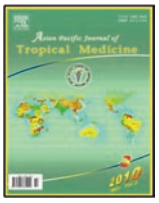
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading

Mechanism of the decreased food consumption and weight gain in rats following consumption of aqueous extract of the calyx of *Hibiscus sabdariffa* during pregnancy

Eghosa E Iyare[1*], Olufeyi A Adegoke[2], Uchenna I Nwagha[3]

[1]*Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*
[2]*Department of Physiology, College of Medicine, University of Lagos, Idiaraba, Lagos, Nigeria*
[3]*Department of Physiology/Obstetrics and Gynaecology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*

ARTICLE INFO

Article history:
Received 8 December 2009
Received in revised form 7 January 2010
Accepted 10 January 2010
Available online 20 March 2010

Keywords:
Food consumption
Fluid consumption
Plasma sodium
Hibiscus sabdariffa
Mechanism
Pregnancy
Weight gain

ABSTRACT

Objective: To investigate the possible mechanisms of the decreasing fluid and food consumption following *Hibiscus sabdariffa* (HS) consumption. **Methods:** On the 1st day of pregnancy, rats were randomly divided into three groups with six animals per each group. One group was given tap water, one was given with extract at 0.6 g/100 mL while the third group was given with extract at 1.8 g/100 mL as their drinking solution. All groups received normal rat chow and drinking solution *ad libitum*. Fluid& food intake and weight were measured daily throughout pregnancy and Na^+ concentration in plasma was determined on the 18th day of pregnancy.**Results:** Results showed decreased fluid and food consumption, decreased weight gain and increased sodium ion concentration in plasma of rats with HS extract compared with the control group. **Conclusions:** Consumption of aqueous extract of the calyx of HS during pregnancy decreases food consumption and weight gain through mechanisms that may depend on Na^+ in HS content and elevating Na^+ concentration.

1. Introduction

Hibiscus sabdariffa L. (HS) (family: Malvaceae) is an annual, erect, bushy and herbaceous sub-shrub that grows up to 8 ft (2.4 m) tall, with smooth or nearly smooth, cylindrical and typically red stem. The botanical features have been excellently described by Ross[1]. In folk medicine, extracts of HS are widely believed to be effective in the treatment of a variety of ailments[2-4]. The effectiveness of HS in the treatment of these ailments have been attributable to the various constituents of HS like flavonoids, anthocyanins and organic acids and sodium ions (Na^+), vitamins A and C and iron (Fe)[5-11].

A sweetened aqueous extract of HS (zobo drink) is gradually becoming a national drink as it is commonly produced, sold and consumed indiscriminately in Nigeria irrespective of their physiological state. It is consumed not necessarily for medicinal purposes, but as a substitute for carbonated drinks because of the folkloric belief that it makes them "feel lighter".

We [12,13] and others[14-16] have confirmed this folkloric belief in both pregnant and non-pregnant rats through mechanisms not yet fully understood. The present study was therefore designed to investigate the possible mechanisms for the decreased fluid and food consumption following HS consumption.

2. Materials and methods

2.1. Experimental animals

Thirty-six in-bred virgin female Sprague-Dawley rats aged between 10-12 weeks and weighing (125±5.5)g with two consecutive regular 4-day estrus cycle were used for this study. These rats were housed individually in cages under standard environmental conditions. The estrous cycles were monitored and male rats of proven fertility were introduced into the cages of the female rats that were expected to get into the estrous phase within 12 hours to

*Corresponding author: Dr Uchenna I Nwagha, Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus
Tel: +2348033128233
E-mail: uchenna.nwagha@unn.edu.ng, uchenwagha@yahoo.com

allow for mating. The 1st day of pregnancy was taken as the day when sperm were seen in the vaginal smear of the rats. On the 1st day of pregnancy, animals were randomly divided into three groups with twelve animals per each group. One group (control) was given by tap water. One was given with extract at 0.6 g/100 mL while the third group was given with extract at 1.8 g/100 mL as drinking solution.

All groups received normal rat chow and drinking solution *ad libitum*. Fluid&food intake and weight were measured daily throughout pregnancy.

Throughout the course of the study, the animals did not exhibit any clinical signs of drug toxicity. All procedures used in this study conformed with the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals[17] and were approved by the Departmental Committee on the Use and Care of Animals.

