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Original Research Article

Effects of Acute Administration of Aqueous Extract From Sepals of *Hibiscus Sabdariffa* on Renal Excretion of Water In Albino Rats

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ABSRTACT

The effect of acute administration of extract of *Hibiscus sabdariffa* on renal excretion of water has been investigated. Sprague Dawley rats of either sex weighing between 150 - 250 g were anesthetized with a mixture of 25 % urethane and 1 s% alphachloralose, and their femoral vein was cannulated intravenous administration of drugs, the bladder was annulated for timely collection of urine. Acute intravenous administration of the extract of *Hibiscus sabdariffa* (5 mg/kg/hr) increased urine output ($p<0.05$), glomerular filtration rate ($p<0.001$), and renal excretion of $Na^+$and K^+. On the other hand, acute oral administration of extract of *Hibiscus sabdariffa* (5mg/kg/hr) had little effect on urine output, glomerular filtration rate, and renal excretion of Na^+ and k^+. Indomethacin, a prostaglandin synthesis inhibitor, had no alterations on the impact of *Hibiscussabdariffa*on renal excretion of water. L- N^G-Nitro argenine methyl ester (L-NAME) did not alter the ability of *Hibiscus sabdariffa* to promote renal excretion of water. This suggests that the action of *Hibiscus sabdariffa* does not involve the synthesis of prostaglandins, nor is it mediated through the stimulation of endothelium-dependent relaxation factors.

Keywords: Urine output, Glomerular filtration rate, renal excretion, Creatinine clearance, *Hibiscus sabdariffa.*

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Introduction

Plants have served as valuable lead compounds for developing many drugs and chemicals available in the market today. Their medicinal use has been based on experience and superstitions. [10]

Hibiscus sabdariffa, a member of the Malvaceae family, commonly called Roselle or Zoboin Northern Nigeria, is a specie of flowering plant in the genus Hibiscus that is native to Africa. The erect plant, usually up to 3 m tall with brilliant red flowers that become succulent with unique flavor used to make jams and beverages, has interesting medicinal uses. The leaves, sepals, and flowers are rich sources of vitamin C and are used traditionally to treat cough, wounds, and high blood pressure. [9] The mechanism by which *Hibiscus sabdariffa* lowers blood pressure was demonstrated, [8] where water extracted from the petalswasseen to have a direct relaxant effect on an isolated aorta of a rat. This work aimed to examine the impact and the possible mechanism of action of the aqueous extent of sepals of *Hibiscus sabdariffa* on renal excretion of water.

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Materials and Methods

Animals

Sprague Dawley rats of both sex and weight between 150 - 200 g were obtained from laboratory Animals house, Pharmacology Department, University of Calabar and acclimatized for 2 weeks. Animals were on rat feed until they were used for the study.

Plant Materials

Dry sepals of *Hibiscus sabdariffa* were purchased from Bogobiri in Calabar in the month of May, 2021. Plant material was authenticated at the Department of Plant and Ecological studies (Botany), University of Calabar where a voucher specimen number BOT/HERB/UCC/074 was deposited. Dried plant material, 64 g was weighed and boiled in 600 ml distilled water for 10-15 minutes. It was then allowed to stand for about 30 minutes. Filtered and the filtrate evaporated to dryness using a freeze dryer. The dry extract was stored in a capped bottle until required. A stock solution 10 mg/ml was freshly prepared on each experiment day.

Equipment Reagents

Polythene catheters, ported, rubber tracheal cannula, dissecting sets and board, funnel, filter paper, clamps, syringes and needles, animal weighing balance, volumetric flasks, UV Spectrophotometer, Heparin, normal saline, indomethacin, L^{NG} – Arginine monomethyl ester, urethane, alpha chloralose, all purchased from Sigma Aldrich.

Sample Preparation

Extract

10mg/ml stock solution was made as stock from were required concentration for injection per time was prepared.

Anesthesia

Anesthesia used was a mixture of 25% urethane and 1% alpha chloralose.

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Indomethacin
10mg/ml indomethacin was prepared in normal saline.

