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ANTI-HYPERLIPIDEMIC AND ANTI-WEIGHT GAIN EFFECTS OF KHAYA TEA ON HIGH-FAT-DIET RATS

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ABSTRACT: Tea extracts are used in many over-the-counter preparations claiming to promote weight loss. The rationale for this usage includes reports that khaya extract inhibit the digestion/absorption of carbohydrate and fat into gastrointestinals tube of rats. The investigators in this study tested the potential of increasing doses of three extracts concentrations (3.3mg/Kg of khaya tea, 6.6mg/Kg of khaya tea, and 9.9mg/Kg of khaya tea) to induce weight loss, steatorrhea, and blood lipid alterations in rats ingesting a high-fat diet. During the 90 days on the HFD, the animals were treated with 3.3mg/Kg, 6.6mg/Kg and 9.9mg/Kg of body weight of khaya tea. The time course of the body weight and obesity-related biochemical parameters were evaluated. The animals were fed with a standard diet (SD, n= 6) or high-fat diet (HFD, n= 6) for 90 days. After 90 days of treatment with 3.3mg/Kg, 6.6mg/Kg and 9.9mg/Kg of body weight, Khaya tea suppressed the increases average 37.43% of the changes in body weight gain (expressed as % of initial body weight) ($P< 0.05$) and decreased the serum triglycerides and low-density lipoprotein (LDL)-cholesterol concentrations at both doses (from 69±2 to 50.3 ±2.7 mg/dL, from 27±1 to 23.5 ±1 mg/dL; $P< 0.05$, respectively) after they had been increased by the HFD. The abdominal lipids content was also decreased by the diet containing khaya tea (from 7.30 ±0.11 to 5.52 ±0.16 mg/dL; $P< 0.05$). These results suggest that khaya tea could be a potentially therapeutic alternative in the prevention of obesity caused by a HFD.

KEYWORDS: *KHAYA senegalensis*, Khaya Tea, anti-hyperlipidemic effect, anti-weight gain effect, bioactives components (phenols and polyphenols) of KT, High-Fat-Diet (HFD) rats.

1 INTRODUCTION

One of the most important strategies in the treatment of obesity includes the development of nutrient digestion and absorption inhibitors, in an attempt to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanisms [1], [2].

In addition to polyphenols such as alkaloids, saponins, flavonoids, tannins and DPPH anti-radical activity, Khaya bark is also rich in phenols, and some phragmalin limonoids such as khayanolides, khayanosides, 2,6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydroxyseneganolide [3], [4].

Khaya is a tree of the family Meliaceae consisting of seven species which originated from tropical Africa. *Khaya senegalensis* is present in Mauritania, Senegal and right up to Northern Uganda and in Cameroon. Its bark is often used to treat certain illnesses such as malaria, head-aches, fever, smallpox, diarrhea, lumbago (back pain), rheumatism, wounds, etc [4]. Khaya tea, a beverage produced from extracts of the bark of Khaya tree is even said to be effective in weight loss therapies of Cameroonian population.

This studies reporting the biological effects of khaya tea, especially with respect to the antioxidant and inhibition properties of the constituents of *KHAYA senegalensis* bark's extracts in animal models. The effect of some polyphenols and others bioactifs components in the inhibition of digestive enzymes has been investigated [5], this is the first report on the

inhibitory activity of khaya tea against porcin pancreatic lipase (LPP). So, the aim of the present study were to examined, the preventive effects of khaya tea on the development of obesity in rat fed a high-fat diet (HFD).

2 METHODS AND PROCEDURES

2.1 KHAYA TEA PREPARATION AND ADMINISTERED DOSES

The extract of Khaya tea has been prepared [3]. The administered doses refitting on results of Abubakar [6] which stipule that for 28 days, for the exctract bark's concentration of *Khaya senegalensis* inferior than 10 mg (KT)/ kg of body weight, there isn't sub- chronictoxicity (ASAT, ALAT, creatinin). Therefore, the animals were treated with 3.3mg/Kg, 6.6mg/Kg and 9.9mg/Kg of body weight of khaya tea in later on.

2.2 PREPARATION OF ATORVASTATIN SOLUTION

The artorvastatin pill (10 mg/kg) has been bought at the pharmacy and has been administrated to animals by dissolution of one pill of atorvastatin in 10 ml distilled water.

