

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Gestational Outcome in Rats That Consumed Aqueous Extract of *Hibiscus sabdariffa* During Pregnancy

E.E. Iyare and O.A. Adegoke

Department of Physiology, College of Medicine, University of Lagos, Idiaraba, Lagos State, Nigeria

Abstract: The present study was designed to investigate the effect of consumption of aqueous extract of *Hibiscus sabdariffa* (HS) during pregnancy on fluid and food intake, weight gain during pregnancy, length of gestation, litter size and birth weight. Eighteen in-bred virgin female Sprague-Dawley rats aged between 10-12 weeks and weighing 125 ± 5.5 g (mean \pm SEM) with two consecutive regular 4-day estrus cycle were used for this study. These rats were randomly assigned to one of three groups on gestational day one. Group A (control) were given tap water to drink throughout pregnancy, while groups B and C were given 0.6g-extract/100ml and 1.8g-extract/100ml respectively to drink throughout pregnancy. All groups had normal rat chow and their respective drinking solution *ad libitum*. Mean fluid and food intake and total pregnancy weight gain were significantly reduced in the HS group. There was no significant difference in length of gestation among the various groups but there was a significant reduction in litter size and a significant increase in litter birth weight in HS groups (B and C) compared with control group (A). There were no apparent malformations at birth.

Key words: *Hibiscus sabdariffa*, fluid and food intake, pregnancy weight gain, litter size and birth weight

INTRODUCTION

Extracts of *Hibiscus sabdariffa* (HS) (family: Malvaceae) are widely believed in folk medicine to be effective in the treatment of a variety of ailments including hypertension, hyperlipidaemia, obesity and diabetes (Perry, 1980; Watt *et al.*, 1962; Oliver, 1962). Its antihypertensive (Obiefuna *et al.*, 1993; Adegunloye *et al.*, 1993; 1996; Haji-Faraji and Haji-Takhani, 1999; Onyenekwe *et al.*, 1999; Odigie *et al.*, 2003; Mojiminiyi *et al.*, 2007; Ajay *et al.*, 2007; Herera-Arellano, 2007), hypolipidaemic (Farombi and Ige, 2007; Hirunpanich *et al.*, 2006; Carvajal - Zarrabal *et al.*, 2005), anti-obesity (Alarcon-Aguilar *et al.*, 2007; Kim *et al.*, 2007) and anti-diabetic (Farombi and Ige, 2007; Lans, 2006) effects have been confirmed and the possible mechanisms have also been delineated.

These effects have been attributable to the various constituents of HS like flavonoids, anthocyanins and organic acids and Na^+ , vitamins A and C and Fe (Fuleki and Francis, 1968; Clydesdale, 1979; Duke and Francis, 1973; Morton, 1987; Daffalah and Al-Mustafa, 1996; Appel, 2003; Adigun *et al.*, 2006).

A sweetened aqueous extract of HS (zobo drink) is gradually assuming the position of a national drink in Nigeria as it is commonly produced, sold and consumed by both males and females, young and old. It is consumed, not necessarily for medicinal purposes but as a substitute for carbonated drinks without regards to the physiological state of the body. Women have even been observed consuming zobo drink during pregnancy. Extract of HS has been reported to decrease fluid and food intake through a mechanism not yet fully understood (Ojokoh, 2006; Iyare and Adegoke, 2008).

Mojiminiyi *et al.* (2000) have shown that rats that consume aqueous extract of HS have hypernatremia possibly through its diuretic action. Hypernatremia and water deprivation have been shown to cause dehydration-anorexia and corresponding decreased food consumption (Ross and Desai, 2005). Decreased food intake in pregnant animals has been shown to cause foetal malnutrition and the attendant developmental sequelae (Armitage *et al.*, 2005a, b; Gluckman and Hanson, 2004a,b; Barker *et al.*, 1993). There is paucity of reports on the effect of consumption of aqueous extract of HS on gestational outcome.

The present study was designed to investigate the effect of consumption of aqueous extracts of HS during gestation on gestational outcome and to examine the possible mechanism by which the effect(s) is (are) mediated.

MATERIALS AND METHODS

Experimental animals: Eighteen in-bred virgin female Sprague-Dawley rats age between 10-12 weeks and weighing 125 ± 5.5 g (mean \pm SEM) 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 allow for mating. Day 1 of pregnancy was taken as the day sperm were seen in the vaginal smear of the rats. On day 1 of pregnancy, animals were divided randomly into three groups of six animals each. Group A (control)

was given tap water to drink. Group B was given 0.6g-extract/100ml while Group C was given 1.8g-extract/100ml as their drinking solution.

