

EFFECT OF CONSUMPTION OF HIBISCUS SABDARIFFA AND AZADIRACHTA INDICA
PRENATALLY AND POSTNATALLY ON MILK CREAMATOCRIT AND EARLY GROWTH OF
OFFSPRING

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Abstract

Background: Aqueous extracts of *Hibiscus sabdariffa* Linn. (HS) and *Azadirachta indica* A. juss (AI) are widely used in Nigeria for medicinal purposes and have been shown to affect postnatal growth of offspring. Breast milk creatinocrit is a simple accurate inexpensive technique and useful measure of lipid and energy content of breast milk. No data has shown effects of these extracts on breast milk creatinocrit and early postnatal growth of offspring. This study was designed to investigate effects of HS and AI on breast milk creatinocrit and early postnatal growth of offspring.

Materials and Methods: 40 pregnant rats weighing 150-200g were used for this study. They were divided into control, HS and AI groups. These groups were subdivided into two of low and high doses. Administration was by gavage and commenced on day 1 of pregnancy and ended on postnatal day 21. Litter weight and length were recorded at birth, postnatal days 7-21. Breast milk was collected from 4 dams in each group at postnatal days 7-21 for milk creatinocrit.

Results: Results showed that aqueous extract of HS and AI caused progressive increase in milk creatinocrit during lactation. This increase peaked greatly in the third week of lactation. There was also a progressive increase in BMI of pups in extract treated group that became significantly greater at weaning.

Conclusion: In conclusion, consumption of aqueous extracts of HS and AI during pregnancy and lactation may accelerate the postnatal growth of the offspring through mechanisms that may depend on increased breast milk creatinocrit.

Keywords: *Hibiscus sabdariffa*, *Azadirachta indica*, Breast milk creatinocrit, early postnatal growth

Introduction

Medicinal plants over the years have been part and parcel of human society for the combat of diseases. These plants have been reliable sources of food because they provide essential nutritional values, medicinal properties and notable physiological effect to life (Dalziel, 1973).

Hibiscus Sabdariffa Linn (HS) and *Azadirachta indica* A. juss (AI) are used as medicinal and food ingredients in many parts of the world including Nigeria. Both plants are highly sourced as food vegetable particularly because of their health promoting and diseases-preventing properties strongly suspected to be due to the presence of many phytochemicals in them (Ijeomah *et al.*, 2012). These phytochemicals in the two plants have been found to be protective and preventive against many degenerative diseases and pathological process such as in ageing (Burns *et al.*, 2001).

HS (family: Malvaceae) commonly known as zobo in Nigeria, is an annual herbaceous shrub, cultivated for its flowers, leaves and seeds. It is found in the tropics, subtropics and other parts of the world (Dalziel, 1973) but it is utilized beyond these areas of cultivation globally. In folk medicine this medicinal herb is used for the treatment of hypertension (Wang *et al.*, 2000; Odigie *et al.*, 2003). The plant is also reported to have hepatoprotective, anti-hyperlipidemic, anticancer and antioxidant properties (Mahadevan and Shivali-kamboj, 2009).

AI (family: Meliaceae), commonly referred to as Dogonyaro or neem plant is an evergreen robust tree. It is mostly found in tropic and sub-tropical areas of the world, African and Asia (Ejoba, 2012). The tree occurs in medium to large size and has dark grey bark and a dense rounded row of pinnate leaves (Ogbuewu, 2008). All parts of the neem tree (leaves, flowers, seeds, fruits, roots and bark) are widely used in traditional medicine. AI is used for the treatment of many health-related problems and known to exert anticancer, antioxidant, wound-healing and antimicrobial properties. All parts of this plant are useful and has been used to treat diseases ranging from tooth decay, ulcer, swollen liver, malaria and dysentery (Ogbuewu, 2008; Mossini *et al.*, 2004).