2.3 ANIMALS AND DIETS

Thirty-six male 90-day-old Swiss strain rats (Sw/Uni) (240±40g), free of specific pathogens, were obtained from laboratory of Biophysic, Alimentary Biochemistry and Nutrition (ENSAI) of NGAOUNDERE UNIVERSITY-CAMEROON. The animals were maintained on a 12:12 h artificial light–dark cycle and housed in individual cages. After a random selection, the rats were introduced to the standard diet A0 (SD, n= 6) or HFD (n= 30) for 90 gays. The compositions of the experimental diets are shown in Table 1. Before the 90 days of the HFD, the animals were randomly divided into five subgroups according to the intervention: B0: (group 1 ; n= 6 ; control group) received HFD plus pure water (by gavage), B1 (group 2 ; n= 6) received HFD plus artovastatin (10mg/kg; by gavage), B2 (group 3 ; n= 6) received HFD plus an aqueous extract of khaya tea (3.3 mg/kg ; by gavage), B3 (group 4 ; n=6) received HFD plus an aqueous extract of khaya tea (6.6 mg/kg ; by gavage) and B4 (group 5 ; n=6) received HFD plus an aqueous extract of khaya tea (9.9 mg/kg ; by gavage). All the groups were treated for 90 days and the solutions were administered by intra-gastric gavage. The total food intake by each group was recorded at least three days, and the body weight of each rat was recorded at least three days. At the end of the experiment, the rats have eaten nothing 17 hours before and were deeply anaesthetized by oil's ether and sacrificed. After dissection, the blood were analysed immediatly then, tissues were collected and stored at −80 °C until analyzed. The experiments were performed in accordance with the principles outlined by the Brazilian College for Animal Experimentation.

Table 1. Composition of the modified experimental diets [7]

Nutrient	A0 : negative control	B0 : positive control	B1 : HFD+10mg/Kg (Artovatatin)	B2 : HFD+3.3mg/Kg (KT)	B3 : HFD+6.6mg/Kg (KT)	B4 : HFD+9.9mg/Kg (KT)
	Standard diet (g/kg)	High-fat diet (HFD) (g/kg)	High-fat diet (HFD) (g/kg)	High-fat diet (HFD) (g/kg)	High-fat diet (HFD) (g/kg)	High-fat diet (HFD) (g/kg)
Cornstarch	397.5	115.5	115.5	115.5	115.5	115.5
Casein	200	200	200	200	200	200
Sucrose	100	100	100	100	100	100
Dextrinated starch	132	132	132	132	132	132
Palm oil	—	312	312	312	312	312
Soybean Oil	70	40	40	40	40	40
Cellulose	50	50	50	50	50	50
Mineral mix	35	35	35	35	35	35
Vitamin mix	10	10	10	10	10	10
L-cystine	3	3	3	3	3	3
Choline	2.5	2.5	2.5	2.5	2.5	2.5
Total	1,000	1,000	1,000	1,000	1,000	1,000
Khaya Tea Extract (mg/Kg)	0	0	0	3.3	6.6	9.9

2.4 BIOCHEMICAL ANALYSIS

The serum was obtained by centrifugation of the blood at 800 g for 10 min and ALT, AST, creatinine, the total cholesterol, triglyceride, and high-density lipoprotein-cholesterol concentrations were immediately determined using an automatic analyzer (COBAS-MIRA System of Roche Diagnostics, Indianapolis, IN). Low-density lipoprotein (LDL)-cholesterol was calculated from the formula:

LDL-cholesterol (mg/dl) = total cholesterol − triglyceride/5 − high-density lipoprotein-cholesterol.

2.5 MEASUREMENTS

Food intake was assessed daily and individual body weights were obtained at least three days. Fecal fat was measured at least three days by weighing the rats on scales. A three-day fecal collection was obtained for the measurements. Fecal fat was determined using minor modifications of a standard gravimetric technique [8] Total fecal collection was added to pre-weighed polypropylene tubes. After the weight was determined, a recorded volume of water was added as needed to provide a smooth homogenate via a blender. Three grams of homogenate was transferred to polypropylene tubes with 1 drop concentrated HCl, 5 mL ethanol, and 10 mL hexane. The tubes were shaken for 10 minutes and centrifuged for five minutes. The hexane layer was transferred to pre-weighed glass vials. This extraction procedure was repeated with an additional 10 mL of hexane. The extracts were evaporated to dryness over a steam bath and stored overnight in a desiccator. The dry weights of the two extracts were added to obtain total fecal fat.

2.6 STATISTICAL ANALYSIS

The data were expressed as the mean ±s.e.m. Comparisons among the groups of data were carried out using the one-way ANOVA followed by the Dunnett multiple Comparisons test. The statistical significance for the expression of the analysis was also assessed by ANOVA and the differences identified were pinpointed by an unpaired Student's t-test. An associated probability (Pvalue) of <5% was considered significant.