All groups received normal rat chow and their drinking solution *ad libitum*. Fluid and food intake and dam weights were measured daily throughout pregnancy and at delivery. Gestational length, litter size and weight were also recorded.

Flame photometry: On gestational day 18, blood samples were withdrawn from the orbital sinus in each rat in each group. The blood sample was then put in a heparinized tube and centrifuged. The sodium ion content of the plasma was assessed by flame photometry.

Extraction procedure: Mature dry dark-red calyces of HS were purchased from a local market in Enugu, Nigeria and authenticated by Mr. T.I. 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 (Iyare and Adegoke, 2008). Briefly, 30g of the dry petals of HS was brewed in 400ml of boiled tap water for 45min. The resulting decoction was filtered and evaporated to dryness giving a dark red powder (yield 48.87%).

0.6g and 1.8g of the dark red powder were weighed and dissolved in 100ml of tap water and then given to groups B and C respectively as their drinking solution.

Statistical analysis: The student's t-test for paired data was used to analyze data from the same group of rats. For data comparison between the three groups, 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.

RESULTS

Maternal fluid and food intake: There was a significant reduction ($P < 0.05$) in fluid (Table 1) and food (Table 2) consumption in all trimesters of pregnancy in the HS groups (B and C) compared with control group (A). This reduction commenced in the first trimester (early in pregnancy).

Weight gain during gestation: The term weight, absolute weight gain and the per cent (%) weight gain (defined as $(\text{Term weight} - \text{Pregravid weight}) / \text{Pregravid weight} \times 100$) were significantly reduced ($P < 0.05$) in the HS groups (B and C) compared with the control group (A) (Table 3).

Length of gestation, litter size and weight: There was no significant difference in the length of gestation among the various groups. There was a significant reduction

Table 1: Effect of consumption of Hibiscus sabdariffa during pregnancy on fluid intake per day

| Groups | Mean fluid intake (ml/rat/day) | | |
|----------------|--------------------------------|---------------|---------------|
| | 1st trimester | 2nd trimester | 3rd trimester |
| 0 (A) | 31.0±0.97 | 36.17±1.22 | 29.17±1.45 |
| 0.6g/100ml (B) | 18.33±0.95* | 22.0±1.03* | 23.33±0.99* |
| 1.8g/100ml (C) | 17.33±0.84* | 18.5±0.65P | 24.17±0.54* |

A = Control group (tap water, no HS), B and C = test groups (HS). N = 6 each. Values are expressed as Mean±SEM, * = $P < 0.05$ vs A, P = $P < 0.05$ vs A and B.

Table 2: Effect of consumption of Hibiscus sabdariffa during pregnancy on mean food intake

| Groups | Mean food intake (g/rat/day) | | |
|----------------|------------------------------|---------------|---------------|
| | 1st trimester | 2nd trimester | 3rd trimester |
| 0 (A) | 21.0±1.46 | 27.33±0.95 | 24.17±0.83 |
| 0.6g/100ml (B) | 17.0±0.73* | 22.33±1.20* | 21.0±0.73* |
| 1.8g/100ml (C) | 17.5±1.28* | 17.83±0.79P | 18.5±0.89* |

A = Control group (tap water, no HS), B and C = test groups (HS). N = 6 each. Values are expressed as Mean±SEM, * = $P < 0.05$ vs A, P = $P < 0.05$ vs A and B.

($P < 0.05$) in litter size and a significant increase ($P < 0.05$) in the litter birth weight in the HS groups (B and C) compared with the control group (A) (Table 4). There was no significant difference in litter size between groups B and C even though group C had quantitatively fewer litters.

Plasma Na⁺ concentration: There was significantly elevated ($P < 0.05$) plasma Na⁺ concentration in the HS groups (B and C) compared with the control group (Table 5).

DISCUSSION

HS dams (groups B and C) drank less fluid compared with the control dams (group A) at all the trimesters of pregnancy (Table 1) possibly because the HS solution was not sweetened and hence unpalatable to them. The decreased fluid intake, which commenced early in pregnancy, may have caused a state of water deprivation and a consequent increase in plasma Na⁺ observed in the HS dams (Table 5). This is in agreement with the reports of earlier workers. For example, Mojiminiyi *et al.* (2000) in their investigation of the diuretic property of HS observed that rats that consumed HS had elevated plasma Na⁺. Also, Ross and Desai (2005) reported that water deprivation causes hypernatremia. Aqueous extract of HS has also been shown to be rich in Na⁺ (Adigun *et al.*, 2006) implying that rats consuming HS may be increasing their oral Na⁺ load and thus consequent increased plasma Na⁺. Another possible mechanism for the elevation of plasma Na⁺ may be due to the action of the flavonoid content of HS. Wang *et al.* (2002) reported that flavonoids inhibit 11 β-hydroxysteroid dehydrogenase (11 β-OHSD). This enzyme is found in the kidneys and elsewhere in the

