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Effect of Methanolic Extract of *Hibiscus Sabdariffa* on Antidiuretic Hormone and Some Electrolytes After Salt and Water Loading in Albino Wistar Rats

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ABSTRACT

This study investigated the effect of methanolic extract of *Hibiscus sabdariffa* on antidiuretic hormone and some electrolytes. Twenty albino wistar rats (150 - 180 g) were used in this present study. During the experiment the animals were weighed and randomly selected into four experimental groups (n = 5). Control group: (Group A) was given distilled water and normal rat chow. Experimental Group B received 8.0% NaCl only. Experimental Group C received 10 ml of distilled water. Group D received 8% NaCl plus 200 mg/kg of *Hibiscus sabdariffa* extract. Experimental Group E received 10 ml of distilled water plus 600 mg/kg of *Hibiscus sabdariffa* extract. Results showed no statistically significant difference in plasma Na^+ and K^+ ($P > 0.05$). Statistically significant difference occurred in K^+ ($P < 0.05$) between Group A (4.08 ± 0.21 mEq/L) compared to Group D (3.53 ± 0.56 mEq/L) and Group E (3.30 ± 0.26 mEq/L). There was no statistically significant difference in plasma Cl^- ($P > 0.05$) except between Group A (95.29 ± 4.79 mEq/L) compared to Group B (147.19 ± 3.62 mEq/L) and Group E (89.82 ± 0.82 mEq/L) at $P < 0.05$. There was no statistically significant difference in plasma HCO_3^- ($P > 0.05$). There was statistically significant difference in HCO_3^- ($P < 0.05$) between Group A (18.73 ± 1.86 mEq/L) compared to Group B (26.50 ± 1.06 mEq/L), Group C (15.05 ± 1.23 mEq/L) and Group E (14.06 ± 1.20 mEq/L). Results showed statistically significant difference in ADH level ($P < 0.05$) between Group A (1.85 ± 0.34 pg/ml) compared to Group C (0.86 ± 0.18 pg/ml). Data suggest that at increased dose-concentration, *Hibiscus sabdariffa* extract increased plasma ADH and reduced plasma Na^+, Cl^-, HCO_3^- and K^+ after water loading but slightly decreased plasma Na^+ and Cl^- after salt loading although a slight increase in ADH level. However, the plasma HCO_3^- and K^+ were increased after salt loading in the rats.

Keywords: *Hibiscus sabdariffa*, Antidiuretic Hormone, Sodium Ion, Potassium Ion, Bicarbonate, Chloride, Salt-Loading, Water-Loading.

Introduction

Hibiscus Sabdariffa (Linn) is an annual dicotyledonous herbaceous shrub is well known in Asia and African country. The aqueous calyx extract of *Hibiscus sabdariffa* is consumed as a local drink in Northern Nigeria. Water extract of

Hibiscus flowers were reported to have a relaxation effect on the uterus and lower blood pressure (Franz and Franz, 1988). Studies in both animals (Ali *et al.,* 1991; Adegunloye *et al.,* 1996; Onyenekwe *et al.,* 1999; Odigie *et al.,* 2003) and human models have demonstrated that extracts or infusions affect atherosclerosis, blood sugar, lipids and blood pressure (Chen *et al.,* 2004; Herra-Arellano *et al.,* 2004). Its folk reputation as an antihypertensive agent in Nigeria has been validated by several studies in animals (Odigie *et al.,* 2003; Mojiminiyi *et al.,* 2007) and man (Haji Faraji and Haji Tarkhani, 1999; Herrera-Arellano *et al.,* 2004; 2007). *Hibiscus* sabdariffa have been shown to possess diuretic properties (Mojiminiyi, 2000; Alorcon-Alonso *et al.,* 2012; Jimenez-Ferrer *et al.,* 2012), natriuretic and potassium sparing effects (Jimenez-Ferrer *et al.,* 2012). Continuous expansion of the extracellular volume (Ledingham, 1953; Borst and Borst, 1963; Guyton *et al.,* 1974) and ingestion of salt (Dahl, 1972; Oliver *et al.,*1975) have been implicated in essential hypertension and studies have indicated that elevation in plasma arginine vasopressin are often present in even mildly hypertensive subjects (Cowley *et al.,* 1981; 1985) , therefore, this study was carried out to test the diuretic action of the extract by evaluating anti-diuretic hormone and some electrolytes after salt loading and water loading in rats.