2.2. Sodium estimation

On the 18th day of gestational, 6 rats in each group were anaesthetized by chloroform inhalation. The thoracic cavity was quickly opened and the heart exposed. Blood samples were withdrawn by cardiac puncture using a 19G hypodermic needle. The needle was removed and the blood ejected into a heparin-containing specimen bottle. The blood was immediately spun at 3 000 rpm for 10 minutes in a centrifuge. The plasma was then gently withdrawn using a Pasteur pipette and stored at −20 ℃. The sodium ion content of the plasma was assessed by flame photometry.

2.3. Extraction procedure

Mature dry dark-red calyces of HS were purchased from a local market in Lagos, Nigeria and authenticated by Mr TI Adeleke of the department of Pharmacognosy, University of Lagos, Nigeria where a voucher specimen number PCG H455 was deposited. The extraction procedure used in our laboratory was as described previously[12,13]. Briefly, 30 g of the dry petals of HS was brewed in 400 mL of boiled tap water for 45 mins. The resulting decoction was filtered and evaporated to dryness giving a dark red powder (yield 48.87%).

0.6 g and 1.8 g of the dark red powder were weighed and dissolved in 100 mL of tap water and then given to HS groups as drinking solution.

2.4. Statistical analysis

Results were expressed as mean±standard error of mean (Mean±SEM). For data comparison, the one way analysis of variance (ANOVA) was used followed by a post-hoc Student's Newman-Keuls test. $P<0.05$ was taken as statistically significant.

3. Results

3.1. Fluid and food intake

Rats in the HS groups (0.6 g/100 mL and 1.8 g/100 mL) drank less fluid compared with the control group at all the trimesters of pregnancy ($P<0.05$) (Table 1). The decreasing fluid consumption was not dose-dependent except in the 2nd trimester when the fluid consumption in rats with high dose of HS was significantly lower than that of the low dose HS rats.

The food consumption in HS group were significantly reduced compared with that of the control group at all trimesters of pregnancy ($P<0.05$) (Table 1). The decreasing food consumption was also not dose dependent except during the 2nd trimester when the food consumption in the high dose HS group was lower than that of the low dose HS group ($P<0.05$).

3.2. Weight gain

The weight gain of the rats with aqueous extract of HS during pregnancy in the first trimester was significantly lower than that of the control rats ($P<0.05$). In the third trimester, there was no significant difference among the three groups. In the second trimester, only the weight gain of the high-dose (1.8 g/100 mL) HS rats was significantly lower than that of the control group. The weight gain of the low-dose HS rats was not significantly different from that of the control group, even though quantitatively lower (Table 2).

The rate of weight gain of the rats with aqueous extract of HS during pregnancy was significantly lower ($P<0.05$) than that of the control rats in all trimesters of pregnancy (Table 2). The decreased rate of weight gain was not dose dependent as there was no difference in the rate weight gain between the rats in the two HS groups in all trimesters of pregnancy.

3.3. Plasma Na^+ concentration

There was a significant increase in the concentration of Na^+ in the plasma of HS rats compared with the control group ($P<0.05$). There was no difference between the two groups (Figure 1).

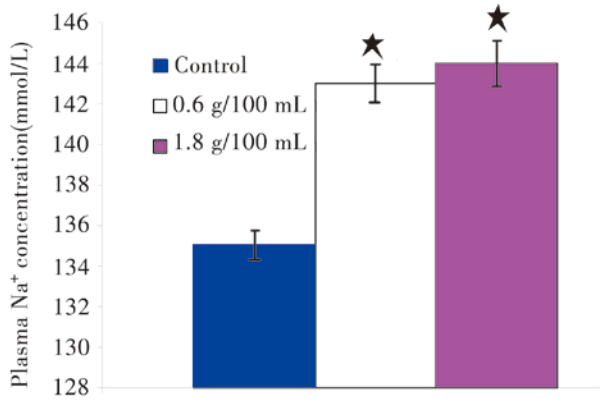


Figure 1. Plasma Na^+ concentration following consumption of *Hibiscus sabdariffa* during pregnancy.
★: $P<0.001$ compared with control, n=6/group

Table 1
Effect of consumption of *Hibiscus sabdariffa* during pregnancy on fluid and food intake.

Index		Control	0.6 g/100 mL extract	1.8 g/100 mL extract
Mean fluid intake (mL/day)	1st trimester	31.00±0.97	18.33±0.95*	17.33±0.84*
	2nd trimester	36.17±1.22	22.00±1.03*	18.50±0.65*△
	3rd trimester	29.17±1.45	23.33±0.99*	24.17±0.54*
Mean food intake (g/day)	1st trimester	21.00±1.46	17.00±0.73*	17.50±1.23*
	2nd trimester	27.33±0.95	22.33±1.20*	17.83±0.79*△
	3rd trimester	24.17±0.83	21.00±0.73*	18.50±0.89*△

Versus control: * $P<0.001$; Versus 0.6 g/100 mL extract group: △ $P<0.01$.