L-NAME
Stock solution of 50 mg/ml was prepared from where the required concentration per time was prepared.

Experiment
Anesthesia: Before each experiment, the rats were weighed and anesthetized intraperitoneally at a dose of 0.5 ml/100g body weight. Supplementary doses of anesthesia were given during the experiment as necessary. About 15 minutes after the induction of anesthesia, the animal was placed on the dissecting board with limbs held in place. A midline incision was made from the chin to the upper part of the sternum, exposing the underlying connecting tissues. Cannulation of the trachea, femoral, vein and bladder was carried out. The trachea was cannulated to improve alveoli ventilation,the femoral vein was used for infusionof drugs, and the bladder was catheterized for timed urine collections. The urine was collected into sample bottles at an interval of 2 hours for intravenous administration of the extract and an interval of 3 hours for oral administration of the extract.
Blood samples, 5ml, were collected from the abdominal vein or aorta into heparinized bottles. This was centrifuged at 3000 rpm for 10 min. The plasma collected was for creatinine clearance determination which was determined by making a 1:500 dilution of urine collected. 3ml of each diluted urine sample was added to 2 ml of alkaline picrate in different cuvettes and allowed to stand for 15 min. After that, absorbance was read at 525 nm using a spectrophotometer. Similarly, plasma creatinine was determined by adding 1 ml of plasma, 3 ml of distilled water, 2 ml of 5 % sodium tungstate solution, and 2ml of 0.6 N H_2SO_4 in a centrifuge bottle and centrifuged for 15 min at 3000 rpm. 3 ml of supernatant, 3ml of distilled water, and 2 ml of alkaline picrate was put in different cuvettes, and absorbance was read at 525 nm.

Calculation
Urine creatinine Uc (mg/100 ml)

$$\frac{\text{Reading of unknown urine sample } x \text{ dillution factor x volume of total urine}}{100}$$

Plasma creatinine PCr in mg/100ml =

$$\frac{\text{Reading of unknown plasma sample}}{\text{Dilution factor}}$$

creatinine clearance Cr.

$$Cr = \frac{\mathbf{Ucr\ x\ V}}{\mathbf{Pcr}}$$

Experimental protocol
Acute oral administration of extract of Hibiscus sabdariffa.
This was necessary to establish the effect of acute administration of *Hibiscus sabdariffa* on urine output, glomerular filtration rate, and renal excretion of Na^+ and K^+. Animals were fasted for 24 hours to ensure their stomach was empty before the experiment. A cannula was passed into the esophagus through the mouth and held in place.
2 ml/100g/hour of normal saline was infused through the esophageal cannula for 3 hours, and urine was collected over 3 hours from the cannulated bladder as previously described. The urine collected served as control and creatinine clearance. Na^+ and K^+ were determined from each sample of urine. The animal was then allowed for about 1 hour without injecting anything until urine output was restored to normal. 5 mg/kg/1 hr. of the extract was then infused through the esophageal cannula, and urine was collected from the bladder for 3 hours. Creatinine clearance, Na^+ and K^+, were determined from each urine sample.

Acute intravenous administration of Hibiscus Sabdariffa.
This was designed to establish the effect of acute intravenous administration of *Hibiscus sabdariffa* on urine output, glomerular filtration rate, and renal excretion of Na^+ and K^+. Each animal was anesthetized, dissected, and cannulated as previously described. Each animal received 2 ml/100g/hr. Normal saline through the femoral vein and urine was collected for 2 hours from the bladder. The urine served as control, and creatinine clearance Na^+ and K^+ were determined from each urine sample. The animals were allowed for about 1 hour without administering anything until the urine output returned to normal. 5 mg/kg/hr of the extract was administered through the femoral vein, and urine was collected through the bladder for a period of 2 hours. Creatinine clearance, Na^+ and K^+, was determined from each sample collected.

