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MATERNAL CONSUMPTION OF AN AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* DURING LACTATION ACCELERATES POSTNATAL WEIGHT AND DELAYS ONSET OF PUBERTY IN FEMALE OFFSPRING

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Summary: The present study was designed to investigate whether maternal consumption of aqueous extract of *Hibiscus sabdariffa* (HS) during lactation will affect the postnatal growth and onset of puberty in the female offspring. Eighteen in-bred virgin female Sprague-Dawley rats aged between 10-12 weeks and weighing 125 ± 5.5g (mean ± SEM) with two consecutive regular 4-day estrus cycles were randomly assigned to one of three groups of 6rats/group. One group had tap water (Control); another had 0.6g/100ml while the third group had 1.8g/100ml in their drinking water throughout lactation (21 days). Results showed that HS consumption during lactation significantly ($P<0.05$) decreased maternal fluid and food intake, increased postnatal weight gain and delays the onset of puberty in the female offspring.

Keywords: *Hibiscus sabdariffa*; postnatal weight gain; onset of puberty

Introduction

The period of lactation (the first 21 days of life), when the mother can pass along contaminants to her offspring, represents a critical period of reproductive development for the rat pups as the male and female reproductive systems undergo substantial development in the first three weeks of life (Pelletier, 2001; Rodriguez et al, 2002; Cyr *et al*, 2002). Kennedy and Mitra (1963) have earlier reported that rat pups undernourished during lactation have delayed puberty onset. This suggests a link between nutrition and the onset of puberty.

The metabolic pathway linking nutrition with the neuroendocrine reproductive system was unclear until the discovery of the hormone leptin (a 167-amino acid peptide hormone) (Zhang *et al*, 1994) which has been postulated to be the metabolic signal that triggers puberty (Bronson and Manning, 1991; Campfield *et al*, 1996; Chehab *et al*, 1997) by interacting with the GnRH neuron (Odell, 1992; Lee, 1995) and causing changes in the frequency and magnitude of GnRH pulses which has been shown to herald the onset of puberty (Wu *et al*, 1996; Terasawa, 1995). Since puberty confers reproductive competence, the existence of a metabolic pathway linking nutrition with the neuroendocrine reproductive system ensures that scarce energy resources are not wasted on reproductive efforts that are unlikely to succeed since one complete 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).

Extracts of *Hibiscus sabdariffa* (HS) have been used in folk medicine in the treatment of several complaints including high blood pressure, liver diseases and fever (Ali et al, 2005; Tseng *et al*, 1997; Usoh *et al*, 2005). "Zobo" drink (a sweetened water extract of the dry petals of HS) is commonly produced, sold and consumed in Nigeria without caution by both males and females. It is consumed as a substitute for carbonated drinks and fruit juices and not necessarily for medicinal reasons. Some people have been observed consuming *zobo* drink during lactation. Extract of HS has also been reported to decrease fluid and food intake following subchronic administration through a mechanism not yet fully understood (Orisakwe *et al*, 2003; Orisakwe *et al*, 2004; Ojokoh, 2006). Thus, when aqueous extract of HS is administered to lactating rats, it may lead to decreased food consumption in these rats and a consequent poor nutrition of the neonates with the attendant developmental sequelae (Barker et al, 1993; Gluckman and Hanson, 2004; Armitage *et al*, 2005a and b).

The present study was designed to investigate whether or not maternal consumption of aqueous extract of HS during lactation has effect of postnatal body weight gain and the onset of puberty in the offspring.

Materials and Methods

Experimental animals

Eighteen in-bred virgin female Sprague-Dawley rats aged between 10-12 weeks and weighing 125 ± 5.5g (mean ± 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 12 hours to allow for mating. Day 1 of pregnancy was taken as the day sperm were seen in the vaginal smears of the rats. From day 1 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 were given 0.6g-extract/100ml and 1.8g-extract/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 and 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. Pups 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 vagina opening occurs (Engelbregt et al, 2000).

