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Effect of Consumption of Aqueous Extract of *Hibiscus sabdariffa* and *Azadirachta indica* during Pregnancy and Lactation on Body Weight Changes in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author IEE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EEE and COE managed the analyses of the study. Author EEE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Extracts of *Hibiscus sabdariffa* (HS) and *Azadirachta indica* (AI) are widely used in Nigeria for medicinal purposes and have also been shown to affect weight changes anecdotally through mechanisms not yet defined. There are reports of decreased food consumption and weight gain in rats consuming HS extracts as the drinking solutions but there is paucity of data on the effect of these two extracts, administered by gavage, on weight changes during pregnancy and lactation. This study was therefore designed to investigate this in relation to food and fluid intake. 40

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pregnant rats weighing 150-200 g were used for this study. They were divided into three groups: control, HS and AI groups. HS and AI groups were subdivided into two subgroups of low and high doses. Extract administration was orally by gavage and commenced on day 1 of pregnancy and ended on postnatal day 21. Food and fluid consumption were monitored throughout pregnancy and lactation. The results showed that the aqueous extract of HS and AI increased consumption of food and fluid during pregnancy and lactation, increased maternal weight gain during pregnancy and lactation. From the results of the present study, it can be concluded that consumption of aqueous extracts of HS and AI during pregnancy and lactation increased fluid and food intake and weight gain of dams with a possible potential to accelerate weight loss or decrease postpartum weight retention during lactation.

Keywords: Hibiscus sabdariffa; Azadirachta indica; pregnancy; lactation; weight changes.

1. INTRODUCTION

Hibiscus sabdariffa and *Azadirachta indica* are used as medicines and food ingredients in many parts of the world including Nigeria [1-3]. Both plants are highly sourced as food vegetable particularly because of their health promoting and diseases-preventing properties which is strongly suspected to be due to the presence of many phytochemicals in them[1]. These phytochemicals like alkaloid, saponin, glycoside, tannin, phenol, flavonoid, steroid, reducing sugar, nimbidin, sodium nimbidate, nimbin, gedunin, delphinidin 3-sambubioside and protocatechuic acid in the two plants have been found to be protective and preventive against many degenerative diseases and pathological process such as in ageing [2].

Hibiscus sabdariffa (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 [3] but it is utilized beyond these areas of cultivation globally. In folk medicine this medicinal herb is used for the treatment of hypertension [4,5]. The plant is also reported to have hepatoprotective, anti-hyperlipidemic, anticancer and antioxidant properties [6].

Azadirachta indica which is commonly referred to as Dogonyaro or neem plant is an evergreen robust tree belonging to the family meliaceae. It is mostly found in tropic and sub-tropical areas of the world, African and Asia [7]. The tree occurs in medium to large size and has dark grey bark and a dense rounded row of pinnate leaves [8]. All parts of the neem tree (leaves, flowers, seeds, fruits, roots and bark) are widely used in traditional medicine. *Azadirachta indica* is used for the treatment of many health related problems and also known to exert anticancer, antioxidant, wound-healing and antimicrobial properties [8]. All parts of this plant are useful and has been used to treat diseases ranging from tooth decay, ulcer, swollen liver, malaria and dysentery [9,10].

Extracts of HS and AI are widely used in Nigeria for medicinal purposes and have also been shown to affect weight changes anecdotally through mechanisms not yet defined. There are reports of decreased food consumption and weight gain in rats consuming HS extracts as the drinking solutions [11,12] but there is paucity of data on the effect of these two extracts, administered by gavage, on weight changes during pregnancy and lactation. This study was therefore designed to investigate this in relation to food and fluid intake.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identifcation and Extract Preparation

Matured calyces of HS were purchased from a local market in Enugu and fresh matured leaves of AI were harvested from *Azadirachta indica* tree located in the premises of University of Nigeria, Enugu campus. Both plant samples were identified and authenticated by Mr. Onyeukwu CJ 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 [13]. Briefly, 30 g 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 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 60^0c for 48hours using soxhlet extractor according to a previously described method [14]. 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.

2.2 Phytochemical Analysis

The standard method of Trease and Evans [15] were used in the analysis of the phytochemcal components of calyces of HS and leaves of AI. The qualitative and quantitative phytochemical analyses of the extracts showed the following components in the Tables below.