Influence of L-NAME on the effect of Hibiscus sabdariffa on renal excretion of water.
This was designed to investigate whether L-NAME will block the effect of *Hibiscus sabdariffa* on renal excretion water.
After being anesthetized, dissected, and annulated, each animal received 2 ml/100g/hr of normal saline through the femoral vein, and urine, which served as control, was collected for 2 hours from the bladder. Creatinine clearance, Na^+ and K^+ were determined from each sample. 100 mg/kg of L-NAME was then injected into the femoral vein after 30min. Extract 5 mg/kg/hrwas infused through the femoral vein, and urine was collected for 2 hours from the bladder. Creatinine clearance, Na^+ and K^+, were determined for each sample.

Influence of Indomethacin on the effect of Hibiscus sabdariffa on renal excretion of water.
This was designed to know whether Indomethacin will block the effect of *Hibiscus sabdariffa* on renal excretion of water.
After being anesthetized, dissected, and annulated, each animal received 2ml/100g/hr of normal saline through the femoral vein, and urine, which served as control, was collected for 2 hours fromthe bladder. Creatinine clearance, Na^+ and K^+ were determined from each sample. 100 mg/kg of Indomethacin was then injected into the femoral vein after 30min. 5 mg/kg/hr of the extract was infused through the femoral vein, and urine was collected for a period of 2 hours from bladder. Creatinine clearance, Na^+ and K^+, were determined for each sample.

Statistical analysis
Statistical test of significance was performed at 95 % confidence level. The paired t - test was used to calculate the level of significance and unpaired t-test was used to compare data from different groups of animals. All results were expressed as means ± standard error of the mean (SEM), (X ± SEM)

Results and Discussion

Acute oral administration of extract increased urine output (2.0 ml) compared to saline administration (1.4 ml). This increase was higher 5.6 ml intravenous administration of the same dose. Glomerularfiltrate rate increased slightly (33 µL/min) with oral administration while intravenous administration significantly increased glomerular filtration rate (124 µL/min). The mean value of Na^+ excreted in urine was slightly higher in the oral administration of extract compared with control. On the other hand, with the intravenous administration, there was a significant output (19.0×10^{-5}) compared to 7.4×10^{-5} for oral administration. The K^+ excretion was not as significant in the oral administration compared with the intravenous 21.4×10^{-5} mmol/100 g). Administration of L-NAME and Indomethacin orally and intravenously did not alter the effects of extract or the GFR or Na^+ and K^+ excretion.
The result suggests that acute oral administration of *Hibiscus sabdariffa* has little effect on renal excretion of water as compared to the intravenous administration of the extract at the same dose. L-NAME and Indomethacin did not alter the outcome of *Hibiscus sabdariffa* on renal excretion of water. This result conforms to the report of Oliver,

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1960 who observed that aqueous extract of *Hibiscus sabdariffa* caused profuseexcretion of urine. *Hibiscus sabdariffa* is capable of causing diuresis, and even though its mechanism of action has not yet been determined, it has been established that agents are causing diuresis to do so either by increase in the excretion of sodium chloride and an accompanying volume of water or by increase in the excretion of potassium. [9] Oral administration of *Hibiscus sabdariffa* was not as effective in increasing excretion of urine Na^+ and K^+, this can be attributed to the first pass effect as experienced during oral administration of drugs. Renal excretion of Na^+ is dependent on several factors, which include an increase in aldosterone level in the blood. [7] this leads to Na^+reabsorption. *Hibiscus sabdariffa* may effectively inhibitthe reabsorption of Na^+thereby promoting urinary excretion of Na^+. On the other hand, inhibition of K^+transport in proximal tubule epithelial cells will prevent K^+reabsorption from tabular fluids, and stimulation of principal cells in the late distant tubule and cortical collecting duct will result in excretion of K^+ into tubular lumen. [3] This could suggest why *Hibiscus sabdariffa* effectively promotes K^+ excretion by simply opening K^+ channels of the epithelial cells in the kidney. L-NAME can constrict vascular bed, reduce renal blood flow and produce a hypertensive response in animals. [6] It inhibits nitric oxide synthesis. [2] Nitric oxide is synthesized by vascular endothelium responsible for vasodilator tone essential for regulating blood pressure. Stimulation of the release of endothelium-dependent relaxation factor will cause an increase in renal blood flow, leading to an increase in GFR with a resulting increase in urine output. [1] On the contrary, from the result, *Hibiscus sabdariffa* could not have acted through stimulation of endothelium-dependent relaxation factor because inhibition of Nitric oxide (NO) by L-NAME did not affect either GFR or urinary excretion of Na^+ and K^+.

