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Research Article

Effect of *Hibiscus sabdariffa* L. Dried Calyx Ethanol Extract on Fat Absorption-Excretion, and Body Weight Implication in Rats

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The effect of *Hibiscus sabdariffa* L. (Hs) calyx extract on fat absorption-excretion and body weight in rats, was investigated. Rats were fed with either a basal diet (SDC = Control diet) or the same diet supplemented with Hs extracts at 5%, 10% and 15% (SD_5, SD_{10} and SD_{15}). Only SD_5 did not show significant increases in weight, food consumption and efficiency compared to SD_C. The opposite occurred in SD_{15} group which showed a significant decrease for these three parameters. The SD_{10} responses were similar to SD_{15}, with the exception of food consumption. In both SD_C and SD_5 groups, no body weight loss was observed; however, only in the latter group was there a significantly greater amount of fatty acids found in feces. A collateral effect emerging from the study is that components of Hs extract at the intermediate and greater concentrations used in this experiment could be considered possible antiobesity agents.

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1. Introduction

The scientific groups carrying out studies on *Hibiscus sabdariffa* L. family Malvaceae calyx ethanol extract in order to ascertain its physiological activity-structure relationship are generally located in areas where it is used in food applications and traditional medicine [1]. Advances in these studies can be divided into three lines: therapeutic effects on lipid metabolism [2–8]; antihypertensive effects [9–13]; apoptotic effects in gastric carcinoma cells [14, 15]. In several of the studies, [2, 4, 6, 8–11, 13] no extract standardization is reported, although a tendency is observed to overcome this deficiency. Besides the calyx, *H. sabdariffa* L. leaf has also been subjected to this type of scientific studies, particularly leaf ethanolic extracts, which have been found to influence lipid metabolism [16–20].

Hs calyx extract contains hibiscus acid, or (+)-hydroxycitric acid, known as (+)-HCA [21–23]. Its isomer, (−)-hydroxycitric acid or (−)-HCA, the active ingredient principle present in *Garcinia indica* and *Garcinia cambogia* fruits, is an inhibitor of citrate lyase [24, 25], and because of this, it has been proposed as an anti-obesity agent [25–30]. Tee et al. [31] reported that hydroxycitric acid (no isomer specified), present in Hs calyx extract, inhibits fat production from carbohydrates in experiments carried out on rats. In addition, Carvajal-Zarrabal et al. [6] suggested that racemization of (+)-HCA to (−)-HCA by the intestinal flora may be a possible explanation to warrant the significant decrease in triacylglycerols in the experiment carried out on rats supplemented with Hs extract. This proposal agrees with the generalization made by Borriello et al. [32], which establishes that phytochemicals absorbed in the intestine can be transformed by colonic bacteria, resulting in serum components different from the original phytochemicals.

Therapeutic effects reported in studies carried out on animal models supplemented with Roselle, *Hibiscus sabdariffa* L. extracts include its influence on lipid metabolism as

Table 1: Composition of basal and experimental diets. Experimental diets are Basal diet plus *Hibiscus sabdariffa* ethanol calyx extract (Diet formulated according to AIN-93 G formulation).

Ingredient	Basal diet (g)
Cornstarch	35.5
Casein	20.0
Dextrinized cornstarch	13.2
Sucrose	10.0
Cellulose	5.0
Mineral mix AING-93 G	3.5
Vitamin mix AING-93 G	1.0
L-Cysteine	0.30
Choline bitartrate	0.25
Ter-butylhydroquinone	0.014
Lard	10.0
Cholesterol	1.0
Cholic acid	0.25

well as its antihypertensive and apoptotic actions. The aim of the present study was to research the action of Hs calyx extract on fat absorption, excretion, and body weight, as no information was encountered referring specifically to these aspects of lipid metabolism.

