

EFFECT OF CONSUMPTION OF AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* AND *AZADIRACHTA INDICA* DURING PREGNANCY AND LACTATION ON BODY WEIGHT CHANGES **IN RATS.**

RUNNING TITLE: AQUEOUS *HIBISCUS SABDARIFFA*, *AZADIRACHTA INDICA* AND WEIGHT CHANGES

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 pregnant rats weighing 150-200g 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

22 monitored throughout pregnancy and lactation. The results showed that the
23 aqueous extract of HS and AI increased consumption of food and fluid during
24 pregnancy and lactation, increased maternal weight gain during pregnancy and
25 lactation. From the results of the present study, it can be concluded that
26 consumption of aqueous extracts of HS and AI during pregnancy and lactation
27 increased fluid and food intake and weight gain of dams with a possible
28 potential to accelerate weight loss or decrease postpartum weight retention
29 during lactation.

30 **Keywords:** Hibiscus sabdariffa, Azadirachta indica, pregnancy, lactation,
31 weight changes.

32 INTRODUCTION

33 *Hibiscus Sabdariffa* and *Azadirachta indica* are used as medicines and food
34 ingredients in many parts of the world including Nigeria¹⁻³. Both plants are
35 highly sourced as food vegetable particularly because of their health promoting
36 and diseases-preventing properties which is strongly suspected to be due to the
37 presence of many phytochemicals in them¹. These phytochemicals like alkaloid,
38 saponin, glycoside, tannin, phenol, flavonoid, steroid, reducing sugar, nimbidin,
39 sodium nimbidate, nimbin, gedunin, delphinidin 3-sambubioside and
40 protocatechuic acid in the two plants have been found to be protective and

41 preventive against many degenerative diseases and pathological process such
42 as in ageing².

43 *Hibiscus sabdariffa* (family: malvaceae) commonly known as zobo in Nigeria,
44 is an annual herbaceous shrub, cultivated for its flowers, leaves and seeds. It is
45 found in the tropics, subtropics and other parts of the world³ but it is utilized
46 beyond these areas of cultivation globally. In folk medicine this medicinal herb
47 is used for the treatment of hypertension^{4,5}. The plant is also reported to have
48 hepatoprotective, anti-hyperlipidemic, anticancer and antioxidant properties⁶.

49 *Azadirachta indica* which is commonly referred to as Dogonyaro or neem plant
50 is an evergreen robust tree belonging to the family meliaceae. It is mostly
51 found in tropic and sub-tropical areas of the world, African and Asia⁷. The tree
52 occurs in medium to large size and has dark grey bark and a dense rounded row
53 of pinnate leaves⁸. All parts of the neem tree (leaves, flowers, seeds, fruits, roots
54 and bark) are widely used in traditional medicine. *Azadirachta indica* is used
55 for the treatment of many health related problems and also known to exert
56 anticancer, antioxidant, wound-healing and antimicrobial properties⁹. All parts
57 of this plant are useful and has been used to treat diseases ranging from tooth
58 decay, ulcer, swollen liver, malaria and dysentery^{8,10}.

59 Extracts of HS and AI are widely used in Nigeria for medicinal purposes and
60 have also been shown to affect weight changes anecdotally through mechanisms

61 not yet defined. There are reports of decreased food consumption and weight
62 gain in rats consuming HS extracts as the drinking solutions^{11,12} but there is
63 paucity of data on the effect of these two extracts, administered by gavage, on
64 weight changes during pregnancy and lactation. This study was therefore
65 designed to investigate this in relation to food and fluid intake.

66

67 **MATERIALS AND METHOD**

68 **PLANT COLLECTION, IDENTIFICATION AND EXTRACT** 69 **PREPARATION**

70 Matured calyces of HS were purchased from a local market in Enugu and fresh
71 matured leaves of AI were harvested from *Azadirachta indica* tree located in the
72 premises of University of Nigeria, Enugu campus. Both plant samples were
73 identified and authenticated by Mr. Onyeukwu, C.J. of the Department of Plant
74 Science and Biotechnology, University of Nigeria, Nsukka where voucher
75 specimens (numbers UNH No 75f and UNH No. 521^A respectively) were
76 deposited.

77 The extraction procedure used for HS was as described previously¹³. Briefly,
78 30g of the dry petals of HS was brewed in 400ml of boiled tap water for
79 45min. The resulting decoction was filtered and evaporated to dryness giving a
80 dark red paste with percentage extraction yield of 47%. The leaves of AI were

81 washed and air-dried. The dried leaves were homogenized using an electric
82 blender. The powder was exhaustively extracted in distilled water at 60⁰c for
83 48hours using soxhlet extractor according to a previously described method¹⁴.
84 The resulting decoction was also filtered and evaporated to dryness giving a
85 black paste with percentage extraction yield of 19.5%. Both extracts were stored
86 in the refrigerator for preservation until use.