Indomethacin, a prostaglandin synthesis inhibitor,did not affect the action of *Hibiscus sabdariffa*. Prostaglandin synthesis inhibitors specifically inhibit the cyclooxygenase pathway, and renal functions are dependent on prostaglandins. [5] Prostaglandins cause dilation of renal vessels and increase renal excretion of Na^+, thus increasing dieresis. [4] From the results, *Hibiscus sabdariffa* did not act through stimulation of prostaglandins because on inhibition of prostaglandin synthesis using Indomethacin. There was still a remarkable increase in urine output GFR and urinary excretion of Na^+ and K^+ when *Hibiscus sabdariffa* was administered intravenously.

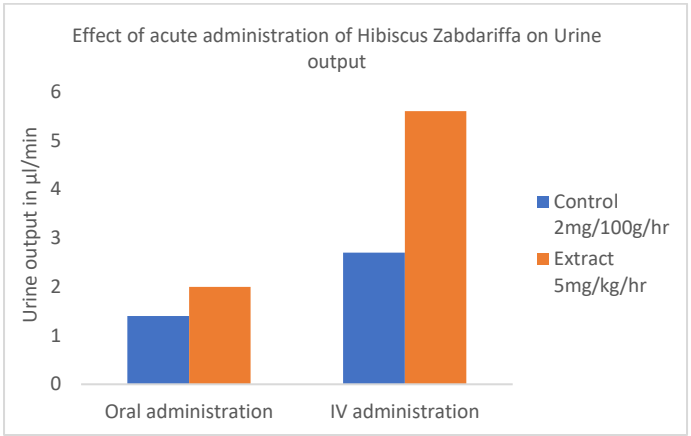

Fig 1: Effect of acute administration of *H. sabdariffa* on urine output

Table 1: Effect of acute administration of *Hibiscus sabdariffa* on Na^+ electrolyte excretion (n=6)

	Oral administration	IV administration
Control 2mg/100g/hr	6.4 x 10^{-5}	7.6 x 10^{-5}
Extract 5mg/kg/hr	7.4 x 10^{-5}	19.0 x 10^{-5}

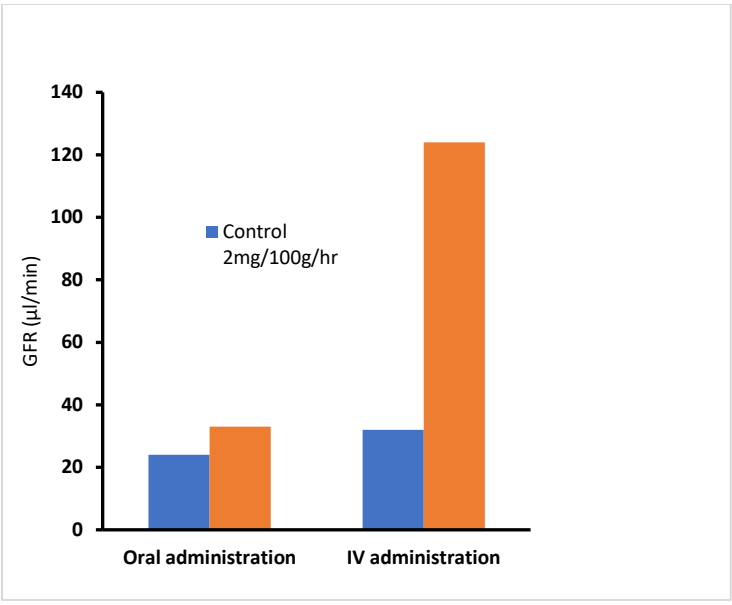

Fig. 2: Effects of acute administration of Hibiscus sabdariffa on GFR (µl/min)

Table 2: Effect of acute administration of *Hibiscus sabdariffa* on K^+ electrolyte excretion

