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# Morphologic Changes in the Utero-ovarian Tissues of the Offspring of Rats Exposed to Aqueous Extract of *Hibiscus sabdariffa* During Lactation

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**ABSTRACT**

*Zobo*, a sweetened aqueous extract of *Hibiscus sabdariffa* (HS), is commonly produced and consumed by Nigerians of all ages irrespective of gender and physiologic state as a substitute for carbonated drinks or fruit juices. In this study, we intended to investigate the morphologic changes induced by HS on the utero-ovarian tissues of offspring of Sprague-Dawley (SD) rats whose mothers drank HS during lactation. Eighteen pregnant rats were used for this study. These rats were given *ad libitum* food and water throughout pregnancy. On the day of delivery the rats were randomly divided into three groups of six animals each: Group A continued with the tap water (control) while groups B and C received 0.6g/100ml and 1.8g/100ml aqueous extract of HS respectively as their drinking fluid throughout 21 days postpartum. Results show that the pups of the HS dams had delayed onset of puberty and heavier uteri and ovaries compared with the control. The HS pups also had fewer mature follicles in the ovary and diffuse infiltrates of eosinophils in the endometrium. It was concluded that HS had estrogenic effects on the uterus and inhibitory effects on growth of follicles in the ovaries.

**Key words:** *Hibiscus sabdariffa*, Morphologic changes, Endometrium, Ovarian tissue, Lactation, Sprague-Dawley rats.

Extracts of *Hibiscus sabdariffa* (HS) have been used in traditional medicine in the treatment of several disease states such as hypertension, hepatic disorders and febrile conditions (Ali et al 2005, Tseng et al 1997, Usoh 2005). In Nigeria, '*zobo'*, a sweetened aqueous extract of the dry petals of HS, is commonly produced, sold and consumed as a substitute for carbonated drinks and fruit juice by men, women (even during pregnancy and lactation!) and children. There is paucity of data on the covert or overt effects of the constituents of this drink on the human body especially in critical physiologic states.

In rats as well as mice, coordinated actions of estrogen and progesterone direct uterine cell proliferation and differentiation (Wang et al 2005). Many natural and synthetic compounds mimic estrogen and influence uterine functions by interacting with nuclear estrogen receptors or other signaling molecules. These compounds include environmental xenoestrogens and dietary phytoestrogens (Wang et al 2005). The estrogenic compounds also act as endocrine disrupters causing developmental and reproductive disturbances (Wang et al 2005). Some of the phytochemical constituents of HS include flavonoids, polysaccharides and organic acids and they have been postulated to be responsible for the observed pharmacological effects (Iyare and Iyare 2007).

Estrogens are female sex steroid hormones that have a plethora of effects on a wide range of tissues ranging from the uterus to the brain and skeleton (Pollard 1997). In the uterus of the adult female rats, the luminal and the eosinophil leukocytes are rich in cytoplasmic estrogen receptors (Lee 1982, Maddox et al 1983). An effect of estrogen in the uterus of rats is the invasion of eosinophil granulocytes into the endometrium and the myometrium (Pollard 1997, Schubert 1998). Indeed, the early uterine responses to estrogens look a lot like an inflammatory response characterized by edema and leukocyte recruitment, and analogies have been drawn between the two processes (Pollard 1997). Progesterone prevents the estrogen induced eosinophilia (Schubert 1998). Estrogen in both the adult rat and mouse uterus also stimulates epithelial cell proliferation, increases vascular

permeability and water imbibition leading to increased uterine weight (Pollard 1997). In addition, estrogens also induce the onset of vaginal opening in rats and this is considered a good marker of the onset of puberty in female rats (Iyare and Adegoke 2008, Engelbregt et al 2000, Frisch et al 1975).

The eosinophils in the uterus of rats are rich in cytoplasmic estrogen receptors (Lee 1982) and they migrate from the blood to the uterus under estrogen stimulation, redistribute through uterine extravascular compartment, degranulate in the organ and release agents that are involved in several parameters of estrogen action (Soto et al 1989) such as rapid increases in uterine oedema (Rojas and Steinsapir 1983), uterine blood flow(Stormshak and Bishop 2008) and weight gain (Pollard 1997).