87 **PHYTOCHEMICAL ANALYSIS**

88 The standard method of Trease and Evans¹⁵ were used in the analysis of the
89 phytochemical components of calyces of HS and leaves of AI. The qualitative
90 and quantitative phytochemical analyses of the extracts showed the following
91 components in the tables below.

92 **Table 1: The phytochemical analysis of the aqueous extract of *Hibiscus***
93 ***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
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94 + = Slight; ++ = Moderate

95 **Table 2: The phytochemical analysis of the aqueous extract of *Azadirachta***
 96 ***indica* leaves**

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

97 + = Slight; ++ = Moderate

98

99 **EXPERIMENTAL ANIMALS**

100 40 inbred virgin albino rats aged between 10-12 weeks weighing 150–200g with
 101 two consecutive regular 4-day estrus cycles were used for this study. The rats
 102 were housed in cages and acclimatized for 2 weeks and maintained under
 103 standard environmental conditions and were also allowed free access to food
 104 (grower pelleted feed) and water.

105 10 male rats of proven fertility were introduced into the cages in the ratio of 1: 4
106 to allow for mating. Day 1 of pregnancy was taken as the day sperm was seen in
107 the vaginal smear of the rats¹⁶.

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

112 **Group A (n=16):** This group was administered *Hibiscus sabdariffa* extract in
113 two doses: low dose (1.5g/Kg body weight) and high dose (3g/Kg body
114 weight)¹⁷.

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

118 **Group C (n=8):** This group was the control group and was administered water.
119

120 **MEASUREMENT OF MATERNAL BODY WEIGHT AND FLUID AND** 121 **FOOD INTAKE**

122 The maternal body weight was measured daily during pregnancy and lactation
123 to determine the effect of the two extracts on body weight of the pregnant rats

124 during the 3 weeks of both pregnancy and lactation. Food and fluid intake were
125 also measured during these periods. These measurements were done using a
126 digital electronic compact balance (S. METTLER, CHINA) and a plastic beaker
127 and recorded to the nearest unit.

128 This work was approved by the College of Medicine Research and Ethics
129 Committee, University of Nigeria, Enugu Campus, Enugu with protocol number
130 040/02/2016.

131

132 STATISTICAL ANALYSIS

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

137

138 RESULTS

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

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

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

Periods	<i>Hibiscus sabdariffa</i>			<i>Azadirachta indica</i>		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
1 st week	20.1±0.19	26.9±1.24 ^{ab}	23.96±0.45 [*]	20.10±0.19	27.48±0.48 [*]	26.64±0.84 [*]
2 nd 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}
3 rd 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}

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

148 **Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and**
 149 ***Azadirachta indica* on fluid intake during pregnancy**

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

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

Periods	<i>Hibiscus sabdariffa</i> (HS)			<i>Azadirachta indica</i> (AI)		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
1 st 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}
2 nd 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}
3 rd 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}

159 * = P <0.05 vs control, P = p<0.05 vs low dose

160 Low dose HS (1.5g/kg bwt) and high dose HS (3.0g/kg bwt)

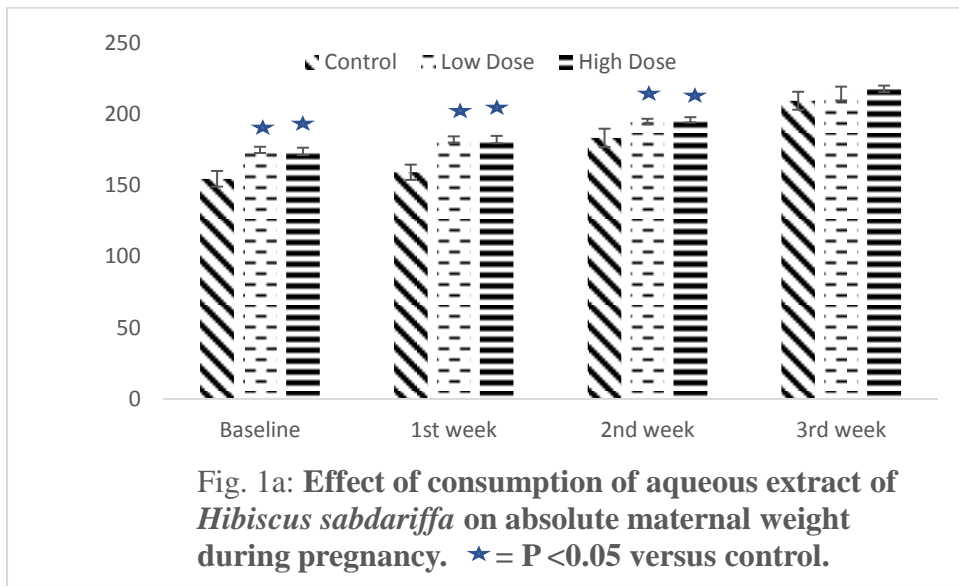
161 Low dose AI (200mg/kg bwt) and high dose AI (400mg/kg bwt)

