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Effect of Lactational Exposure to an Aqueous Extract of *Hibiscus sabdariffa* on Body Mass Index at Onset of Puberty in Female Sprague - Dawley Rats

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Abstract: The present study aimed to investigate whether or not maternal consumption of aqueous extract of *Hibiscus sabdariffa* (HS) during lactation has any effect on the body weight and body mass index at onset of puberty in the female offspring. 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. On the day of delivery, the dams and their pups were randomly assigned to one of three groups of 6 rats/group. One group had tap water (control), another had 0.6g-HSextract/100ml in their drinking water while the third group had 1.8g-HSextract/100ml in their drinking throughout lactation. Results showed that maternal consumption of aqueous extract of HS during lactation at the doses tested, delayed onset of puberty and elevated body weight and body mass index at onset of puberty in female offspring.

Key words: *Hibiscus sabdariffa*, body mass index, onset of puberty, lactation

INTRODUCTION

Nutritional perturbations have been observed to affect the reproductive performances of many species. This knowledge has been applied to the rearing of domestic species for at least a century. Nutritional perturbations, when applied during lactation, have demonstrated long-term programming of the function of specific organ systems (Hoet and Hanson, 1999; Guzman *et al.*, 2006) including the reproductive system.

Kennedy and Mitra (1963) showed that female rats undernourished during lactation had delayed onset of puberty accompanied by a significantly lower body weight. This is not surprising because with food scarcity, these animals are forced to set priorities, shutting off reproductive functions for the survival of the species (Wade and Schneider, 1992; Wade *et al.*, 1996; Glass *et al.*, 1986) since one reproductive cycle of ovulation, conception, pregnancy and lactation is one of the most energy consuming activities a female mammal can undertake, particularly in species that bear multiple young (Engelbregt *et al.*, 2001).

In Nigeria, a sweetened aqueous extract of *Hibiscus sabdariffa* (HS) (family: Malvaceae), generally called "zobo" drink, is commonly produced, sold and consumed by both adults and children, male and female alike usually as a substitute for carbonated soft drinks because of the immense cost difference and the ready availability of HS. HS extract has been shown to decrease fluid and food intake (Iyare and Adegoke, 2008; Ojokoh, 2006; Orisakwe *et al.*, 2004) and causes diuresis in rats (Mojiminiyi *et al.*, 2000) and anecdotal reports by those who consume zobo drink seem to strengthen these observations. It therefore follows that

when aqueous extract of HS is administered to lactating animals, it may lead to decreased food consumption in these animals and a consequent poor lactation that may result in decreased nutrient delivery to the suckling neonates.

It is not yet established whether or not maternal consumption of aqueous extract of HS during lactation has any effect on the body weight and body mass index at onset of puberty in the female offspring. The present study was therefore designed this.

MATERIALS AND METHODS

Experimental animals: 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 housed individually in cages under standard environmental conditions. The estrus 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 estrus phase within twelve hours to allow for mating. Day one of pregnancy was taken as the day sperm were seen in the vaginal smear of the rats. From day one of pregnancy till delivery, animals had *ad libitum* access to food and water. On the day of delivery, the dams and their pups were divided randomly into three groups of six dams each. The first group (control) was given tap water to drink throughout lactation while the second and third groups (HS groups) were given 0.6g-HSextract/100ml and 1.8g-HSextract/100ml respectively to drink throughout lactation. All groups received normal rat chow *ad libitum*. Fluid and food intake and dam weights were measured

daily throughout lactation. Each dam in each group was allowed 9 pups to nurse throughout the lactational period so as to eliminate the effect of undernutrition or overnutrition of some of the pups. After 21 days, the pups were weaned to tap water. After weaning, the female pups were kept in groups of three per cage. Pup's weight were recorded at birth, weaning and weekly thereafter till onset of puberty. Pubertal development starts soon after weaning, so from postnatal day 30 onwards, the young female rats were inspected daily for vaginal opening since onset of puberty is defined as the age (in days) at which vaginal opening occurs (Engelbregt *et al.*, 2000).

Extraction procedure: Mature dry dark-red calyces of HS were purchased from a local market in Lagos, 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: Results of the present study show a significant reduction in fluid and food intake by the HS groups compared with the control group throughout lactation (Tables 1 and 2).

The fluid intake was not dose dependent (since there was no difference between fluid intakes in the HS groups) whereas the decreased food intake appeared to be dose dependent. There was no difference in fluid intake between the first and second week of lactation in the control and rats given 1.8g-HSextract/100ml while rats given 0.6g-HSextract/100ml drank more fluid in the second week compared with the first week. Food intake in the second week was not different from the first in rats given 1.8g-HSextract/100ml while control and 0.6g-HSextract/100ml rats consumed more food in the second week compared with the first week.