Table 1. The phytochemical analysis of the aqueous extract of *Hibiscus sabdariffa* calyces

Constituent	Units	Qualitative	Quantitative
Alkaloid	%	+	0.105
Saponin	%	++	1.083
Flavonoid	%	++	26.256
Steroid	mg/l	++	0.3113
Glycoside	mg/l	++	1.5640
Reducing sugar	mg/l	+	17.5

+ = Slight; ++ = Moderate

Table 2. The phytochemical analysis of the aqueous extract of *Azadirachta indica* leaves

Constituent	Units	Qualitative	Quantitative
Alkaloid	%	++	0.13
Tannin	%	++	1.625
Saponin	%	++	0.297
Flavonoid	%	+	7.289
Phenol	mg/l	+	8.748

+ = Slight; ++ = Moderate

2.3 Experimental Animals

40 inbred virgin albino rats aged between 10-12 weeks weighing 150–200 g 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 [16].

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 *Hibiscus sabdariffa* extract in two doses: low dose (1.5 g/Kg body weight) and high dose (3 g/Kg body weight) [17].

Group B (n=16): This group was administered *Azadinachta indica* in two doses also: low dose (200 mg/Kg body weight) and high dose (400 mg/Kg body weight) [18].

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

2.4 Measurement of Maternal Body Weight and Fluid and Food Intake

The maternal body weight was measured daily during pregnancy and lactation to determine the effect of the two extracts on body weight of the pregnant rats during the 3 weeks of both pregnancy and lactation. Food and fluid intake were also measured during these periods. These measurements were done using a digital electronic compact balance (S. METTLER, CHINA) and a plastic beaker and recorded to the nearest unit.

2.5 Statistical Analysis

The data were analyzed statistically using SPSS version 20.0. Result 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.

3. RESULTS

3.1 Effect of Consumption of Aqueous Extract of *Hibiscus sabdariffa* and *Azadirachta indica* on Food Intake during Pregnancy

Result showed significant increases in food intake in the low dose and high dose HS and AI groups when compared with control group.

Table 3. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and *Azadirachta indica* on food intake during pregnancy

Periods	*Hibiscus sabdariffa*			*Azadirachta indica*		
	Control	Low dose	High dose	Control	Low dose	High dose
1st week	20.1±0.19	26.9±1.24*b	23.96±0.45*	20.10±0.19	27.48±0.48*	26.64±0.84*
2nd week	20.16±0.22	22.86±0.57*	24.81±0.77*p	20.16±0.22	27.57±0.80*	33.10±0.67*p
3rd week	20.31±0.74	21.74±1.05	27.18±0.52*p	20.31±0.74	24.15±0.91	30.54±1.00*p

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

Table 4. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and *Azadirachta indica* on fluid intake during pregnancy

Periods	*Hibiscus sabdariffa (HS)*			*Azadirachta indica (AI)*		
	Control	Low dose	High dose	Control	Low dose	High dose
1st week	24.43±0.20	23.61±0.34*	24.26±0.28	24.43±0.20	24.84±0.33	28.9±0.25*p
2nd week	25.57±0.36	24.47±0.16*	24.26±0.19*	25.57±0.36	27.57±0.80*	28.86±0.63*p
3rd week	23.1±0.38	23.78±0.47	25.43±0.20*p	23.1±0.38	25.42±0.50*	28.76±0.64*p

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

3.2 Effect of Consumption of Aqueous Extract of *Hibiscus sabdariffa* and *Azadirachta indica* on Fluid Intake during Pregnancy

Result showed decrease in fluid intake ($p<0.05$) in the 1st and 2nd week of pregnancy but significant increase in the 3rd week was observed only in the high dose HS. Low dose AI showed no significant difference ($p>0.05$) in fluid intake in the 1st and 2nd week of pregnancy when compared with control but increased significantly in the 3rd week of pregnancy. High dose AI showed significant increases in the three weeks of lactation in fluid intake when compared with control.

3.3 Effect of Consumption of Aqueous Extract of *Hibiscus sabdariffa* and *Azadirachta indica* on Maternal Weight during Pregnancy

The results showed a progressive significant increase in maternal weight gain in the low and high dose HS groups as pregnancy progressed (Fig. 1a and 1b). These increases were however lower than those of the control group except at week one in which case the increases were higher. This suggests that consumption of aqueous extract of HS during pregnancy decreases pregnancy weight gain.