	Oral administration	**IV administration**
Control		
2mg/100g/hr	14.1 x 10^{-5}	11.4 x 10^{-5}
Extract 5mg/kg/hr	14.7 x 10^{-5}	21.4 x 10^{-5}

Table 3: Influence of L-NAME on the effect of acute administration of *Hibiscus sabdariffa* on Na^+ and K^+ electrolyte excretion
n=6

	Na^+	**K^+**
Control		
2mg/100g/hr	7.3 x 10^{-5}	6.5 x 10^{-5}
Extract		
5mg/kg/hr	17.0 x 10^{-5}	15.6 x 10^{-5}
Extract +		
L-NAME	15.0 x 10^{-5}	17.2 x 10^{-5}

Table 4: Influence of Indomethacin on the effect of acute administration of *Hibiscus sabdariffa* on Na^+ and K^+ electrolyte excretion
n=6

	Na^+	**K^+**
Control		
2mg/100g/hr	7.8 x 10^{-5}	5.5 x 10^{-5}
Extract		
5mg/kg/hr	20.0 x 10^{-5}	17.4 x 10^{-5}
Extract +		
Indomethacin	19.8 x 10^{-5}	17.0 x 10^{-5}

Table 5: Influence of L-NAME on the effect of acute administration of *Hibiscus sabdariffa* on Urine output and GFR

n=6

	Urine output (µl/min)	GFR(µl/min)
Control 2mg/100g/hr	2.9	31
Extract 5mg/kg/hr	5.8	126
Extract + L-NAME	4.7	121

Table 6: Influence of Indomethacin on the effect of acute administration of *Hibiscus sabdariffa* on Urine output and GFR

n=6

	Urine output (µl/min)	GFR(µl/min)
Control 2mg/100g/hr	2.7	34
Extract 5mg/kg/hr	6	128
Extract + L-NAME	5.8	126

Conclusion

It is evident from this study that acute intravenous administration of extract of the sepals of *Hibiscus sabdariffa* led to diuresis. This diuretic effect could be associated with increases in glomerular filtration rate and urinary excretion of Na^+ and K^+ as recorded in this work. *Hibiscus sabdariffa* on renal function is neither mediated through stimulation of endothelium-dependent relaxation factor nor the synthesis of prostaglandins.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Badr, K. F. Murrary, J.J. Brayer, M.D. Takahashi, K.Inagami, T., and Harris, R.C. Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. *Journal Clinical Invest.* 1989; 83: 336-342.
2. Baylis, C. Mitruka, B., and Dang, A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J of Clinic Investigation.* 1992; 90: 278-281.
3. Crayen, M. L., and Thoenes, W. Architecture and cell structure in the distal nephron of the rat kidney. *Cytobiol. Eur. J. Cell Biol.* 1978; 17:197.
4. Dunn, M. J., & Hood, V. L. Prostaglandins and the kidney. *Am. J. Physiol,* 1977; 233(3), 169-184.
5. Gerber, J. G., Kaler, R. T. and Nies, A. S. The effect of PGI_2 PGE_2, and 13, 14-dihydro PGE_2 on the baroreceptor mechanisms of renin release in dog. *Circ. Res.* 1979; 44: 796-799.
6. Moncada, S., and Higgs, A. A. The L-arginine- nitric oxide pathway. *The New England Journal of Medicine,* 1993; 399: 2002-2012.
7. Mulrow, P. J., and Forman, B. H. The effect of mineral ocorticcids on renal tubules. *American Journal of Medicine.* 1972; 53:561-52.
8. Obiefuna, P. C. M. Owolabi, O.A, Adegunloye, B. J., Obiefuna, I.P., and Sofola, O.A. The petal extract of *Hibiscus sabdariffa* produces relaxation of rat isolated aorta *International Journal of Pharmacognosy,* 1993;31: 1-6.
9. Oliver, B. Medicinal plants in Nigeria. *Nigerian College of Arts and Technology*, 1960; pp. 16-42.
10. [Sofowora In: Medicinal plants and traditional medicine in African ed. Sofowora. John Wiley. 1982; Pp. 66-76.