Table 3: Effect of consumption of Hibiscus sabdariffa during pregnancy on weight gain

| Groups | Pregavid wt (g) | | wt gain (g) | % wt gain |
|----------------|-----------------|--------------|-------------|-------------|
| | Term wt (g) | Absolute | | |
| 0 (A) | 124.36±3.73 | 227.5±5.40 | 103.13±2.37 | 83.05±2.24 |
| 0.6g/100ml (B) | 122.5±8.05 | 211.67±9.28* | 89.17±2.20* | 73.31±4.38* |
| 1.8g/100ml (C) | 122.5±2.3 | 202.5±4.5* | 80.0±5.1* | 65.5±4.9* |

wt = weight, A = Control group (tap water, no HS), B and C = test groups (HS). N = 6 each. Values are expressed as Mean ± SEM, * = P<0.05 vs A.

Table 4: Effect of consumption of Hibiscus sabdariffa during pregnancy on length of gestation, litter size and litter birth weight

| Groups | Length of gestation(days) | Litter size (n) | Litter weight (g) |
|----------------|---------------------------|-----------------|-------------------|
| | | | |
| 0.6g/100ml (B) | 22.15±1.07 | 6.50±0.76* | 6.00±0.14* |
| 1.8g/100ml (C) | 21.75±0.75 | 4.67±0.88* | 6.11±0.12* |

A = Control group (tap water, no HS), B and C = test groups (HS). N = 6 each. Values are expressed as Mean ± SEM, * = P<0.05 versus control group (A).

body and catalyzes the conversion of the active glucocorticoids to the inactive forms. In the kidney glucocorticoids can combine with the mineralocorticoid receptors to mediate a mineralocorticoid effect (Na⁺ reabsorption). Thus, inhibition of 11 β-OHSD by HS flavonoids increases the concentration of active glucocorticoids at the mineralocorticoid receptors. This results in increased Na⁺ reabsorption in the kidneys and consequent elevation in plasma Na⁺ concentration.

The reduced fluid intake in the HS dams and the accompanying plasma hypernatremia may have caused dehydration-anorexia (Ross and Desai, 2005) with the resultant decrease in food consumption (Table 2) and decreased pregnancy weight gain (Table 3) in these dams.

The decreased litter size (Table 4) observed in the HS dams, in this study, may be due to a possible interaction of the HS constituents (especially the flavonoids) with the endometrium and/or blastocyst in a concentration dependent manner (Nivsarkar *et al.*, 2005) to decrease the number of implantation sites.

Since maternal malnutrition during pregnancy has been shown to result in low birth weight (Barker, 2000; Seckl, 1998; Philips *et al.*, 1998; Lesage *et al.*, 2001; Fowden and Forhead, 2004), the reduced maternal food intake induced by HS consumption, surprisingly, did not result in low birth weight. This may be due to a variety of reasons. Firstly, more nutrients and space may have been available to the reduced fetuses in the HS dams compared with the control dams. Secondly, the developing fetuses of the HS dams may have recruited adaptive mechanisms to protect their growth (Gluckman and Hansen, 2004a,b) and/or the malnutrition in the HS dams that commenced early in pregnancy, may have caused placental development that favoured increased nutrient delivery to the developing foetuses (Woodall *et al.*, 1996; Osgerby *et al.*, 2002; Fowden *et al.*, 2006,

Table 5: Effect of consumption of Hibiscus sabdariffa during pregnancy on plasma Na⁺ concentration

| Na ⁺ (mmol/L) | | |
|--------------------------|----------------|----------------|
| 0 (A) | B (0.6g/100ml) | C (1.8g/100ml) |
| 135.0±0.73 | 143.0±0.93* | 144.0±1.15* |

A = Control group (tap water, no HS), B and C = test groups (HS). N = 6 each. Values are expressed as Mean±SEM, * = P<0.05 vs A.

Thirdly, the constituents of HS may have directly influenced the growth of the fetuses. For example, vitamin C (Jain *et al.*, 2008; Wu *et al.*, 2008; Yajnik, 2006), vitamin A and Fe (Ceasay *et al.*, 1997; Yajnik, 2006) and flavonoids (Hilakivi-Klarke *et al.*, 1998) have been shown to have direct growth promoting effect.

In conclusion, the results of this study seem to justify the need for the exercise of caution in the use of aqueous extract of HS in pregnancy and since birth weight is not a reliable index for the degree of developmental compromise, the assessment of postnatal growth and health in adulthood in these pups will be instructive.

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