Materials and Methods

Twenty albino wistar rats (150 - 180 g) were obtained from the animal house of the Department of Zoology, University of Nigeria, Nsukka and maintained in a temperature of 25^0C - 30^0C, 12 hours light and 12 hours dark cycle (approved institutional guidelines that are in compliance with international laws and policies). All animals were given free access to tap water and regular pelleted rat chow during the experiments. During the experiment the animals were weighed and randomly selected into four experimental groups (n = 5). Control group: (Group A) was given distilled water and normal rat chow. Experimental Group B received 8.0% NaCl only (Sofola *et al.,* 2002; Mojiminiyi *et al.,* 2007). Experimental Group C received 10 ml of distilled water. Group D received 8% NaCl plus 200 mg/kg of *Hibiscus sabdariffa* extract. Experimental Group E received 10 ml of distilled water plus 600 mg/kg of *Hibiscus sabdariffa* extract.

Acute Toxicity Test

The LD_{50} on *Hibiscus sabdariffa* was carried out employing the method by Lorks (2000). This test involved a total of ten rats and carried out in 2 stages. Animals received oral administration of methanolic extract of *Hibiscus sabdariffa*.

Stage 1 involved six rats. They were grouped into 3 groups (n=2). Group 1 received 10mg/kg of methanolic extract of *Hibiscus sabdariffa*. Group 2 received 100 mg/kg of methanolic extract of *Hibiscus sabdariffa*. Group 3 received 1000 mg/kg of methanolic extract of *Hibiscus sabdariffa*. The animals were constantly monitored for a period of 5 hours, then the next 6 hours and then over a period of 24 hours. No death was recorded.

Stage 2 was grouped into 4 (n = 1). Group 1 received 1500 mg/kg, Group 2 received 2500 mg/kg, Group 3 received 3500 mg/kg, and Group 4 received 5000 mg/kg of methanolic extract of *Hibiscus sabdariffa* respectively. All animals were monitored again over a period of 24 hours. No death was also recorded.

Extract Preparation

Fresh calyces of *Hibiscus sabdariffa* were bought from Elele market in Rivers State, Nigeria. They were sorted to remove debris and dust particles. Large quantities of the calyces were then collected and sun dried for seven days. After drying, they were milled with a mortar and pestle to get a coarse powder used for the extraction. About 200g of the grounded form was soaked in 1000 ml of methanol and placed in a mechanical shaker for 48 hours before filtering with a white handkerchief into a clean bottle. The filtrate was then concentrated to dryness at 50^0C in an electric oven (gallenkamp). It produced a semi-solid mass when dried and stored in an air tight container in the refrigerator below 10^0C. 1.1 g of the extract is then measured using an electric weighing balance and then dissolved into 11 ml of distilled water to give a stock solution of 100mg/ml. The extract was administered orally using a 2 ml syringe without needle. This was done carefully to prevent damage of the alimentary canal of the mice.

Sample Collection

At the end of two days experiment, the animals were sacrificed under anesthesia with the use of chloroform. Blood was obtained via cardiac puncture and was put in a labeled EDTA anticoagulant bottle for determination of antidiuretic hormone and some serum electrolytes.

Statistical Analysis

Results are expressed as mean ± SEM. Statistical significance of the differences observed between control and experimental groups (ANOVA) was evaluated by Turkey's multiple comparison at $P < 0.05$.

Results

Results in figure 1 showed no statistically significant difference in plasma Na^+ ($P > 0.05$) between Group A (141.44 ± 1.87 mEq/L) compared with Group B (179.40 ± 6.13 mEq/L), Group C (127.43 ± 4.33 mEq/L), Group D (148.36 ± 6.72 mEq/L) and Group E (123.92 ± 7.08 mEq/L). There was no statistically significant difference in plasma Cl^- ($P > 0.05$) between Group A (95.29 ± 4.79 mEq/L) compared with Group C (118.73 ± 11.87 mEq/L) and Group D (136.44 ± 17.54 mEq/L) respectively. However, statistically significant difference was observed ($P < 0.05$) between Group A (95.29 ± 4.79 mEq/L) compared to Group B (147.19 ± 3.62 mEq/L) and Group E (89.82 ± 0.82 mEq/L) respectively.