There were also progressive significant increases in maternal weight gain in the low and high dose AI groups as pregnancy progressed (Fig. 2a and 2b)). These increases were however lower than those of the control group except at week one in which case there was no difference between the control value and those of the extracts. This suggests that consumption of aqueous extract of AI during pregnancy decreases pregnancy weight gain.

3.4 Effect of Consumption of Aqueous Extract of *Hibiscus sabdariffa* on Food Intake during Lactation

Result showed significant increase in food intake in the low dose HS whereas, there was a significant decrease ($p<0.05$) in the high dose HS when compared with control. The food consumed by the low dose HS group was also significantly greater than that of the high dose HS group. In the AI groups, there was no difference in the amount of food consumed among the groups in the first two weeks of lactation but the food consumed in the third week in both low and high dose AI groups were significantly greater than that of the control.

The fluid consumption in the low dose HS group was significantly greater than the fluid consumption in both the control and high dose HS groups. There was no difference in the fluid consumption between the control and high dose HS groups. There were progressive increases in the fluid consumption in both low and high dose AI groups and these fluid consumptions were significantly greater than that of the control throughout the three weeks of lactation.

3.5 Effect of Consumption of Aqueous Extract of *Hibiscus sabdariffa* and *Azadirachta indica* on Maternal Weight during Lactation

Results showed no significant difference in maternal weight gain during lactation in both low dose and high dose HS groups compared with control group except the 2nd and 3rd week of the high dose group that was significantly lower than both low dose and control groups (Table 6a). This may suggest that the high dose HS accelerated weight loss during lactation. For the AI, result showed no significant difference in maternal weight gain among the three groups during lactation except the low dose group that was significantly lower than that of the control in the 1st week of lactation (Table 6b).

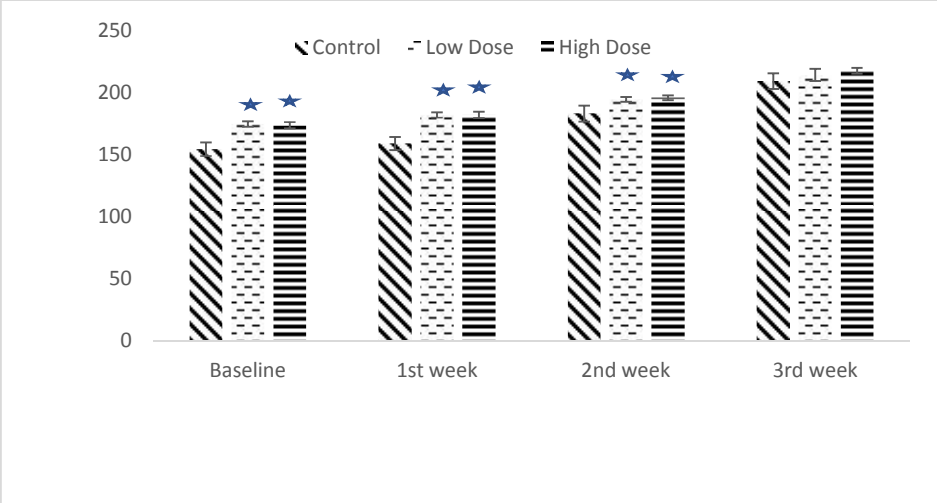

Fig. 1a. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* on absolute maternal weight during pregnancy
**= P <0.05 versus control*