2. Materials and Methods

2.1. Plant Material and Extract Preparation. The air dried calyces along with a sample of the flowering plant of *Hibiscus sabdariffa* were acquired from the local market in Veracruz, Mexico. The sample was authenticated as *Hibiscus sabdariffa* L. by Prof. Sergio Avendaño and registered as O.Carvajal 001 at the Herbarium of the Ecology Institute A.C., Xalapa, Veracruz. Mexico. The calyces (135 g) were placed in a flask and 500 mL (96%) ethanol were added. The content was left for 8 days, with an occasional shaking to increase the extraction capacity. The macerated substance was filtered and concentrated in a rotary evaporator at 38 °C. The solid mass obtained from the evaporated extract was approximately 7.5% w/v. The concentrated plant extract was stored at 4 °C until used. Its viscosity at 25 °C was 1200 c.p.

2.2. Animals and Diets. The experimental protocol for animal experiments was approved by the Animal Ethic Committee, Chemical-Biology Area, University of Veracruz (Program: *Hibiscus sabdariffa* Part I 2004–2008). Forty Male Sprague-Dawley rats (6 weeks old, 250 to 350 g in weight) were purchased from Harlan Teklad, Co. (Mexico City) and individually housed in stainless steel mesh cages in a temperature-controlled room (22–25 °C regulated by an electronic timer) with a 12-hours light/dark cycle. They had free access to food and non-ionized water throughout the feeding period. The basal diet was prepared according to the American Institute of Nutrition [33] and is shown in Table 1. Lard (10 g/100 g diet) was employed as the source of dietary fat, and cholesterol and cholic acid were added at 1 and 0.25 g/100 g diet, respectively. The experimental diet is the basal diet plus ethanol dried extract of *Hibiscus sabdariffa* calyces at levels of 5, 10, and 15 g of extract/100 g diet. Animals were fed the basal diet for one week in order to develop an atherogenic condition (cholesterol ≥ 220 mg/dL, atherogenic index defined as total cholesterol, cHDL/cHDL ≥ 2.5). Thereafter, they were divided into four groups (10 rats each). The control group (SD_C) was maintained on the basal diet and three groups of rats, designated as SD_5, SD_{10}, SD_{15}, received the respective experimental diet for 4 weeks. The parameters that were quantified are directly related to the digestive process and its effects; as this lasts approximately 3 hours, its effect on body weight can be measured with confidence and reproducibility within an experimental period of 4 weeks. Diets were prepared once a week and stored in powdered form at 4°C until feeding. Body weight and food intake were measured daily. Feces were collected during the last 5 days and freeze-dried. At the end of the experimental period, diets were withdrawn for at least four hours.

2.3. Determination of Fat in Feces. Fecal fats were extracted according to the method of Jeejeebhoy et al. [34]. Briefly, 1 g freeze-dried feces was acidified with 2 drops concentrated HCl to release free fatty acids, and sequentially extracted with solvent No.1 (heptane: diethyl ether: 95% ethanol = 1 : 1 : 1) and solvent No.2 (heptane: diethyl ether: 95% ethanol: water = 1 : 1 : 1 : 1). The lipid extract was vacuum dried (Gallenkamp Oven Mod. UAF- 570-0300) and weighed. The apparent absorption rate (%) of dietary fat was calculated as 100 × [amount of daily fatty acid intake − amount of fecal fatty acids excreted]/[amount of daily fatty acid intake]. To identify the individual fatty acids excreted into the feces, the lipid extract was saponified with an ethanol-KOH solution, and the saponified fatty acids were acidified with an HCl solution, diluted with an equal volume of H_2O, and methylated with an H_2SO_4/MeOH solution (1 : 115) as described by Ikeda et al. [35]. Fatty acids were determined by gas chromatography (Hewlett Packard 5890, Palo Alto, CA.) with pentadecanoic acid as an internal standard. All chemicals used were analytical grade.

2.4. Statistical Analysis. The obtained data were expressed as mean ± standard deviation of means ($\bar{x}$ ± SD). A one-way analysis of variance (ANOVA) was used to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where a significant difference was indicated. Minitab version 12 statistical software was used.