The present study was designed to investigate the morphologic changes induced by HS on the utero-ovarian tissues of offspring of Sprague-Dawley (SD) rats whose mothers drank HS during lactation.

## MATERIALS AND METHODS

### Extraction procedure:

Mature dry dark-red calyces of HS were purchased in a local market in Enugu, Nigeria. It was authenticated at the Department of Botany, University of Nigeria, Nsukka, Nigeria and 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 Iyare 2006a,b, 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.

The concentrations in the exposed groups (groups B and C) were derived as follows: 10mls of filtrate was added to 48mls of tap water to make approximately 0.6g/100ml tap water (group B) while 10mls was added to 9mls of tap water to make approximately 1.8g/100ml tap water (group C).

### Animals and treatment:

Eighteen in-bred virgin female Sprague-Dawley (SD) rats age between 10-12 weeks and weighing 125±5.5g (mean ±SEM) with two consecutive regular 4-day estrous cycles were used for this study. They were housed individually in cages under standard environmental conditions. The estrous cycles were monitored and males 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 spermatozoa were seen in the vaginal smear of the rats. From day 1 of pregnancy through delivery, animals were given food and water *ad libitum.* On the day of delivery, the dams were divided randomly into three groups of six animals each (A, B, and C). Group A had tap water only while groups B and C had 0.6g/100ml and 1.8g/100ml HS respectively in their drinking water throughout lactation (0-21 days postpartum). All groups had normal rat chow *ad libitum*. The pups were weaned and after 21 days they were inspected daily for vaginal opening. The age at vaginal opening (onset of puberty) was recorded and body weight and length measured. Thereafter, the animals were sacrificed and the uteri and ovaries were removed, trimmed of fat, weighed and fixed in 10% fomalin for histopathological study.

### Histopathologic examination:

The uteri and ovaries were removed and fixed in 10% formalin at room temperature. The tissues were processed using standard histopathological procedures of alcohol dehydration and embedding in paraffin wax. Serial sections of 3-5ìu were prepared and stained with haematoxylin eosin (H&E). This was assessed at magnifications of x10, x20, and x40 objectives and photographed by Bresser mikrocular, elektronisches ocular.

## RESULTS

The HS groups (groups B and C) had significantly heavier body weights, body length

and body mass index than the unexposed (control) group at both concentrations (Table 1). The ovaries and uteri were also significantly heavier in the HS group compared with the control at both concentrations (Table 2). The HS groups also had fewer well formed follicles in the ovary than the control (Figs. 1a and 1b respectively) and also had delayed onset of puberty (Table 1).

**Histopathological findings:**

Control group: The endometrium was lined by cuboidal cells with deeply chromatic nuclei with sub epithelial halo (Fig 2b). The endometrial glands were round to oval in shape and lined by cuboidal cells. The stroma was relatively dense and cellular with oval shaped stromal cells having deeply chromatic nuclei (Fig 2b). The ovaries contained many well formed follicles with scanty but highly vascularized stroma (Fig 1a).

HS group: The endometrium was lined by markedly tortuous stratified columnar epithelium with vesicular nuclei. Foci of apoptosis were noted. The endometrial glands were round to oval and lined by the same type of epithelial cells (Fig 2a). The stroma was loose with plump stromal cells having vesicular nuclei. The stroma was diffusely infiltrated by numerous eosinophils (Fig 3). The ovaries had markedly reduced numbers of well formed follicles surrounded by focally dense, oedematous and vascular stroma (Fig 1b).