162 **Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and**
 163 ***Azadirachta indica* on maternal weight during pregnancy**

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

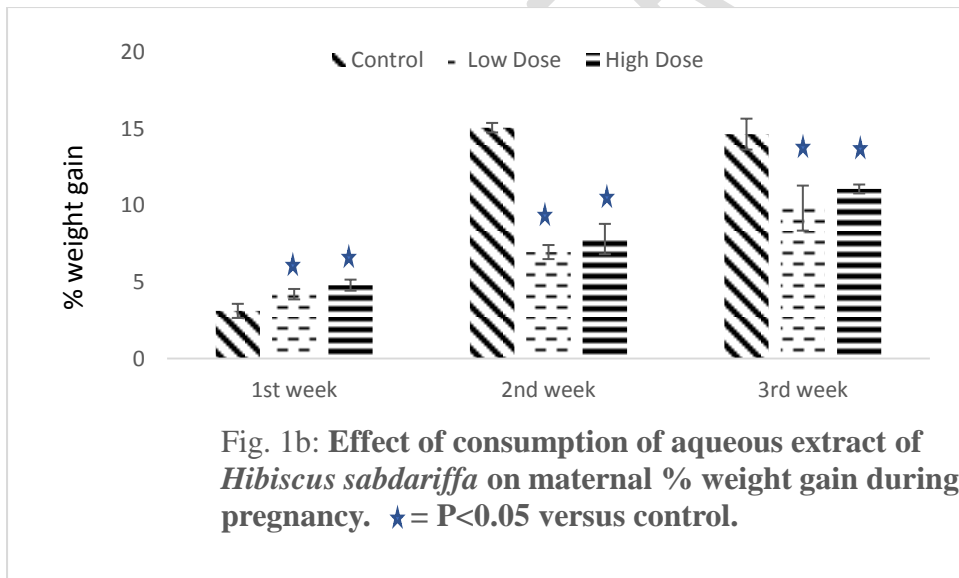
170 There were also progressive significant increases in maternal weight gain in the
 171 low and high dose AI groups as pregnancy progressed (Fig. 2a and 2b)). These
 172 increases were however lower than those of the control group except at week
 173 one in which case there was no difference between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