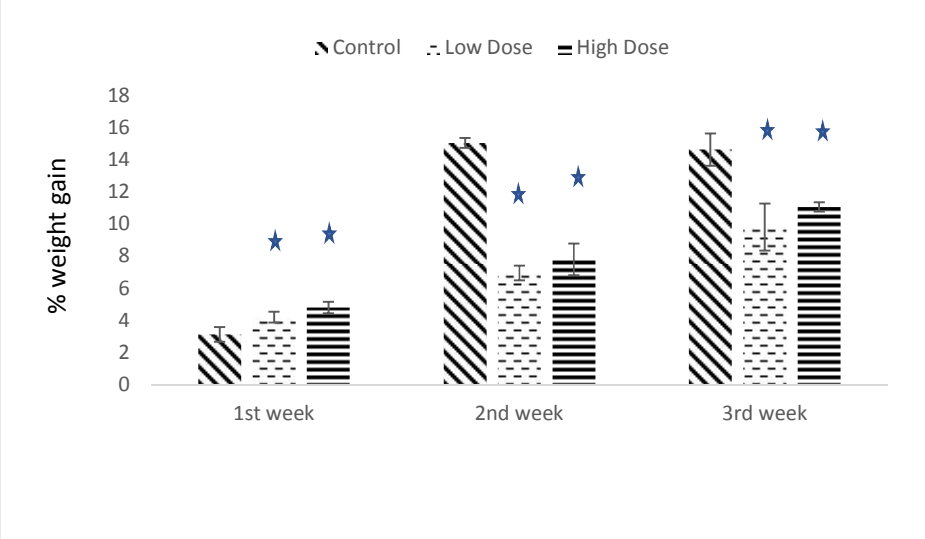

Fig. 1b. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* on maternal % weight gain during pregnancy
**= P<0.05 versus control*

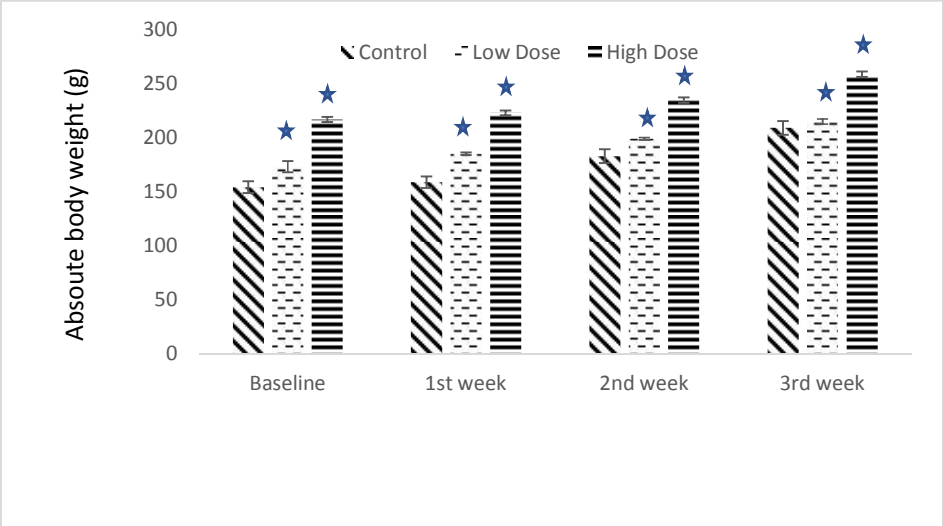

Fig. 2a. Effect of consumption of aqueous extract of *Azadirachta indica* on absolute maternal weight during pregnancy
**= P<0.05 versus control*

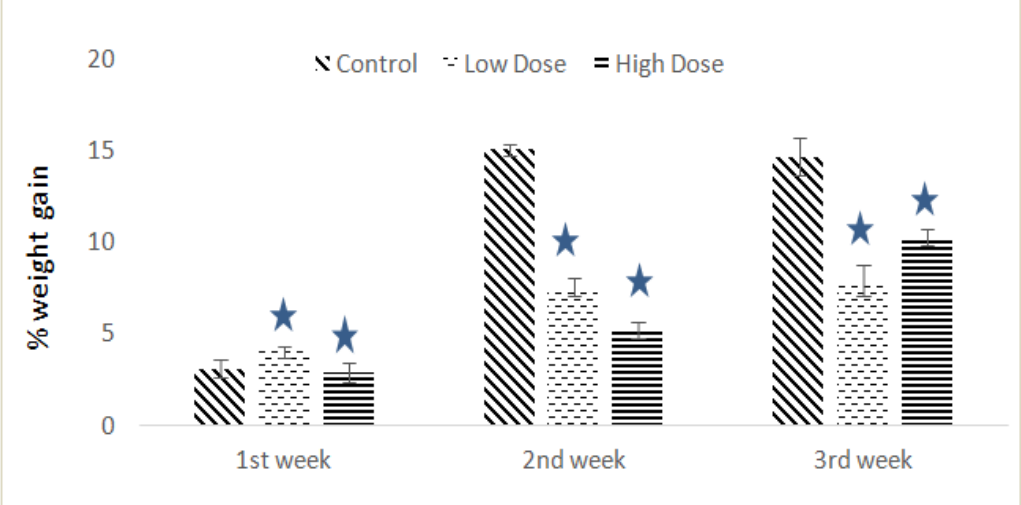

Fig. 2b. Effect of consumption of aqueous extract of *Azadirachta indica* on maternal % weight gain during pregnancy
**= P<0.05 versus control*