Breast milk is the normative standard for infant feeding and nutrition because it has been associated with a reduction in the incidence of gastrointestinal tract infection, respiratory tract infection and otitis media (American Association of Paediatrics, 2012; World Health Organization, 2013). It is important to note that breast milk is widely accepted to be the optimal source of nutrition for the newborn infants because it contains appropriate amount of protein, lipid, carbohydrate, micronutrients, trace elements, vitamins, minerals, digestive enzyme and hormones required for early postnatal growth of offspring, development and immune protection. However, it is now universally recognized that there is no commercial formula that can equal breast milk. The relationship of breast milk feeding with a significant reduction in the incidence of necrotizing enterocolitis (NEC) has been suggested in preterm infants (Sullivan, 2010). Additionally, there is extensive evidence that offspring who had been breastfed with breast milk show lower risk of same non-communicable diseases later in life (American Association of Paediatrics, 2012; World Health Organization, 2013). Indeed, breast milk given to infant at the early postnatal stage has been associated with lower risk of obesity, lower levels of arterial blood pressure, lower total and LDL-blood cholesterol level in adulthood and lower risk of developing type-2 diabetes (American Association of Paediatrics, 2012; World Health Organization, 2013). Breast milk is not only associated with improved parameters of growth in offspring but also with a better neuronal-behavioral development (Mortensen *et al.*, 2002; American Association of Paediatrics, 2012). Breast milk creamatocrit is the cream column separated from the milk by centrifugation and expressed as a percentage of the length of the total milk column. It is a simple method of estimating the calorific value of breast milk (Lucas, 1978).

Infant growth during the early postnatal period is obviously the result of the development of complex functional, structural and metabolic mechanisms, which are however not yet entirely clarified (Patel and Srinivasan, 2010). However, this period is characterized by a great plasticity with critical windows in which any inadequate insult or intervention may cause both positive and adverse postnatal growth and development (Ostadalova and Babicky, 2012). The present study was thus designed to determine the effect of consumption of aqueous extract of HS and AI during pregnancy and lactation on breast milk creamatocrit and early postnatal growth of offspring.

Materials and Method

Plant Collection, Identification and Extract Preparation

Matured calyces of HS were purchased from a local market in Enugu and fresh matured leaves from AI tree located in the premises of University of Nigeria, Enugu campus were harvested. Both plant samples were identified and authenticated by Mr. Onyeukwu, C.J. of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where voucher specimens (numbers UNH No 75f and UNH No. 521^A respectively) were deposited. The extraction procedure used for HS was as described previously (Iyare and Adegoke, 2011). Briefly, 300g of the dry calyces of HS was brewed in 4000ml of boiled tap water for 45min. The resulting decoction was filtered and evaporated to dryness giving a dark red paste with percentage extraction yield of 47%. The leaves of AI were washed and air-dried. The dried leaves were homogenized using an electric blender. The powder was exhaustively extracted in distilled water at 60^oc for 48hours using soxhlet extractor according to a previously described method (Biu *et al.*, 2010). The resulting decoction was also filtered and evaporated to dryness giving a black paste with percentage extraction yield of 19.5%. Both extracts were stored in the refrigerator for preservation until use.

Phytochemical analysis

The standard method of Trease and Evans (2002) were used in the analysis of the phytochemical components of calyces of HS and leaves of AI. The qualitative and quantitative phytochemical analyses of the extracts are presented in Table 1.

Experimental animals

40 inbred virgin albino rats aged between 10-12 weeks weighing 150–200g with two consecutive regular 4-day estrus cycles were used for this study. The rats were housed in cages and acclimatized for 2 weeks and maintained under standard environmental conditions and were also allowed free access to food (grower pelleted feed) and water. 10 male rats of proven fertility were introduced into the cages in the ratio of 1: 4 to allow for mating. Day 1 of pregnancy was taken as the day sperm was seen in the vaginal smear of the rats (Benie *et al.*, 2003).

On day 1 of pregnancy the rats were randomly divided into 3 groups: A, B and C with groups A and B further subdivided into two subgroups of low and high doses. Extract administration also commenced on day 1 of pregnancy and ended on postpartum day 21.

Group A (n=16): This group was administered HS extract in two doses: low dose (1.5g/Kg body weight) and high dose (3g/Kg body weight) (Iyare and Adegoke, 2008).