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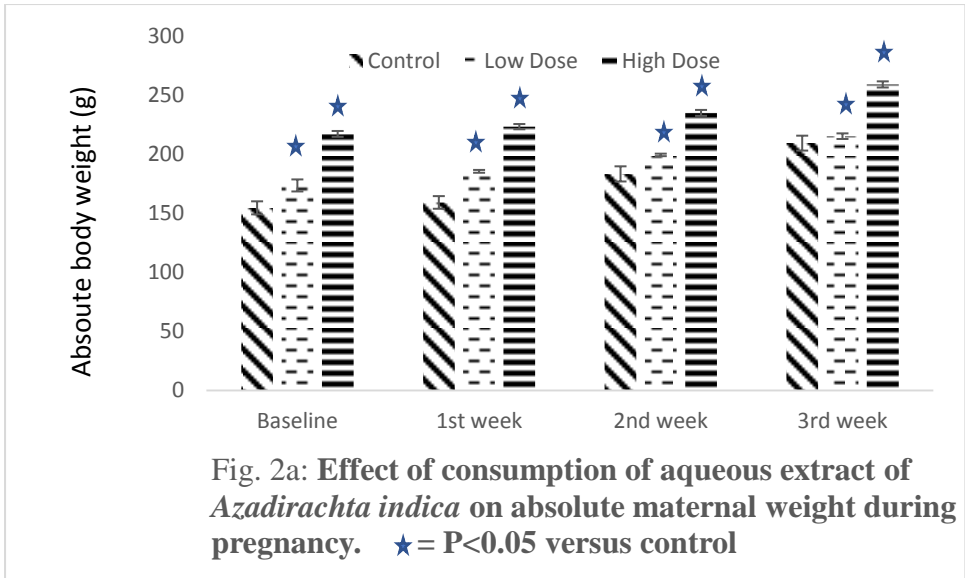
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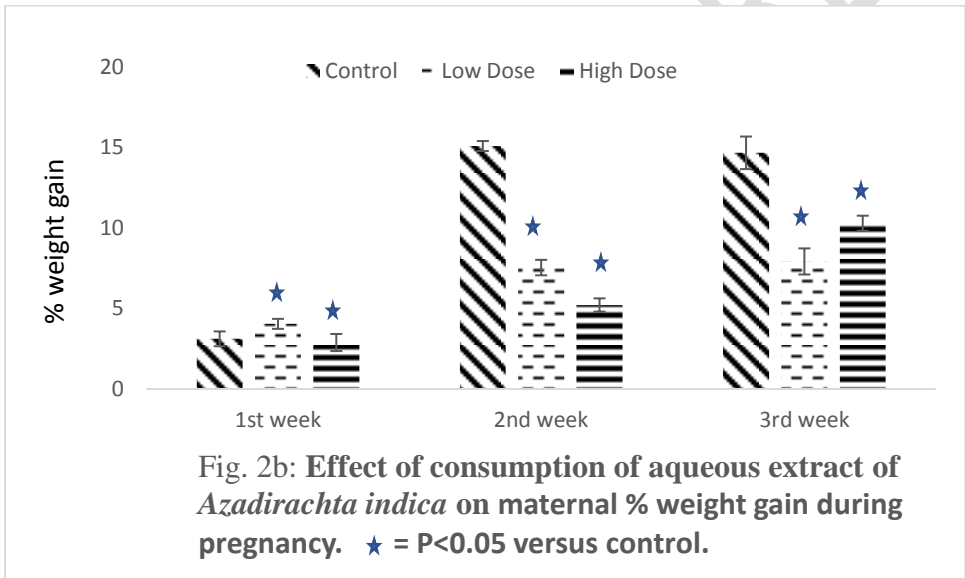
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186 **Effect of consumption of aqueous extract of *Hibiscus Sabdariffa* on food**
 187 **intake during lactation**

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

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

Periods	<i>Hibiscus sabdariffa</i>			<i>Azadirachta indica</i>		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
1 st week	25.22 ± 2.34	31.30 ± 2.16*	24.65 ± 1.49 ^P	25.22 ± 2.34	29.84 ± 1.92	27.56 ± 2.38
2 nd 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
3 rd 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*

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

200

201 **Table 5b: Effect of consumption of aqueous extract of *Hibiscus Sabdariffa***
 202 **and *Azadirachta indica* on fluid intake during lactation**

203 The fluid consumption in the low dose HS group was significantly greater than
 204 the fluid consumption in both the control and high dose HS groups. There was

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

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

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

212

213 **Effect of consumption of aqueous extract of *Hibiscus sabdariffa* and**
 214 ***Azadirachta indica* on maternal weight during lactation**

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

221 groups during lactation except the low dose group that was significantly lower
 222 than that of the control in the 1st week of lactation (Table 6b).

223 **Table 6a: Effect of consumption of aqueous extract of *Hibiscus sabdariffa***
 224 **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*			
1 st 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
2 nd 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* ^{aa}
3 rd week	190.60±7.85	216.86±4.67* ^p	226.10±1.25* ^p	6.90±1.28 ^a	6.54±0.90	3.88±0.77* ^α

225 Low dose HS (1.5g/kg bwt) and high dose HS (3.0g/kg bwt)
 226 Low dose AI (200mg/kg bwt) and high dose AI (400mg/kg bwt)
 227 * = P<0.05 vs Control; a = P<0.05 vs 1st week; α = P<0.05 vs Low Dose
 228

229 **Table 6b: Effect of consumption of aqueous extract of *Azadirachta Indica***
 230 **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*			
1 st 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
2 nd 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
3 rd 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

231 Low dose HS (1.5g/kg bwt) and high dose HS (3.0g/kg bwt)

232 Low dose AI (200mg/kg bwt) and high dose AI (400mg/kg bwt)
233 * = 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
234

235

236 **DISCUSSION**

237 ***Hibiscus sabdariffa* and Food and fluid intake during pregnancy and** 238 **lactation**