Table 5a. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and *Azadirachta indica* on food intake during lactation

Periods	*Hibiscus sabdariffa*			*Azadirachta indica*		
	Control	Low dose	High dose	Control	Low dose	High dose
1st week	25.22±2.34	31.30±2.16*	24.65±1.49P	25.22 ±2.34	29.84±1.92	27.56±2.38
2nd week	33.64±2.57	45.08±4.04*	27.27±1.76*P	33.64±2.57	36.86±2.21	39.26±3.01
3rd week	38.53±1.99	49.04±2.86*	32.40±2.27*P	38.53±1.99	49.19±2.25*	48.35±3.25*

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

Table 5b. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and *Azadirachta indica* on fluid intake during lactation

Periods	*Hibiscus sabdariffa*			*Azadirachta indica*		
	Control	Low dose	High dose	Control	Low dose	High dose
1st week	26.31±1.72	31.29±2.00*	25.19±1.30P	25.81 ±1.57	31.67±1.59*	33.05±2.30*
2nd week	35.72±2.67	46.14±2.01*	30.33±1.53P	33.47±1.61	41.95±2.52*	49.14±3.10*P
3rd week	41.12±1.38	54.31±2.44*	39.00±2.16P	40.52±2.18	58.76±2.29*	59.29±3.47*

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

Table 6a. Effect of consumption of aqueous extract of *Hibiscus sabdariffa* on maternal weight during lactation

Periods	Maternal absolute weights			Maternal % weight gains		
	Control	Low dose	High dose	Control	Low dose	High dose
Baseline	164.72±6.11	188.38±3.98*	206.91±1.13*			
1st week	172.43±6.42	195.33±4.17*	215.14±1.03*	4.67±0.53	3.69±0.25	4.00±0.27
2nd week	177.87±6.46	203.90±4.91*	217.81±1.59*	3.32±1.10	4.71±2.04	1.23±0.45*αa
3rd week	190.60+7.85	216.86+4.67*p	226.10+1.25*p	6.90±1.28a	6.54+0.90	3.88+0.77*α

*Low dose HS (1.5 g/kg bwt) and high dose HS (3.0 g/kg bwt); Low dose AI (200 mg/kg bwt) and high dose AI (400 mg/kg bwt); * = P<0.05 vs Control; a = P<0.05 vs 1st week; α = P<0.05 vs Low Dose*

Table 6b. Effect of consumption of aqueous extract of *Azadirachta indica* on maternal weight during lactation

Periods	Maternal absolute weights			Maternal % weight gains		
	Control	Low dose	High dose	Control	Low dose	High dose
Baseline	164.72±6.11	184.14±5.51*	238.62±4.14*			
1st week	172.43±6.42	198.77±2.43*	215.14±1.03*	4.67±0.53	3.68±0.23*	3.97±0.58
2nd week	177.87±6.46	211.19±1.66*	226.86±3.00*	3.32±1.10	6.59±1.60	3.71±1.20
3rd week	247.86±3.88	256.81±4.50*p	269.95±4.80*p	6.90±1.28	7.49±1.45	5.15±0.65

*Low dose HS (1.5 g/kg bwt) and high dose HS (3.0 g/kg bwt); Low dose AI (200mg/kg bwt) and high dose AI (400 mg/kg bwt); * = P<0.05 vs Control; a = P<0.05 vs 1st week; α = P<0.05 vs Low Dose; p =P<0.05 vs Low Dose*

4. DISCUSSION

4.1 *Hibiscus sabdariffa* and Food and Fluid Intake during Pregnancy and Lactation

The present study revealed significant increase in food intake in the test group when compared with control and also decrease in fluid intake ($p<0.05$) in the 1st and 2nd week of pregnancy but significant increase in the 3rd week was observed only in the HS high dose. Earlier reports [11,17] have shown that the aqueous extract of HS decreases food and fluid intake during pregnancy through a mechanism not yet fully understood. The differences between the findings of the earlier report and the observation of this study could be as a result of the differences in the mode and route of administration and the duration at which the dams received the plant extract. In earlier reports [11,13,17] the extract was administered as the drinking solution only, but in the present study the extract was administered orally, and water given *ad libitum*. The extract is hypertonic and thus stimulates the sensation of thirst when administered orally. This sensation of thirst may have been responsible for the increase fluid intake observed in the present study. Also, the extract of HS is rich in Na^{+17} which also increases the sensation of thirst. With increased fluid intake and consequent hydration, there is the abolition of the decreased food intake induced by the dehydration-anorexia following consumption of HS [17]. The present study also showed that maternal consumption of aqueous extract of HS during lactation caused significant increase in food intake in the low dose group and significant decrease ($p<0.05$) in the high dose group when compared with the control group. HS low dose also caused significant increase in fluid intake while the high dose showed no significant difference ($p>0.05$) when compared with control. The observation from the present study was different from the observation of Iyare and