Extraction Procedure

Mature calyces of HS were purchased from a local market in Enugu, Nigeria, and authenticated at the Department of Botany, University of Nigeria, Nsukka, Nigeria. The extraction procedure used was as described previously (Adegunloye et al, 1996) but with slight modification. 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%).

Statistical analysis

The Student's *t-test* for paired data was used to analyse 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 extract groups) whereas the decreased food intake was dose dependent. There was no difference in fluid intake between the first and the second week of lactation in the control and rats given 1.8g/100ml while rats given 0.6g/100ml drank more fluid in the second week compared with the first week. Food in the second week was not different from the first in rats given 1.8g/100ml while control and 0.6g/100ml rats consumed more food in the second week compared with the first week.

Fluid intake in the third week was not different from the first week in the control group while groups 0.6g/100ml and 1.8g/100ml rats drank 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/100ml rats that consumed more food in the third week compared with the second week.

Table 1: Effect of maternal exposure to aqueous HS on volume of fluid (ml) consumed during lactation.

Group	Period of Lactation		
	1st week	2nd week	3rd week
0.6g/100ml	133.04 ± 4.15*	165.56 ± 11.21*$P	169.42 ± 4.77*$
1.8g/100ml	133.29 ± 4.14*	128.77 ± 2.88*	169.44 ± 1.64*$H
Control	224.68 ± 23.38	263. 19 ± 22.69	211.59 ± 5.77H

*N=6 each. Values are expressed as Mean ± SEM. *= P<0.05 compared with Control group. $ = P<0.05 compared with 1st week. P = P<0.05 compared with 1.8g/100ml group. H = P<0.05 compared with 2nd week.*

Table 2: Effect of maternal exposure to aqueous HS on food consumption (g) during lactation

Group	Period of Lactation		
	1st week	2nd week	3rd week
0.6g/100ml	127.4 ± 4.25*P	159.9 ±3.32*$P	152.57 ± 5.37*P$
1.8g/100ml	116.78 ± 4.01*	117.43 ± 3.37*	127.29 ± 5.37*$H
Control	156.87 ± 4.13	195. 65 ±8.06$	179.73 ± 5.52$

*N=6 each. Values are expressed as Mean ± SEM, *= P<0.05 compared with Control group, $ = P<0.05 compared with 1st week, P = P<0.05 compared with 1.8g/100ml group, H = P<0.05 compared with 2nd week.*

Table 3: Effect of HS on maternal weight changes during lactation.

.Maternal weight changes (g)	Groups		
	0.6g/100ml	1.8g/100ml	Control
Pregravid weight	128.8 ± 8.6	122.5 ± 2.3	125.0 ± 4.5
PND 0	177.5 ± 0H	167.5 ±7.8H	180.0 ± 12.7H
PND 7	185.5 ± 2.5PH	172.9 ± 5.7H	189.8 ± 6.9H
PND 14	200.5±0.5PH	183.8±7.8H	194.0±9.5H
PND 21	202.0±3.5PH	185.1±7.1H	183.7±8.3H

N=6 each. Values are expressed as Mean ± SEM. PPD = postpartum day, P = P<0.05 compared with PPD 0.H = P<0.05 compared with pregravid weight

Maternal Body Weight

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 group 0.6g/100ml dams that showed significant weight increases compared with postpartum day 0 (Table 3).

Table 4: Effect of Lactational exposure to HS on absolute postnatal weight of offspring.

weight changes (g)	Groups		
	0.6g/100ml	1.8g/100ml	Control
PND 0 (Birth)	5.72 ± 0.09	5.67 ± 0.14	5.61 ± 0.14
PND 21(weaning)	26.89 ± 1.8*	28.61 ±1.31*	22.78 ± 0.65
PND 28	41.83 ±2.46*	44.44 ± 1.34*	32.78 ± 0.77
PND 35	76.67±1.44P*	62.22±0.98*	48.33±0.93
PND 42	80.56±5.8*	82.5±0.63*	55.83±1.44

*N=9 each. Values are expressed as Mean ± SEM. PPD = postpartum day, * = P<0.05 compared with Control; P = P<0.05 compared with 1.8g/100ml.*

Offspring Postnatal Weight

Results show a significantly increased body weights at postnatal days 21, 28, 35 and 42 in the HS group compared with the control group (Table 4). There was no difference between the body weights of groups 0.6g/100ml and 1.8g/100ml offspring at all periods of measurement (except at PND 35 when group 0.6g/100ml offspring were bigger than 1.8g/100ml offspring), suggesting no dose dependent effect.