Group B (n=16): This group was administered AI in two doses also: low dose (200mg/Kg body weight) and high dose (400mg/Kg body weight) (Iyare and Obaji, 2014).

Group C (n=8): This group was the control group and was administered water.

This work was approved by the Research and Ethics Committee of the Directorate of Research and Publications, College of Medicine, University of Nigeria, Enugu Campus, Enugu with protocol number 040/02/2016. Approval date was 28/02/2016.

Measurement of litter size, length and weight

On the day of delivery, litter sizes, lengths and weights were recorded using a stainless metre rule (WINS FISH BRAND, CHINA) and a digital electronic compact balance (S. METTLER, CHINA). Litter weights and length were subsequently recorded at postnatal days 7, 14 and 21.

Breast milk collection and creatocrit determination

Breast milk was collected from 4 dams in each subgroup at postnatal days 7, 14 and 21 according to the method of Paul et al., (2015) but with slight modification. On each day, dams were separated from their pups for 3 hours prior to milking to allow accumulation of the breast milk. Subsequently the dams were injected with 0.1ml of oxytocin (10iu/ml, oxytocin Bengen, WdT eG, Garbsen, Germany) intraperitoneally and after 1 minute the milking process commenced. The mammary glands of the dams were massaged slightly to induce milk ejection reflex. The dams were milked by gently applying pressure to the base of the mammary gland until milk became visible. All teats of each dam were repeatedly milked for a period of 10 to 15 min. The milk was collected in a plain sample container. Milk volume collected ranged between 0.4ml-0.5ml per dam. The expressed breast milk was collected from the sample container into an 80iu/ml non-heparinized micro haematocrit tube (Vasekaer 6-8 dk-2730 Harlev, Denmark) through capillary action. The haematocrit tube was immediately placed in a haematocrit machine (Hawksley England) and Centrifuged at 3000 rpm for 15min until the lipid or cream part separated from the aqueous part of the milk. After centrifugation the haematocrit tube was placed vertically on an haematocrit reader (Hawksley England) and the length of the packed cream layer at the top of the tube was read directly within one hour. The cream layer was expressed as percentage of milk column to indicate the creatocrit value (Chatterjee *et al.*, 1986; Du *et al.*, 2017).

Statistical analysis

The data were analyzed statistically using SPSS version 20.0. Results were expressed as mean \pm standard error of means (SEM) and an analysis of variance followed by a post-hoc Student-Neuman-Keuls' test. $P < 0.05$ was considered statistically significant.

Results

Table 1: The phytochemical analyses of the aqueous extracts of *Hibiscus sabdariffa* calyces and *Azadirachta indica* leaves

Constituents	Units	<i>Hibiscus sabdariffa</i>		<i>Azadirachta indica</i>	
		Qualitative	Quantitative	Qualitative	Quantitative
Alkaloid	%	+	0.105	++	0.13
Saponin	%	++	1.083	++	0.297
Flavonoid	%	++	26.256	+	7.289
Tannin	%			++	1.625
Steroid	mg/ml	++	0.3113		
Glycoside	mg/ml	++	1.564		
Reducing sugar	mg/ml	+	17.5		
Phenol	mg/ml			+	8.748

+ = Slight; ++ = Moderate

Result showed a progressive increase in breast milk creatinocrit value that peaked in the third week of lactation in both extract groups when compared with control except low dose HS that was lowest in the 3rd week (Table 2).

Table 2: Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and *Azadirachta indica* on breast milk creatinocrit value

Periods	<i>Hibiscus sabdariffa</i>			<i>Azadirachta indica</i>		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
1 st week	27.50±0.35	34.50±0.35*	25.19±1.30	27.50±0.35	31.00±0.71*	35.50±0.35*
2 nd week	28.50±0.35	36.00±0.00*	35.50±0.35*	28.50±0.35	33.00±0.71*	30.00±0.00*
3 rd week	32.00±0.71	26.50±0.35*	35.00±0.00*	32.00±0.71	38.50±0.35*	44.50±0.35*

*= p <0.05 vs control; Low dose HS (1.5g/kg bwt) and high dose HS (3.0g/kg bwt); Low dose AI (200mg/kg bwt) and high dose AI (400mg/kg bwt)