**Table 1: Effect of maternal consumption of *Hibiscus sabdariffa* during lactation on some body parameters of the offspring at onset of puberty.**

| Body parameters | Group | | |
|---|---|---|---|
| | A | B | C |
| Age (days) | 43.11 1.84 | 49.671.55* | 48.781.70* |
| Weight (g) | 58.89 1.96 | 116.115.70* | 100.284.92* |
| Body length (cm) | 12.9 0.15 | 15.600.24*[P] | 14.810.18* |
| Body mass index (BMI) ($g/cm^2$) | 0.35 0.006 | 0.470.01* | 0.450.01* |

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

**Table 2: Effect of maternal consumption of *Hibiscus sabdariffa* during lactation on ovarian and uterine weights at onset of puberty.**

| Groups | Ovary | Uterus |
|---|---|---|
| A | 0.0170.002 | 0.0960.043 |
| B | 0.0500.002*[P] | 0.1560.012 |
| C | 0.0620.001* | 0.1640.016 |

*N* = 9 each. Values are expressed as M±SEM, * = P<0.05 compared with Control, [P] = P<0.05 compared with 1.8g/100ml

## Figures

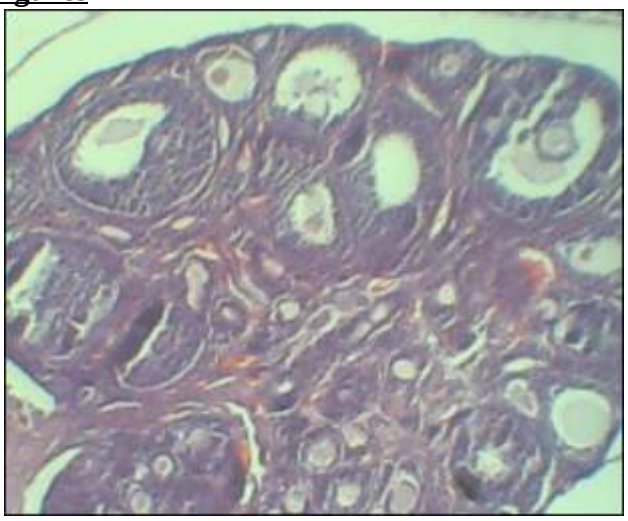

**Fig. 1a:** Ovary of offspring of Control rats showing many well formed follicles

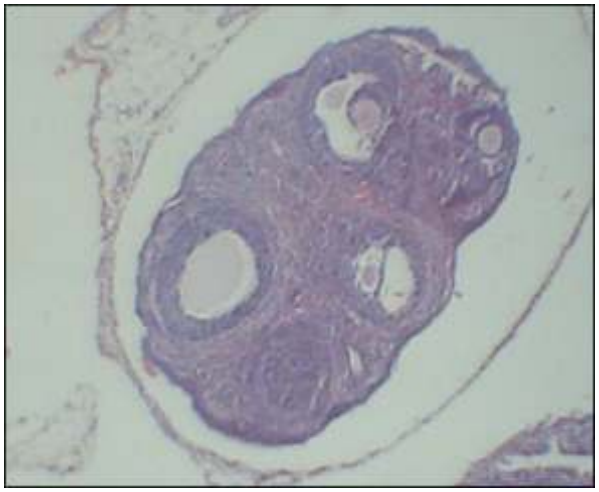

Fig. 1b: Ovary of offspring of HS rats showing scanty follicles

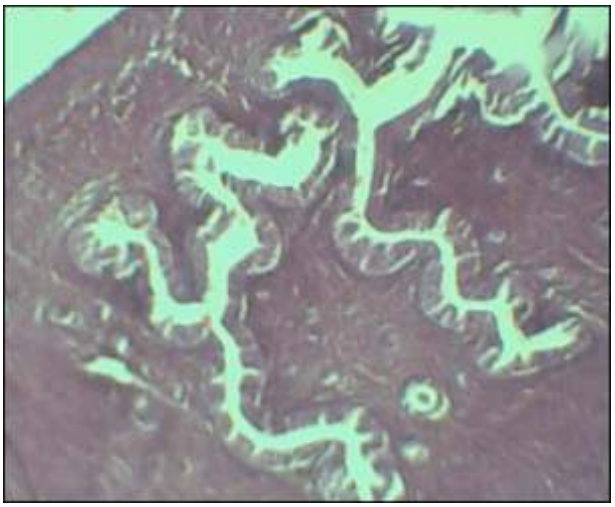

Fig. 2a: Endometrium of offspring of HS rats.