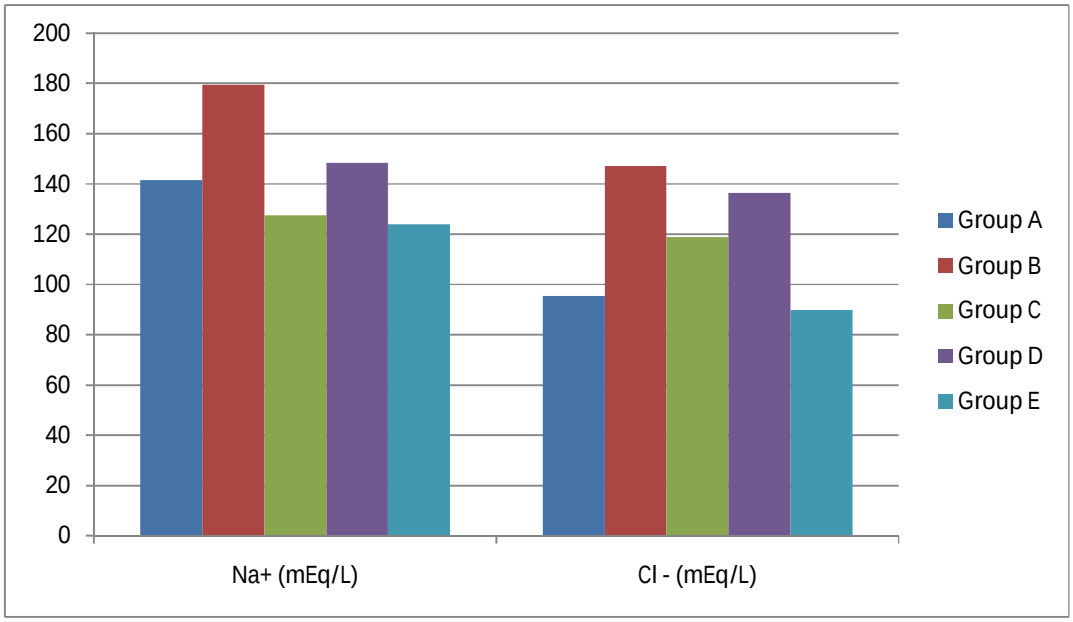

Figure 1: Effect of Methanolic Extract of *Hibiscus sabdariffa* on Serum Sodium Ion and Chloride

In figure 2, there was no statistically significant difference in K^+ ($P > 0.05$) between Group A (4.08 ± 0.21) compared with Group B (7.30 ± 0.69 mEq/L) and Group C (3.45 ± 0.52 mEq/L). Statistically significant difference occurred in K^+ ($P < 0.05$) between Group A (4.08 ± 0.21 mEq/L) compared to Group D (3.53 ± 0.56 mEq/L) and Group E (3.30 ± 0.26 mEq/L). There was no statistically significant difference in plasma HCO_3^- ($P > 0.05$) between Group A (18.73 ± 1.86 mEq/L) compared with Group D (20.22 ± 2.83 mEq/L). There was statistically significant difference in HCO_3^- ($P < 0.05$) between Group A (18.73 ± 1.86 mEq/L) compared to Group B (26.50 ± 1.06 mEq/L), Group C (15.05 ± 1.23 mEq/L) and Group E (14.06 ± 1.20 mEq/L).