Table 2
Effect of consumption of *Hibiscus sabdariffa* during pregnancy onabsolute weight gain and weight gain rate in each trimester.

Index		Control	0.6 g/100 mL extract	1.8 g/100 mL extract
Absolute weight gain (g)	1st trimester	26.3±1.6	21.2±0.7*	19.3±2.1*
	2nd trimester	30.5±1.8	26.2±0.7*	22.5±2.0*△
	3rd trimester	45.6±1.9	41.8±0.9	38.1±6.1
Rate of weight gain (g/day)	1st trimester	3.8±0.2	3.0±0.1*	2.8±0.3*
	2nd trimester	4.4±0.3	3.7±0.1*	3.2±0.3*△
	3rd trimester	6.5±0.3	6.0±0.1	5.4±0.9

Versus control: * $P<0.001$; Versus 0.6 g/100 mL extract group: △ $P<0.01$.

4. Discussion

Rats that were given by aqueous HS during pregnancy in the study drank less fluid compared with the control rats. This may be due to the fact that since the aqueous extract of HS was not sweetened, it may have been unpalatable to these rats, thus resulting in reduced consumption.

The water deprivation following the decreased fluid intake may have cause a state of hypernatraemia in these rats[18] as observed in the study. Also, aqueous extract of HS is rich in Na^+[11] suggesting that consumption of aqueous extract of HS increases the body´s Na^+ load. Mojiminiyi and Coworkers[19] in their investigation of the diuretic action of aqueous extract of HS observed that rats that consumed this extract had elevated Na^+ concentration in plasma.

It is well established that flavonoids inhibit the action of 11 β-hydroxysteroid dehydrogenase type-2 (11 β HSD2)[20-22]. This enzyme, localized to mineralocorticoid target cells in the kidney, colon and parotid glands as well as the pancreas and placenta[23-25], is identified as the enzymatic "gatekeeper" that catalyses the conversion of the active glucocorticoid into the inactive form, thus excluding active glucocorticoids from the non-specific mineralocorticoid receptors which display little inherent specificity for their normal ligand, aldosterone[26,27]. Thus, consumption of aqueous extract of HS rich in flavonoids may lead to the inhibition of the action of this enzyme, thereby allowing glucocorticoids (which normally circulates at concentrations higher than aldosterone) to gain access to the mineralocorticoid receptors[9,11]. This causes hyperstimulation of these receptors resulting in excessive Na^+ reabsorption and retention[28]. This mechanism may also contribute to the elevated Na^+ concentration in the rats with aqueous extract of HS in the study. The hypernatraemia in the rats with aqueous extract of HS (evidenced by the elevated plasma Na^+) may cause dehydration-anorexia in these rats[18] which probably led to the decreased food consumption in the study.

Since injection of hypertonic solution into the gastrointestinal tract (GIT) stimulates thirst and inhibits feeding in rats[29], consumption of aqueous extract of HS, which has been reported to be rich in Na^+[11], may imply ingestion of a hypertonic solution into the GIT. This may stimulate the sensor, postulated to be located in the GIT, to generate signals that provoke the early termination of meal independent of systemic hypertonicity[29]. This also may contribute to the decreased food consumption in the study. The decreased food consumption may be due to the known anorectic effect of the phytoestrogens which are present in the HS extract[30].

The decreased food consumption following water deprivation (whether absolute or relative through ingestion of a hypertonic solution) is a normal physiological homeostatic mechanism protecting against cellular dehydration. This is because reducing food intake during water deprivation allows for the absorption of osmotically sequestered water in the gut[31,32] as well as reducing the obligatory urinary water loss[32].

The observed decreased food consumptionof the pregnant rats with aqueous HS may lead to the decreased weight gain, since malnutrition can cause reduced weight gain in pregnancy[33-35].

From the foregoing, it can be concluded that consumption of aqueous extract of the calyx of HS during pregnancy may decrease food consumption and weight gain mainly through stimulation of Na^+-sensitive gut sensors and systemically mediated dehydration-anorexia induced by the elevated plasma Na^+ concentration.

Conflict of interest statement

We declare that we have no conflict of interest.

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