Age and Body weight of offspring at onset of puberty

There was a significant delay in the onset of puberty and an elevated body weight in the HS groups compared with the control group (Table 5).

Discussion

Since dams that were given aqueous HS extract drank less fluid compared with the control dams during lactation (Table 1), the decreased fluid intake may have caused a state of water deprivation and a consequent plasma hypernatraemia in these dams (Ross and Desai, 2005). Aqueous extract of HS has also been shown to be rich in Na^+ (Adigun *et al*, 2006). This, coupled with the reported diuretic effect of HS (Mojiminiyi et al, 2000) may have caused the plasma hypernatraemia observed by Mojiminiyi and Co-workers (2000).

Table 5: Effect of lactational exposure to HS on some body parameters at onset of puberty

Parameters	Groups		
	0.6g/100ml	1.8g/100ml	Control
Age (days)	49.67 ±1.55*	48.78 ±1.70*	43.11 ±1.84
Weight (g)	116.11 ±5.70*	100.28 ±4.92*	58.89 ±1.96

*N=9 each. Values are expressed as Mean ± SEM. PPD = postpartum day; * = P<0.05 compared with Control.*

The water deprivation in these dams and the accompanying plasma hypernatraemia (osmotic stress) may have caused dehydration-anorexia (Ross and Desai, 2005) with the resultant decrease in food intake (nutrient stress).

We speculate that the decreased food intake in the dams that drank HS may have led to decreased leptin levels in these dams (Maffei et al, 1995; Frederich *et al*, 1995). Since leptin is transferred from the mother to the neonate through breast milk (Houseknecht *et al,* 1997) and contributes to the neonatal leptin surge (Ahima *et al*, 1998) that is necessary for the normal development and maturation of the hypothalamic neurocircuitry that regulates body weight (Bouret *et al*, 2004; Bouret and Simerly, 2004), 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 in accordance with the "thrifty phenotype" hypothesis (Hales and Barker, 1992). That the "thrifty phenotype" offspring are better able to acquire and utilize nutrient and demonstrate an increase risk of obesity as adults (Barker *et al*, 1993) may have accounted for the higher weight increases in the pups from dams that were given HS extract during lactation compared with the control pups. The higher weights in the pups of HS dams may also suggest an abnormal development of the hypothalamic neurocircuitry that regulate body weight (Plagemann *et al*, 2000).

Vagina opening is considered a good marker of the onset of puberty in the female rat (Engelbregt *et al*, 2002). In the present study, there was a delayed onset of puberty (Table 4) in the pups from dams that were given HS extract during lactation through mechanisms that may not be unconnected with the ability of HS extract to decrease fluid and food intake (and consequently creating osmotic and nutrient stresses) since malnutrition during the lactational period has been shown to delay the onset of puberty (Engelbregt *et al*, 2001). Osmotic and nutrient stresses are known to result in elevated glucocorticoid level. Elevated plasma glucocorticoid level causes a permanent resetting of endocrine systems, such as the somatotrophic and hypothalamo-pituitary adrenal axes (Barker, 2000; Seckl, 1998; Edwards et al, 1993; Phillips et al, 1998; Lesage et al, 2001; Fowden and Forhead, 2004) and a delay in the onset of puberty in female offspring (Smith and Waddell, 2000) possibly by causing altered behavior and abnormalities in hypothalamic-pituitary-gonadal function (Wellberg *et al*, 2001).

In conclusion, the results of the present study have shown that maternal consumption of aqueous extract of HS during lactation accelerates offspring postnatal growth and delays the onset of puberty through mechanisms that may depend on the ability of HS extract to decrease maternal fluid and food intake.

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