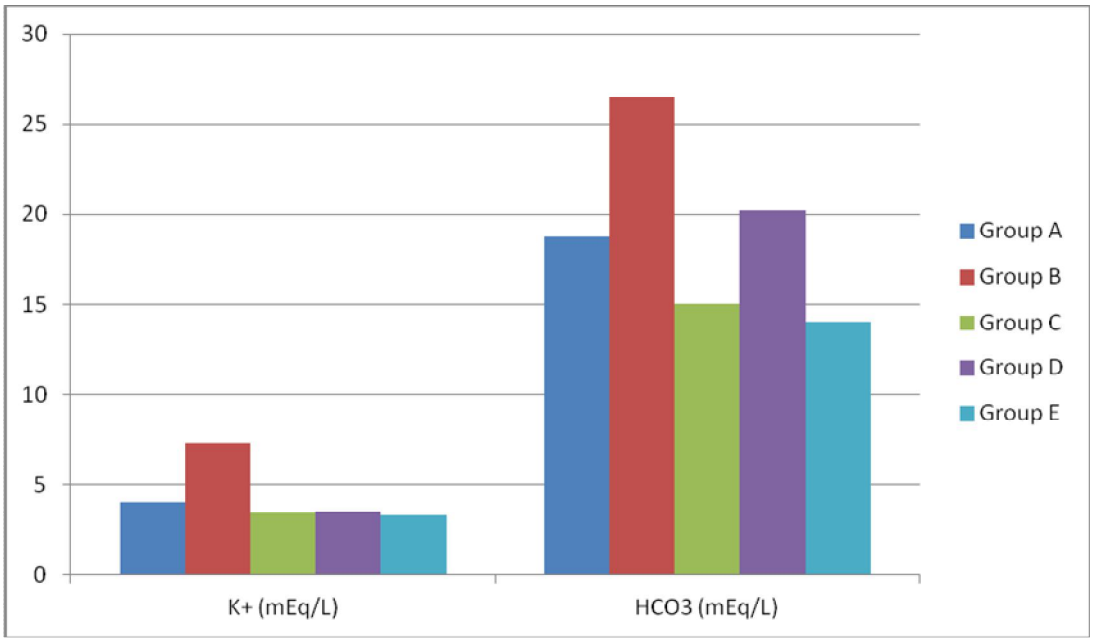

Figure 2: Effect of Methanolic Extract of *Hibiscus sabdariffa* on Serum Potassium Ion and Bicarbonate

In the figure 3, results showed statistically significant difference in ADH level ($P < 0.05$) between Group A (1.85 ± 0.34 pg/ml) compared to Group C (0.86 ± 0.18 pg/ml). However, there was no statistically significant difference in ADH ($P > 0.05$) between Group A (1.85 ± 0.34 pg/ml) compared with Group B (1.50 ± 0.45 pg/ml), Group D (3.88 ± 0.47 pg/ml) and Group E (5.79 ± 0.99 pg/ml).