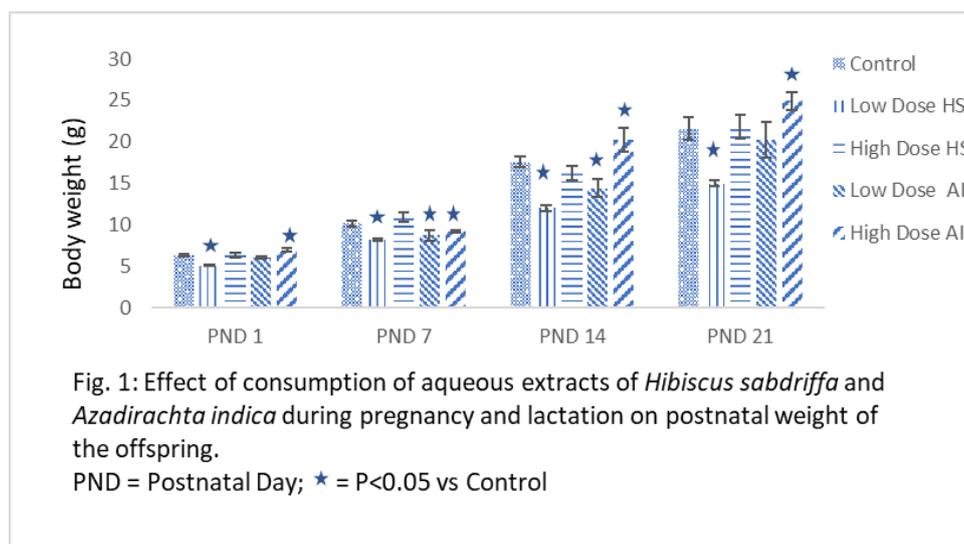
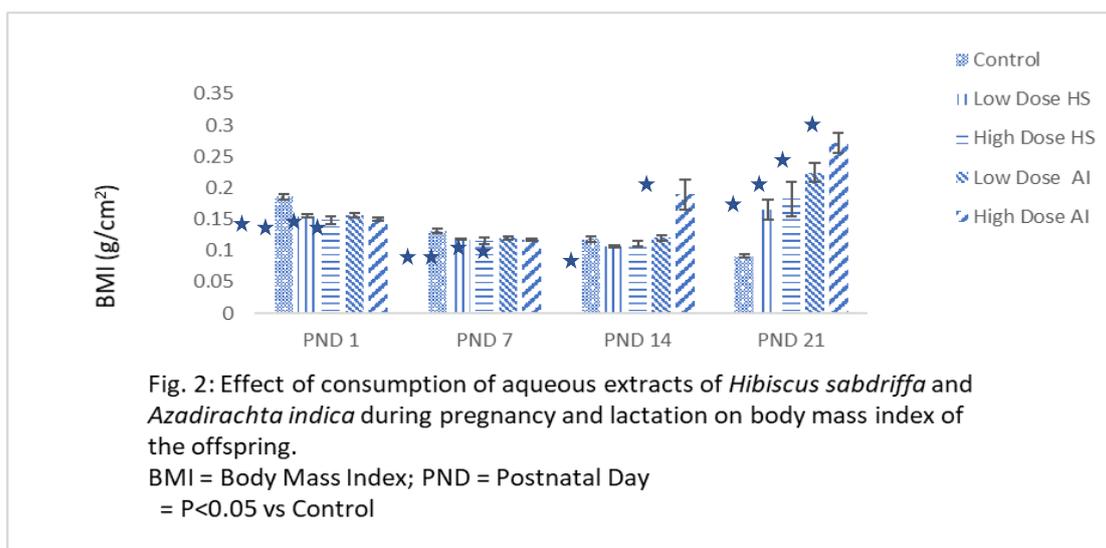


Figure 1: Effect of consumption of aqueous extract of *Hibiscus sabdariffa* during pregnancy and lactation on body weight of offspring

Result showed that the body weights of the low dose HS groups were consistently lower from birth to day 21 compared with both control and high dose groups whereas the body weights of the high dose group were consistently similar with those of the control groups from birth to day 21. For the AI, result showed a consistent decrease in body weight in low dose AI group compared with control except at birth and day 21 were they were similar. That of the high dose showed a consistent increase except at day 7 when it was significantly lower than that of the control.