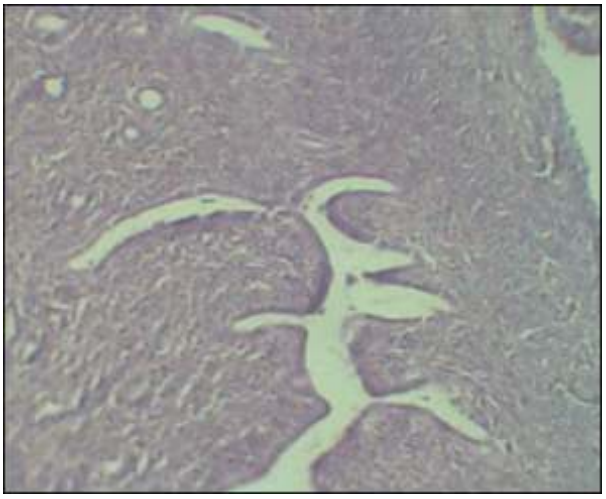

Fig. 2b: Endometrium of offspring of Control rats.

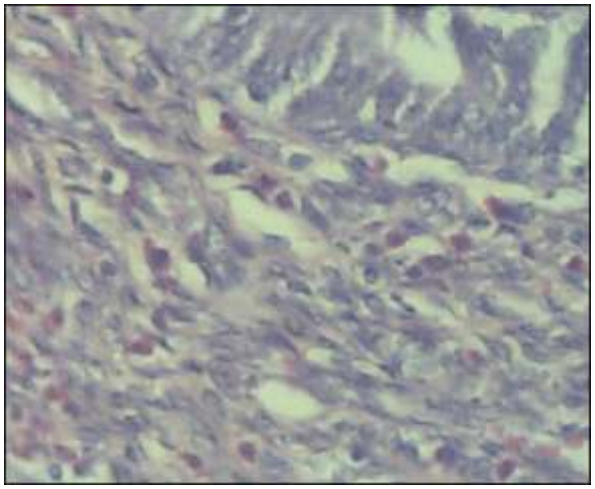

Fig. 3: Uterus of the offspring of HS rats showing eosinophil infiltrates

## DISCUSSION

The findings of increased endometrial epithelial height and tortuosity as well as diffuse infiltration of the stroma with numerous eosinophils, increased uterine and ovarian tissue weights in this study, point to marked estrogenic effects on these tissues (Wang et al 2005, Pollard 1997, Schubert 1998, Frisch et al 1975).

It has been observed that female animals fed phytoestrogen-rich diet displayed significantly delayed puberty onset compared with those fed the Phytoestrogen-free diet (Lund et al 2001).

Since HS is rich in phytoestrogen (Tseng et, 1997, Tsai et al 2002, Hirunpanich et al 2004)

and it was only the lactating dams that consumed HS directly during lactation (and not the offspring), the observation of the estrogenic effects above coupled with the delay in the onset of puberty in the offspring may suggest a programming of these effects by the phytoestrogens (or their metabolites) that were transported from the lactating dams to the suckling neonates through breast milk.

It is known that many natural and synthetic compounds mimic estrogen and influence uterine functions by interacting with nuclear estrogen receptors or other signaling molecules. These estrogenic compounds also act as endocrine disrupters, causing developmental and reproductive disturbances. Estrogens acting through membrane receptors have been found to suppress LH secretion by gonadotropes (Stormshak and Bishop 2008). These findings could also explain the marked reduction in the number of developing ova and the delayed vaginal opening seen in the exposed group compared with the control in this study.

The present study has shown that maternal consumption of HS during lactation facilitates uterine growth and priming while slowing the growth of follicles in the ovaries as well as delaying puberty in the female offspring even though they had increased BMI.

## REFERENCES

Ali BH, Wabel NA, Blunden G (2005). Phytochemical, pharmacological and toxological aspects of *Hibiscus sabdariffa* L: a review. Phytother Res. **16**: 19 (5): 369-75.