Adegoke[11] who reported a reduction in fluid and food intake following administration of the extract during lactation. Again, the differences in the observation may be due to the difference in the mode and route of administration as earlier discussed.

4.2 *Azadirachta indica* and Food and Fluid Intake during Pregnancy and Lactation

The present study revealed significant increase in food intake when compared with control. Low dose AI showed no significant difference ($p>0.05$) of fluid intake in the 1st and 2nd week of pregnancy when compared with control but increased significantly in the 3rd week of pregnancy. AI high dose showed progressive increase in fluid intake when compared with control. It has been noted that if tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed [19]. It is possible that the increased food and fluid intake observed in this study may have been as a result of decreased amount of tannin present in the plant extract used for this study. The basic physiological principles that governs the regulation of nutrient intake appears to be neither the fluctuation of energy level of the body nor energy content of ingested foods but the detection of the degree of depletion and repletion of essential nutrients [20]. Therefore, it could be concluded that the energy need of the dam during the period of pregnancy necessitated the increased food and fluid intake by the hormones Neuropeptide Y which predominately increased carbohydrate intake in rats by a direct action within the central nervous system [21].

There was no significant difference ($p>0.05$) in food intake in the 1st and 2nd week of lactation in the AI low and high doses when compared with control but in the 3rd week there was a significant increase in the AI low and high dose groups when compared with control. Progressive increases in fluid intake was observed as lactation progressed when compared with control. Wang et al. [22] found that condensed tannin from *L. corniculatus* increased milk yield secretion rates of protein and lactose thereby increasing efficiency of milk production. It is therefore possible that due to the nutritional requirement of the dam to meet up with milk production for the offspring the tannin in extract affected food and fluid intake which was evident in the increased value of the breast milk creamatocrit noticed in the 3rd week of lactation. Lactating mothers who do not get enough energy and nutrition are at risk of maternal depletion and in other to prevent this enough food must be made available to the mother. Breastfeeding also increases the mother's need for water this may suggest the reason for the increased fluid intake noticed.

4.3 *Hibiscus sabdariffa* and Weight during Pregnancy and Lactation

The result from this study showed that there was a significant increase in maternal weight in the low and high dose groups in the 1st and 2nd week of pregnancy but no significant difference ($p>0.05$) in the weights in the 3rd week when compared with the control.

Iyare and Adegoke [13] noted that the plant extract caused decrease fluid and food intake that resulted in decreased pregnancy weight gain amongst the dams that consumed the extract. These variations noticed in this study and that of others [11,13,17] may possibly be as a result of differences in the method of administration of the plant extract as discussed above. The increase in maternal weight observed in this study during pregnancy may therefore suggest that the dams got more food and fluid during the period of administration which may have resulted in the increased weight gained. The increased weight gain may also have been due to the increased number of developing foetuses as shown by the increased litter size.

The present study showed an increase in maternal weight during lactation in the HS treated groups that peaked in the 3rd weeks of lactation when compared with control. The increase in weight could also be as a result of increased food and fluid intake in the HS treated group.

4.4 *Azadirachta indica* and Weight during Pregnancy and Lactation

This study revealed significant increase in maternal weight in the A1 400 mg treated dams when compared with control while the A1 200mg dams showed significant increase in maternal weight in the 1st and 2nd week but at the 3rd week there was no significant difference ($p>0.05$) when compared with control. This significant increase in weight may be as a result of increased intake of food and fluid during the period of pregnancy.

The present study also showed an increase in maternal weight during lactation in the AI treated groups that peaked in the 3rd weeks of lactation when compared with control. The increase in weight could be as a result of increase in food and fluid intake in the AI treated group.

5. CONCLUSION

From the results of the present study, it can be concluded that consumption of extracts of HS and AI during pregnancy and lactation increases fluid and food intake and weight gain of dams with a possible potential to decrease postpartum weight gain during lactation.

CONSENT

It is Not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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