3. Results

3.1. Atherogenic Condition, Growth, and Diet Consumption Parameters. Animals fed the basal diet attained an atherogenic condition, their levels of cholesterol and their atherogenic index being 481 ± 82 mg/dL and 6.1, respectively. Table 2 shows growth, food consumption, and weight parameters for both the control group SD_C and the three

Table 2: Growth parameters and fecal weight in rats treated with *Hibiscus sabdariffa* L. extract, A one-way analysis of variance (ANOVA) was used to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where a significant difference was indicated.

Parameters	Dietary groups			
	Control (SD_C)	SD_5	SD_{10}	SD_{15}
Initial body weight (g)	260 ± 7	261 ± 11	338 ± 10	261 ± 2
Final body weight (g)	307 ± 9	306 ± 11	356 ± 11	267 ± 4
Body weight gain (g)	47 ± 7	45 ± 5	18 ± 4**	6 ± 2**
Food intake (g/d)	14.5 ± 0.3	14.2 ± 0.8	13.8 ± 0.6	12.1 ± 1.1*
Food efficiency (g body weight gain/g food intake)	3.2 ± 0.6	3.2 ± 0.3	1.3 ± 0.3*	0.5 ± 0.2**
Fecal dry weight (g/d)	1.25 ± 0.08	1.42 ± 0.12*	0.81 ± 0.05**	0.75 ± 0.09**

Statistical differences ($^*P < .05$; $^{**}P < .01$), when compared with the control group.

Table 3: Apparent fat absorption and fecal fat excretion in rats fed with *H. sabdariffa* L. extract, A one-way analysis of variance (ANOVA) was used to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where a significant difference was indicated.

Parameters	Dietary groups			
	Control SD_C	SD_5	SD_{10}	SD_{15}
Fatty acid absorption (%)	95.1 ± 0.31	91.4 ± 1.01**	93.6 ± 1.83	95.2 ± 0.89
Fatty acid excretion (μmol/d)				
14 : 0	7 ± 1	12 ± 1	5 ± 1**	5 ± 1**
16 : 0	20 ± 4	396 ± 40**	140 ± 7*	89 ± 6*
16 : 1	8 ± 1	10 ± 1	9 ± 1	9 ± 1
18 : 0	289 ± 97	437 ± 58**	55 ± 5**	33 ± 5**
18 : 1	8 ± 1	62 ± 9**	17 ± 2*	14 ± 1*
18 : 2 (n-6)	9 ± 1	18 ± 2**	18 ± 2**	18 ± 1**

Statistical differences ($^*P < .05$; $^{**}P < .01$), when compared with the control group.

experimental groups supplemented with Hs extract (SD_5, SD_{10}, and SD_{15}). No significant differences were observed between the experimental groups and control as regards body weight; however, body weight gain in SD_{10} and SD_{15} groups was significantly less ($P < .01$) than in control group SD_C. Food consumption in the experimental groups (SD_5, SD_{10}, SD_{15}) decreased with Hs extract dose, but this only became significant ($P < .05$) in the SD_{15} group. Food efficiency for the SD_5 group was the same as for control SD_C; however, for SD_{10} and SD_{15} groups, a significant decrease in this parameter was observed ($P < .05$ and $P < .01$, resp.) compared to control. Feces weight (g/d) in all experimental groups varied significantly compared to the control, with a significant increase ($P < .05$) observed in the SD_5 treated group and a significant decrease ($P < .01$) in the SD_{10} and SD_{15} treated groups.