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Figure 2: Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and *Azadirachta indica* during pregnancy and lactation on BMI of offspring

Result showed a significant increase in BMI of the control offspring at birth and at day 7 relative to the low and high dose HS extract groups. At day 14, there appeared a reversal of trend as there was no difference in the BMI between the control group and the high dose HS group. Only the BMI of the low dose group was significantly lower. At day 21, the BMI of both low and high dose HS groups were significantly higher than that of the control group. Result also showed a similar trend in BMI for the AI groups as observed for HS. There was also an observed decrease in BMI in the low and high dose groups of AI at birth and day 7. But on day 14, the low dose AI levelled up and became similar with that of the control while the high dose was increased. At day 21, however, the BMI of both low and high dose AI were significantly higher than that of the control.

Discussion

Breast milk creatocrit

An important variable in the study of breast milk is the lipid concentration which is the major determinant of the energy value because lipids are essential for the formation of cell membranes, hormones and are necessary for proper eye and brain development especially during prenatal period and into the first few years of the child life (Innis and Friesen, 2008). Breast milk creatocrit is the cream column separated from the milk by centrifugation and expressed as a percentage of the length of the total milk column (Vasquez-Roman *et al.*, 2014). It is a simple method of estimating the calorific value of breast milk (Lucas, 1978; Wang *et al.*, 2000). Although the gravimetric method is the gold standard for determining the fat content of breast milk, the creatocrit method is preferable because of the ease of operation and real-time analysis (Du *et al.*, 2017). Herbs and plants have been reported to have lactogenic effect (Goyal *et al.*, 2003; Ouedraogo *et al.*, 2004). There have been reports that some African women with lactation insufficiency particularly those living in villages depend largely on herbal supplements for milk increase or milk induction despite the modern alternative of bottle/formula feeding (Okasha *et al.*, 2008).

Both extracts of HS and AI are rich in glycoside, saponin, steroid, flavonoid and tannin as shown in table 4.2.1 and 4.2.2. These components have been shown to increase breast milk yield (Okasha *et al.*, 2008; Bako *et al.*, 2013). It is possible therefore that these components in the extracts may have been responsible for the observed increase breast milk creatocrit in the present study.

Postnatal growth

The present study showed a significant increase in BMI of the control offspring at birth relative to the HS and AI extract group which. This observation is instructive because anecdotal reports by women who consume these extracts suggest that they do so because of the folkloric belief that these extracts make delivery easy. This observed increase in BMI of the control showed a progressive decrease as lactation progressed until PND 14 of lactation when there appeared a reversal of trend. At PND 14, only the BMI of the low dose HS was significantly lower whereas low dose AI and high dose HS were not significantly different and the high dose AI was significantly higher. Beyond PND 14 there appeared a progressive increase in the BMI of HS and AI extract treated group relative to control. This increase appeared to peak at PND 21. Since the infant is not fully matured at birth and since the process of maturation

continues during the immediate postnatal period (Patel and Srinivasan, 2010), the importance of breastmilk becomes very significant at this period since it is the only source of nutrition for the maturing pups. Larger milk volume and greater energy intake especially from the higher milk fat content from the early days of lactation results in a faster growth in pups. These suggest that the observed effects on the postnatal growth of offspring in this study may be linked to the extract-induced increase in breast milk creatinocrit and by extension, increased fat and energy content. Increased creatinocrit value is an index of increased lipid concentration and increased lipid is rich in calories which is a vital tool for the early postnatal growth and development of offspring.

Conclusion

From the results of the present study, it can be concluded that consumption of aqueous extracts of HS and AI during pregnancy and lactation may accelerate the postnatal growth of the offspring possibly through increased breast milk creatinocrit. Further work needs to be done to ascertain the volume of breast milk consumed by the pups so as to be able to definitively link the accelerated growth with the volume of milk consumed.

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Conflict of Interest: We have no conflict of interest to disclose.

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