Engelbregt MJT, Houdijk MECAM, Popp-Snijders C, Delemarra-van de Waal HA (2000). The effects of intrauterine growth retardation and postnatal undernutrition on onset of puberty in male and female rats. Pediatric Research **48** (6): 803-807.

Frisch RE, Hegsted DM, Yoshinaga K (1975). Body weight and food intake at early estrus of rats on a high-fat diet. PNAS **72**(10): 4172-4176.

Hirunpanich V Utaipat A Morales NP Bunyapraphatsara N Sato H Herunsalee A Suthisisang C (2005). Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* LINN. (Roselle) in vitro using rat low-density lipoprotein (LDL). Biological and Pharmaceutical Bulletin **28**: 481484.

Iyare EE, Iyare FE (2006a). Effect of *in utero* exposure to an aqueous extract of *Hibiscus Sabdariffa* on offspring's postnatal growth in Sprague-Dawley rats. Nigerian Journal of Health and Biomedical Sciences, **5** (1): 27-30.

Iyare EE, Iyare FE (2006b). Lactational exposure to an aqueous extract of *Hibiscus Sabdariffa* accelerates offspring's postnatal growth in Sprague-Dawley rats. Journal of Experimental and Clinical Anatomy, **5** (1) : 6-9.

Iyare EE, Iyare FE (2007). Maternal consumption of aqueous extract of *Hibiscus Sabdariffa* during pregnancy attenuates pregnancy weight gain and postpartum weight loss. Afr. J. Biomed. Res. **10**: 257-261.

Iyare EE, Adegoke OA (2008). Postnatal weight gain and onset of puberty in rats exposed to aqueous extract of *Hibiscus sabdariffa* in utero. Pakistan Journal of Nutrition. **7**(1): 98-101.

Lee SH (1982). Uterine epithelial and eosinophil estrogen receptors in rats during the estrous cycle. Histochemistry and cell biology. **74**(4): 443-452.

Lund TD, Rhees RW, Setchell KD, Lephart ED (2001). Altered sexually dimorphic nucleus of the preoptic area (SDN-POA) volume in adult Long-Evans rats by dietary soy phytoestrogens. Brain Res. **28**;914(1-2):92-9.

Maddox DE, Butterfield JH, Ackerman SJ, Coulam CB, Gleich GJ (1983). Elevated serum levels in human pregnancy of a molecule immunochemically similar to eosinophil granule major basic protein. J. Exp. Med. **158**: 1211-1226.

Pollard JW (1999). Editorial Genetic regulation of estrogen responsiveness. Endocrinology. **140**(2): 553-555.

Rojas AM Steinsapir J (1983). Multiple mechanisms of regulation of estrogen action in the rat uterus: effects of insulin. **Endocrinology**. 112: 586-591

Schubert AK 1998. Effects of progesterone antagonists on eosinophilic granulocytes in the rat uterus. Ann Anat. **180**(5): 401-408

Soto J, Tchernitchin AN Poloni P Voigt G Caro B Agurto M (1989). Effect of ketotifen on the distribution and degranulation of uterine eosinophils in estrogen-treated rats. Inflammation research. **28**(3-4): 198-203.

Stormshak F, Bishop CV (2008). Board-invited review: Estrogen and progesterone signaling: Genomic and nongenomic actions in domestic ruminants. J. Anim Sci. **86**:299-315.

Tsai PJ, McIntosh J, Pearce P, Camden B, Jordan BR (2002). Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L) extract. Food Research International **35**: 351-356.

Tseng TH, Kao ES, Chu CY, Chou FP, LinWu, HW, Wang CJ 1997. Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food and Chemical Toxicology **35**: 1159-1164.

Usoh IF, Akpan EJ, Etim EO Farombi EO, (2005). Antioxidant Actions of Dried Flower Extracts of *Hibiscus sabdariffa* L. On Sodium Arsenite - Induced Oxidative Stress in Rats. Pakistan Journal of Nutrition. **4** (3): 135-141.

Wang H, Tranguch S, Xie H, Hanley G, Das KS, Dey SK, (2005). Variation in Commercial rodent diets induces disparate molecular and physiological changes in the mouse uterus. PNAS **102** (28): 9960-9965.