Fluid intake in the third week was no different from the first week in the control group while groups 0.6g-Hsextract/100ml and 1.8g-HSextract/100ml rats drank

Table 1: Effect of maternal consumption of aqueous extract of Hibiscus sabdariffa on volume of fluid consumed during lactation

Groups	Volume (ml/kg b w/d) of fluid consumed		
	1st week of lactation	2nd week of lactation	3rd week of lactation
Control	224.68±23.38	263.19±22.69	211.59±5.77 [⊘]
0.6g/100ml	133.04±4.15 [*]	165.56±11.21 ^{⊘*}	169.42±4.77 [*]
1.8g/100ml	133.29±4.14 [*]	128.77±2.88 [*]	169.44±1.64 ^{⊘*}

N=6 each. Values are expressed as Mean ± SEM. ^{*} = $P < 0.05$ vs control, [⊘] = $P < 0.05$ vs 1st week, [⊘] = $P < 0.05$ vs 1.8g/100ml group, [⊘] = $P < 0.05$ vs 2nd week.

Table 2: Effect of maternal consumption of aqueous extract of Hibiscus sabdariffa on amount of food consumed during lactation

Groups	Amount (g/kg b w/d) of food consumed		
	1st week of lactation	2nd week of lactation	3rd week of lactation
Control	156.87±4.13	195.65±8.06 [⊘]	179.73±5.52 [⊘]
0.6g/100ml	127.4±4.25 ^{⊘*}	159.9±3.32 ^{⊘*}	52.75±5.37 ^{⊘*}
1.8g/100ml	1116.78±4.01 [*]	117.43±3.37 [*]	127.29±5.37 ^{⊘*}

N=6 each. Values are expressed as Mean ± SEM. ^{*} = $P < 0.05$ vs control, [⊘] = $P < 0.05$ vs 1st week, [⊘] = $P < 0.05$ vs 1.8g/100ml group, [⊘] = $P < 0.05$ vs 2nd week.

more fluid in the third week compared with the first week. Rats in all groups consumed more food in the third week than in the first week. There was no difference between fluid intake in the second and third week in all groups except group 1.8g-HSextract/100ml rats that consumed more food in the third week compared with the second week.

Maternal weight during lactation: There was no significant difference between lactational weight measurements at postpartum days 7, 14 and 21 compared with postpartum day 0 in all groups except 0.6g-HSextract/100ml dams that showed significant weight increases compared with postpartum day 0 (Table 3).

Body parameters at onset of puberty: There was a significant delay in the onset of puberty and an elevated body weight, body length and BMI in the HS groups compared with the control group (Table 4).

Organ weight and weight index at onset of puberty: The absolute weights and the weight index of the heart, liver and spleen were all significantly greater in the offspring of HS groups compared with the offspring of the control group. The absolute weight of the kidney, but not the weight index, was also greater in the offspring of the HS groups compared with the offspring of the control group (Table 5 and 6).

DISCUSSION

In the present study we focused on body weight and BMI at onset of puberty in rats whose mothers drank HS

Table 3: Effect of consumption of aqueous extract of Hibiscus sabdariffa on maternal weight changes during lactation

Groups	Maternal weight changes (g)				
	Pregravid wt	PPD 0	PPD 7	PPD 14	PPD 21
Control	125.0±4.5	180.0±12.7 [§]	189.8±6.9 [§]	194.0±9.5 [§]	183.7±8.3 [§]
0.6g/100ml	128.8±8.6	177.5±0 [§]	185.5±2.5 ^{P&}	200.5±0.5 ^{P&}	202.0±3.5 ^{P&}
1.8g/100ml	122.5±2.3	167.5±7.8 [§]	172.9±5.7 [§]	183.8±7.8 [§]	185.1±7.1 [§]

N=6 each. Values are expressed as Mean±SEM. Wt = weight, PPD = postpartum day.

^P = P< 0.05 vs 1.8g/100ml group, [§] = P< 0.05 vs pregravid wt.

Table 4: Effect of maternal consumption of Hibiscus sabdariffa during lactation on some body parameters of the offspring at onset of puberty

Body parameters	Groups		
	Control	0.6g/100ml	1.8g/100ml
Age (days)	43.11±1.84	49.67±1.55*	48.78±1.70*
Weight (g)	58.89±1.96	116.11±5.70*	100.28±4.92*
Body length (cm)	12.9±0.15	15.60±0.24 ^P	14.81±0.18*
Body mass index (BMI) (g/cm ²)	0.35±0.006	0.47±0.01*	0.45±0.01*

N = 9 each. Values are expressed as Mean ±SEM, * = P<0.05 vs Control. ^P = P<0.05 vs. Control and 1.8g/100ml.