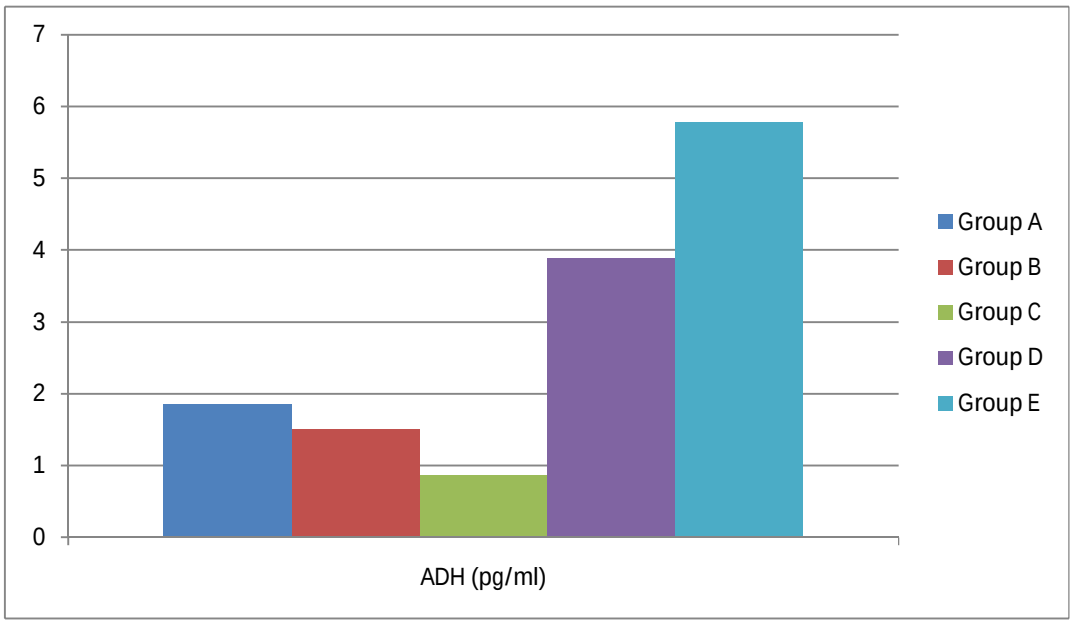

Figure 3: Effect of Methanolic Extract of *Hibiscus sabdariffa* on Serum Antidiuretic Hormone

Discussion

The present study revealed the effect of *Hibiscus sabdariffa* extract on plasma Na^+, Cl^-, K^+, HCO_3^-, and ADH after water-loading and salt-loading in rats. Continuous expansion of the ECF has been implicated in hypertension; result in figure 1 showed that 600 mg/kg of the extract reduced plasma Na^+ and Cl^- after water-loading and also reduced HCO_3^- and K^+ levels. Several studies have shown that *Hibiscus sabdariffa* extract lowers blood pressure in animals (Odigie *et al.,* 2003; Mojiminiyi *et al.,* 2007) and man (Haji Faraji and Haji Tarkhani, 1999; Herrera-Arellano *et al.,* 2004; 2007). Studies have shown that the diuretic, natriuretic and potassium sparing effect of *Hibiscus sabdariffa* extract are due in part to the modulation of aldosterone activity (Jimenez-Ferrer *et al.,* 2012). Report had shown that plasma renin activity was suppressed by the administration of ADH and sodium excretion increased due to a decreased aldosterone activity (Joppich and Weber, 1976). Renin acts enzymatically on angiotensinogen to release angiotensin I two additional amino acids split from angiotensin I to form angiotensin II under the influence of angiotensin-converting enzyme. Angiotensin II further triggers the release of aldosterone. The reduction in Na^+ and Cl^- observed in the study could be due to the effect of the extract on ADH. In order to support our findings, thus, we evaluated the ADH level in the plasma. In figure 3, the plasma level of ADH was high after administering 600mg/kg of the extract indicating that the increased ADH may have suppressed the renin-angiotensin-aldosterone system pathway

thereby leading to decreased plasma Na^+ and Cl^-. From the results in figure 2, it showed that K^+ in the ECF did not increase in contrast to Jimenez-Ferrer et al. (2012). The level of HCO_3^- was also reduced indicating that *hibiscus sabdariffa* extract alters ECF electrolytes.

Studies have shown that *Hibiscus sabdariffa* attenuated the development of salt-induced hypertension associated with high potassium ion content (Mojiminiyi *et al.,* 2012). The result in figure 2 showed that Na^+ and Cl^- levels did not decrease significantly, whereas a slight increase in K^+ and HCO_3^- was observed in salt-loaded rats. The plasma ADH level slightly increased in salt-loaded rats after administration of 200mg/kg of the extract compared to normal control rats. Our findings show that 600mg/kg of extract significantly increased ADH level and reduced the plasma electrolytes. Aldosterone is one of the body's most powerful sodium-retaining hormones, only transient sodium retention occurs when excess amounts are secreted. An aldosterone-mediated increase in ECF volume lasting more than 1 to 2 days leads to an increase in arterial pressure (Guyton and Hall, 2011). It is plausible to suggest that action of the extract is dose-dependent because of the slight increase in ADH observed after administering 200mg/kg of extract which did not exert a strong effect on the electrolytes. Our findings corroborated with studies by Joppich and Weber (1976) and Jimenez-Ferrer *et al.,* (2012) that extract modulated aldosterone activity by the suppressive effect of ADH on renin.

This present study concludes that *Hibiscus sabdariffa* extract is beneficial in the treatment of water-loaded and salt-loaded hypertension due to its effect on ADH production thereby altering plasma electrolytes in a dose-dependent pattern.

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