239 The present study revealed significant increase in food intake in the test group
240 when compared with control and also decrease in fluid intake (p<0.05) in the 1st
241 and 2nd week of pregnancy but significant increase in the 3rd week was observed
242 only in the HS high dose. Earlier reports^{11,17} have shown that the aqueous
243 extract of HS decreases food and fluid intake during pregnancy through a
244 mechanism not yet fully understood. The differences between the findings of
245 the earlier report and the observation of this study could be as a result of the
246 differences in the mode and route of administration and the duration at which
247 the dams received the plant extract. In earlier reports^{11,13,17} the extract was
248 administered as the drinking solution only, but in the present study the extract
249 was administered orally, and water given *ad libitum*. The extract is hypertonic
250 and thus stimulates the sensation of thirst when administered orally. This
251 sensation of thirst may have been responsible for the increase fluid intake
252 observed in the present study. Also, the extract of HS is rich in Na⁺¹⁷ which also

253 increases the sensation of thirst. With increased fluid intake and consequent
254 hydration, there is the abolition of the decreased food intake induced by the
255 dehydration-anorexia following consumption of HS¹⁷. The present study also
256 showed that maternal consumption of aqueous extract of HS during lactation
257 caused significant increase in food intake in the low dose group and significant
258 decrease ($p < 0.05$) in the high dose group when compared with the control
259 group. HS low dose also caused significant increase in fluid intake while the
260 high dose showed no significant difference ($p > 0.05$) when compared with
261 control. The observation from the present study was different from the
262 observation of Iyare and Adegoke¹¹ who reported a reduction in fluid and food
263 intake following administration of the extract during lactation. Again, the
264 differences in the observation may be due to the difference in the mode and
265 route of administration as earlier discussed.

266

267 ***Azadirachta indica* and Food and fluid intake during pregnancy and** 268 **lactation**

269 The present study revealed significant increase in food intake when compared
270 with control. Low dose AI showed no significant difference ($p > 0.05$) of fluid
271 intake in the 1st and 2nd week of pregnancy when compared with control but
272 increased significantly in the 3rd week of pregnancy. AI high dose showed

273 progressive increase in fluid intake when compared with control. It has been
274 noted that if tannin concentration in the diet becomes too high, microbial
275 enzyme activities including cellulose and intestinal digestion may be
276 depressed¹⁹. It is possible that the increased food and fluid intake observed in
277 this study may have been as a result of decreased amount of tannin present in
278 the plant extract used for this study. The basic physiological principles that
279 governs the regulation of nutrient intake appears to be neither the fluctuation of
280 energy level of the body nor energy content of ingested foods but the detection
281 of the degree of depletion and repletion of essential nutrients²⁰. Therefore, it
282 could be concluded that the energy need of the dam during the period of
283 pregnancy necessitated the increased food and fluid intake by the hormones
284 Neuropeptide Y which predominately increased carbohydrate intake in rats by a
285 direct action within the central nervous system²¹.

286 There was no significant difference ($p>0.05$) in food intake in the 1st and 2nd
287 week of lactation in the AI low and high doses when compared with control but
288 in the 3rd week there was a significant increase in the AI low and high dose
289 groups when compared with control. Progressive increases in fluid intake was
290 observed as lactation progressed when compared with control. Wang et al.²²
291 found that condensed tannin from *L. corniculatus* increased milk yield secretion
292 rates of protein and lactose thereby increasing efficiency of milk production. It
293 is therefore possible that due to the nutritional requirement of the dam to meet

294 up with milk production for the offspring the tannin in extract affected food and
295 fluid intake which was evident in the increased value of the breast milk
296 creamatocrit noticed in the 3rd week of lactation. Lactating mothers who do
297 not get enough energy and nutrition are at risk of maternal depletion and in
298 other to prevent this enough food must be made available to the mother.
299 Breastfeeding also increases the mother's need for water this may suggest the
300 reason for the increased fluid intake noticed.

301

302 ***Hibiscus sabdariffa* and Weight during pregnancy and lactation**

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

307 Iyare and Adegoke¹³ noted that the plant extract caused decrease fluid and food
308 intake that resulted in decreased pregnancy weight gain amongst the dams
309 that consumed the extract. These variations noticed in this study and that of
310 others^{11,13,17} may possibly be as a result of differences in the method of
311 administration of the plant extract as discussed above. The increase in maternal
312 weight observed in this study during pregnancy may therefore suggest that
313 the dams got more food and fluid during the period of administration which

314 may have resulted in the increased weight gained. The increased weight gain
315 may also have been due to the increased number of developing foetuses as
316 shown by the increased litter size.

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

321

322 ***Azadirachta indica* and Weight during pregnancy and lactation**

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

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

333 CONCLUSION

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

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