Table 5: Effect of maternal consumption of Hibiscus sabdariffa during lactation on absolute weight of offspring visceral organs at onset of puberty

Groups	Absolute weight (g)				
	Kidney	Heart	Liver	Spleen	Adrenal glands
Control	0.610±0.029	0.243±0.013	2.66±0.16	0.135±0.017	0.020±0.002
0.6g/100ml	1.090±0.026 ^P	0.442±0.012*	5.672±0.205*	0.485±0.076*	0.043±0.002 ^P
1.8g/100ml	0.958±0.037*	0.413±0.012*	5.046±0.304*	0.411±0.099*	0.030±0.003*

N = 9 each. Values are expressed as Mean ±SEM, * = P<0.05 vs Control. ^P = P<0.05 vs Control and 1.8g/100ml.

Table 6: Effect of maternal consumption of Hibiscus sabdariffa during lactation on the weight index of offspring visceral organs at onset of puberty

Groups	Weight index (x 10 ⁻³)				
	Kidney	Heart	Liver	Spleen	Adrenal glands
0 (A)	10.323±0.184	4.047±0.132	44.959±1.872	2.248±0.225	0.340±0.018
0.6g/100ml	9.507±0.349	3.847±0.109	49.24±1.367	4.140±0.554*	0.381±0.028 ^P
1.8g/100ml	9.604±0.184*	4.162±0.139	50.247±1.391*	3.866±0.740	0.299±0.020

N = 9 each. Values are expressed as Mean ± SEM, * = P<0.05 vs Control, ^P = P<0.05 vs 1.8g/100ml.

during lactation. BMI is an easily obtainable, widely accepted measurement showing correlations with percentage body fat (Maffei *et al.*, 1995; Twisk *et al.*, 1998; Blum *et al.*, 1997), absolute fat mass (Horlick *et al.*, 2000) and serum leptin (Maffei *et al.*, 1995; Blum *et al.*, 1997; Horlick *et al.*, 2000).

At onset of puberty, there was a significantly elevated body weight and BMI in offspring of rats that drank HS during lactation compared with the offspring of the control rats. This may suggest that in the offspring of the HS rats, the normal nutritional circumstances and metabolic conditions postnatally did not lead to normal body composition at onset of puberty. The onset of puberty was delayed in offspring of HS rats compared with the offspring of the control rats. This suggests that the onset of puberty in the offspring of HS rats is not related to body weight, BMI or a certain percentage of body fat. This is in contrast with the observations of Frisch *et al.* (1977) and Frisch (1980) who hypothesized

that a certain percentage of body fat is needed to start puberty.

The metabolic pathway to link body fat stores with the neuroendocrine reproductive system was unclear until the discovery of the adipocyte-derived hormone leptin. The finding that leptin circulates in plasma in proportion with body adiposity (Maffei *et al.*, 1995) led to the theory that leptin acts as an "adipostat", a humoral signal carrying information regarding energy reserves. The possibility of a link between leptin and reproduction became apparent when it was observed that a homozygous mutation in the leptin (*ob*) gene was responsible for the obesity syndrome in the obese (*ob/ob*) mouse (Zhang *et al.*, 1994). It has also been suggested that leptin is the signal that informs the brain that metabolic stores are adequate for the initiation of reproductive function (Chehab *et al.*, 1996), thus ensuring that scarce energy resources are not wasted on reproductive efforts that are unlikely to succeed.

Since body weight, BMI and serum leptin levels are highly positively correlated (Butzow *et al.*, 1999; Maffei *et al.*, 1995; Considine *et al.*, 1996; Butte *et al.*, 1997) and since puberty confers reproductive competence, the delayed onset of puberty coupled with high body weight and BMI in the offspring of HS dams may suggest a depression of the leptin-signaling pathway that normally informs the brain that energy resources are adequate to support pregnancy.

We hypothesize that this depression of the leptin-signaling pathway may have been programmed during lactation. This is inferred from the fact that in rodents, neuroendocrine maturation occurs mainly during the early postnatal period, as opposed to primates, in which it takes place during the third trimester of gestation and that the decreased food intake in the HS dams may have led to decreased leptin levels in these dams (Maffei *et al.*, 1995; Frederich *et al.*, 1995). Since leptin is transported from the mother to the neonate through breast milk (Hoggard *et al.*, 1997; Banks *et al.*, 1996), the decreased leptin level may have acted as the metabolic signal to the neonate of the status of maternal energy reserves and by extension, environmental food availability thus inducing in the neonate some of the metabolic adaptations that are designed to enhance postnatal survival under conditions of poor nutrition (Hales and Barker, 1992).

The weaning of the pups from HS dams to ad libitum food and water may represent a deviation from the nutritional plane to which the pups had adapted. This metabolic conflict may have caused the cardiomegaly, hepatomegaly and splenomegaly observed in the pups from the HS dams and consequently the elevated body weight and BMI at onset of puberty.

We conclude that maternal consumption of aqueous extract of HS during lactation at the doses tested, elevated body weight and BMI at onset of puberty in female offspring through mechanism that may depend on disruption of the leptin-signaling pathway.

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