* Fruit 300 mg/kg.BW ;
P4 = Seedless *Abelmoschus esculentus* Fruit 75 mg/kg.BW ;
P5 = Seedless *Abelmoschus esculentus* Fruit 150 mg/kg.BW
P6 = Seedless *Abelmoschus esculentus* Fruit 300 mg/kg.BW

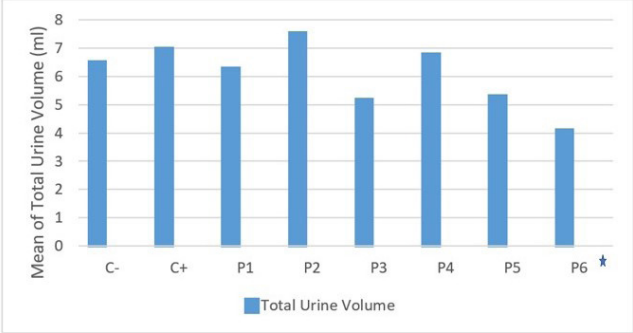

Figure 1: Urine volume after oral adminstration of *Abelmoschus esculentus* ethanol extract
* C- = Na- CMC 0.5% ; C+ = Furosemide ; P1 = Whole *Abelmoschus esculentus* Fruit 75 mg/kg.BW ; P2 = Whole *Abelmoschus esculentus* Fruit 150 mg/kg.BW ; P3 = Whole *Abelmoschus esculentus* Fruit 300 mg/kg.BW ; P4 = Seedless *Abelmoschus esculentus* Fruit 75 mg/kg.BW ; P5 = Seedless *Abelmoschus esculentus* Fruit 150 mg/kg.BW; P6 = Seedless *Abelmoschus esculentus* Fruit 300 mg/kg.BW

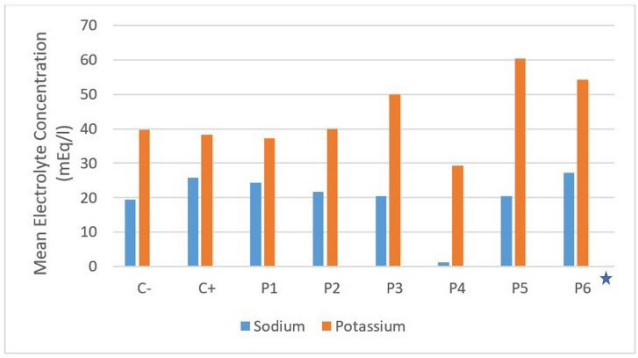

Figure 2: Urine electrolytes after oral adminstration of *Abelmoschus esculentus* ethanol extract
* C- = Na- CMC 0.5% ; C+ = Furosemide ; P1 = Whole *Abelmoschus esculentus* Fruit 75 mg/kg.BW ; P2 = Whole *Abelmoschus esculentus* Fruit 150 mg/kg.BW ; P3 = Whole *Abelmoschus esculentus* Fruit 300 mg/kg.BW ; P4 = Seedless *Abelmoschus esculentus* Fruit 75 mg/kg.BW ; P5 = Seedless *Abelmoschus esculentus* Fruit 150 mg/kg.BW; P6 = Seedless *Abelmoschus esculentus* Fruit 300 mg/kg.BW

of urine electrolytes as doses were increased. Fig. 2 also show increasing potassium urine excretion of whole *Abelmoschus esculentus* (P1- P3) in a dose dependent manner.

DISCUSSION

Phytonutrients found in herbs and natural plants continue to gain popularity and public interest as an alternative to modern medicine in both developed and developing countries (18). Despite the decent amount of promising health benefits of *Abelmoschus esculentus* which makes *Abelmoschus esculentus* oftentimes used as folk medicine, (5) traditional claims are not yet supported by scientific evidence. In this study, ethanolic extract of *Abelmoschus esculentus* showed significant difference in 5th hour urine volume contrary to previous report by Maramag which exhibited no significant difference between furosemide and aqueous extract of *Abelmoschus esculentus* fruit in urine volume increment (16). Results might differ due to the choice of extraction solvent and method. This study used ethanol, a hydroalcholic, to extract *Abelmoschus esculentus*' phytoconstituents. Ethanol is known as a good solvent for flavonoids and polyphenol. Furthermore, ethanol is considered as a less toxic solvent to assess physiological system (19). In this study, the first hour of urine excretion in which *Abelmoschus esculentus* inflicts its effect is similar to furosemide's onset of action yet *Abelmoschus esculentus* exhibited a duration of action of 6 hours according to this study which is slightly longer than furosemide's duration of action of 2-3 hours (14). Ethanolic extract of whole *Abelmoschus esculentus* fruit showed a statistically non-significant 2nd and 3rd hour urine excretion although mechanism is yet unknown due to limitation of data regarding each phytoconstituents' diuretic duration of action.

Although the primary mechanism of diuretic effect induced by phytoconstituents has not yet been reported,

alkaloid and tannin, which are present in ethanolic extract of *Abelmoschus esculentus*, had been previously suggested to be responsible for the diuretic activity of aqueous extract of leaves of Vepris heterophylla (20,21). Diuretic mechanism exerted by phytoconstituents isn't fully known but several hypotheses in previous study stated tubular reabsorption of water and anions inhibitory mechanism (22). Linoleic acid which enhances diuretic effect of found in *Abelmoschus esculentus* seed, thus might explain the higher urine output in *Abelmoschus esculentus* whole fruit compared to seedless fruit (6,23).

In this study, there was no statistically significant difference in excretion of urinary electrolytes by ethanolic extract of *Abelmoschus esculentus* compared to furosemide as a natriuretic and kaliuretic agent. In addition, higher urinary potassium excretion was demonstrated by ethanolic extract of *Abelmoschus esculentus*, compared to furosemide. However, this failed to reach statistical significance. Quercetin and fenol, which affect potassium urine excretion was originally found in skin and seed components of *Abelmoschus esculentus* (24). Quercetin and its derivatives have binding sites on the NKCC1 co-transporter in basolateral membrane of secretory epithelial cells at the level of thick ascending limb in the kidney (25). Contrary to furosemide which binds to NKCC2 co-transporter, NKCC1 co-transporters are more inclined to enhance potassium secretion (15,25). Therefore, it may be possible that treatment of hyperkalemic events due to potassium supplementations and acute tissue or cells breakdown (e.g rhabdomyolisis and hemolysis) (26) may utilize *Abelmoschus esculentus* as kaliuretic agent. Upcoming studies should investigate the difference in kaliuretic effects of compounds of *Abelmoschus esculentus*.

CONCLUSION

This study showed no statistically significant difference in diuretic and electrolyte excretory activities between ethanolic extract of Okra (*Abelmoschus esculentus*) and furosemide. Okra (*Abelmoschus esculentus*) exerted similar diuretic effect to furosemide in terms of onset of action, urine volume and potassium excretion. Moreover, administration along with other diuretics needs electrolytes monitoring.

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