3.2. Apparent Fecal Fat Absorption and Excretion. The results shown in Table 3 reflect the effect of the diet the animals were subjected to in this study, concerning fat absorption and excretion. In the case of SD_5, fat absorption was significantly lower ($P < .01$) compared to control group SD_C. A tendency toward higher excretion was observed in the fecal fatty acid profiles of experimental groups, as compared to control. Specifically, SD_5 group showed an increase for all fatty acids in feces, significantly so for 16:0 (palmitic), 18 : 0 (stearic), 18 : 1 (oleic), and 18 : 2 (n-6) (linoleic). Fatty acids measured in groups SD_{10} and SD_{15} showed an increase in four cases: 16 : 0 (palmitic), 16 : 1 (palmitoleic), 18 : 1 (oleic), and 18 : 2 (n-6) (linoleic); only for palmitoleic was the increase not significant.

4. Discussion

Both experimental (SD_5, SD_{10}, and SD_{15}) and control (SD_C) groups were fed *ad libitum*. Only SD_5 group did not show a significant difference in the three parameters studied: weight gain, food consumption, and efficiency, as compared to control. These results were different between groups SD_5 and SD_{15}, SD_5 showing behavior similar to control, while in SD_{15} a significant decrease in all three parameters was observed. SD_{10} was similar to SD_{15}, except in the case of food consumption. Considering food efficiency comprises both weight gain and food consumption, and that, as a result, dietary components and their effect on body weight are related; it is evident that Hs extracts, at intermediate and greater concentrations used in these experiments (SD_{10} and SD_{15}), by not increasing body weight, reveal themselves as potential antiobesity agents.

On the other hand, the SD_5 group absorbed the least amount of fat, exhibited an increase in all fatty acids in feces resulting from fat hydrolysis, and did not lose weight, the

latter behavior being similar to control. Fatty acid excretion in SD_C, however, was less than in SD_5 group. One possible explanation for this behavior in control and SD_5 groups, where weight gain was similar though with differential lipid excretion, could be due to weight gain in SD_5 group basically through carbohydrate absorption; this assumes that Hs extract components at this level of concentration do not exert an inhibitory effect on pancreatic amylase.

Lower weight gain in SD_{10} and SD_{15} groups, added to their similar total lipid absorption, though different in excreted fatty acid type (greater palmitic, oleic, and linoleic and lower stearic acids as compared to control) seems to indicate that at these concentrations Hs extract components could inhibit pancreatic amylase, as reported by Hansawasdi et al. [36, 37], who identified Hibiscus acid, or (+)-HCA, as responsible for this action. This would consequently prevent polysaccharide unfolding and absorption. Mention should be made of the significant decrease in food consumption observed in the SD_{15} group, possibly related to a dietary palatability problem when Hs extract concentration was increased. Significantly increased C16:0 excretion, observed in all groups, is attributable to Hs extract chemical components. It is relevant to recall that the fat administered in this experiment was lard, rich in triacylglicerols with C16:0 in sn-2 position. Renaud et al. [38] observed that C16:0 interesterification in fat triacylglycerols results in a greater fat secretion in experimental animal feces accompanied by a decrease in TAG and cholesterol levels, including HDL cholesterol. We consider that this result is due to, amongst other factors, the specificity of lingual and gastric lipases which hydrolyse esters in positions 1 and 3, the latter being twice as susceptible as the former. In order to explain the effect of Hs extract chemical components, in their original state or modified by the intestinal flora, on increased C16:0 concentration (20, 7, and 4.5 times in SD_5, SD_{10}, and SD_{15}, respectively, compared to SD_C), as observed in the present study of fecal fat, the following hypotheses are proposed: inactivation of lipases; impediment of 2-monoacylglycerol uptake into the enterocyte, due to a competitive saturation of the specific transport system [39], or interesterification of the C16:0 from sn-2 to sn-1 or sn-3. Validation of these hypotheses requires further study.

It can be concluded that animals kept on a diet supplemented with Hs calyx ethanol extract showed significant C16:0 excretion in feces. The three hypotheses proposed to explain this excretion need subsequent testing and validation. Besides, a collateral effect emerging from the study is that Hs extract components at the intermediate and greater concentrations used in this experiment could be considered possible anti-obesity agents, through their tendency to inhibit α-amylase.

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