3 RESULTS

3.1 EFFECTS OF KHAYA TEA ON WEIGHT GAIN

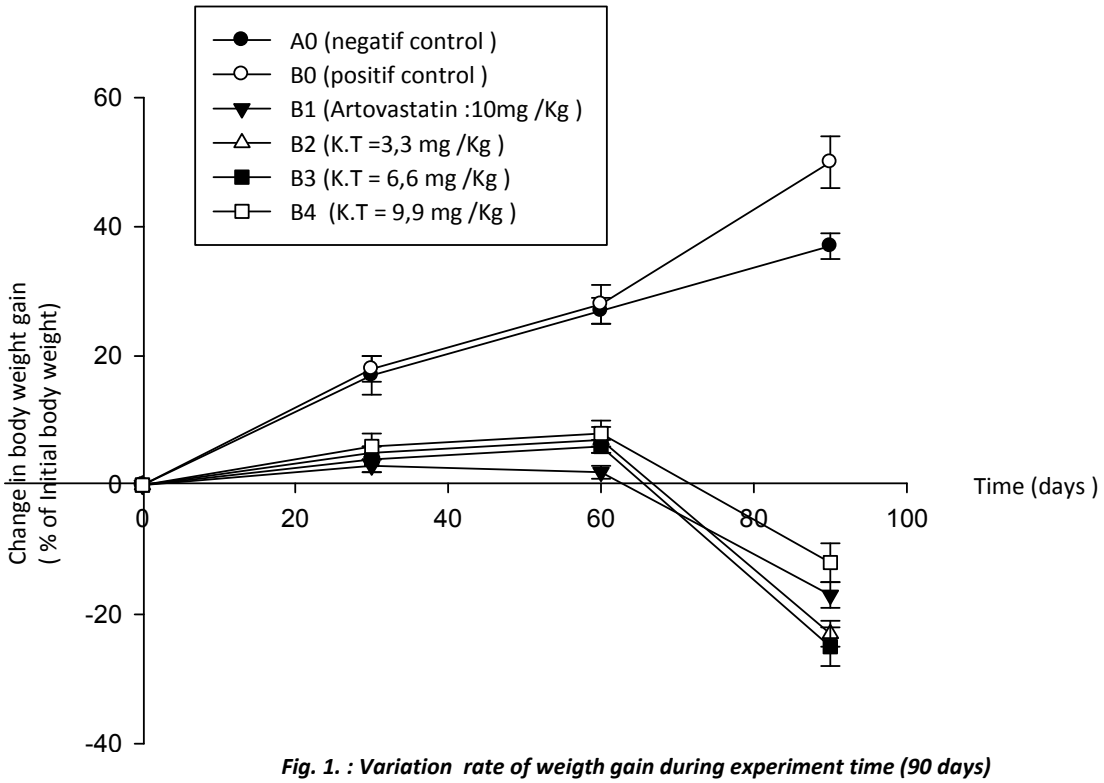

Fig. 1. : Variation rate of weigth gain during experiment time (90 days)

Fig. 1 shows average 37.43% of the changes in body weight gain (expressed as % of initial body weight) for the rats fed the HFD with. The rats fed on HFD for 90 days showed a significantly higher body weight gain than rats fed on the SD (HFD, 99.2% vs. SD, 58.2%; P< 0.05). In rats fed on HFD and treated with KT in the last thirthy days, the body weight gain (HFD + KT 3.3 mg/kg, 35.3% ; HFD + KT 6.6 mg/kg, 47,6 % and HFD + KT 9.9 mg/kg, 29.4% was significantly suppressed as compared to the group fed on HFD with pure water (HFD, 99.2% P< 0.05). It is interesting that three doses of KT suppressed the weight gain. The energy intake per rat differed between the SD and HFD groups throughout the whole experimental period, but it did not differ between the group fed HFD alone and the groups fed HFD plus KT.

3.2 EFFECTS OF KHAYA TEA ON SERUM PARAMETERS, AND ABDOMINAL LIPIDS

Table 2. Serum parameters and abdominal lipids in rats fed experimental diets

Serum lipids (mg /dL)	A0 : SD	B0 : HFD	B1 : HFD+ Artov.	B2 : HFD+ 3.3mg/Kg (KT)	B3 : HFD+ 6.6mg/Kg (KT)	B4 : HFD+ 9.9mg/Kg (KT)
Total cholesterol (mg /dL)	50±1^{a}	56±2^{b}	49±4^{a}	51±1^{a}	60± 3^{b}	44±2^{a}
LDL-cholesterol	26±1^{b}	27±1^{b}	24±2^{a}	23±1^{a}	30±2^{b}	24±1^{a}
HDL-cholesterol	26±1^{d}	13±1^{a}	21±1^{c}	15±1^{b}	14±2^{b}	17±3^{c}
Triglycerides	47±3^{b}	69±2^{c}	50±4^{b}	51±2^{b}	57±4^{b}	43±2^{b}
Abdominal lipids(g)	5.23±0.16^{a}	7.30±0.11^{b}	6.30±0.50^{b}	5.41±0.12^{a}	5.78±0.21^{a}	5.38±0.15^{a}

Data are expressed as means ±s.e.m. (n= 3). Means in the same row not sharing a common superscript are significantly different (P< 0.05).

As shown in table 2, the HFD-induced hyperlipidemia, with no significant increases in total serum cholesterol for HFD+ KT 6.6 mg/Kg group and significant decreases in total serum cholesterol for HFD+ KT 3.3 mg/Kg and for HFD+ KT 9.9 mg/Kg groups, LDL-cholesterol, and triglyceride as compared to the SD group. The animals fed on HFD and treated for the 90 days with Khaya tea (for HFD+ KT 3.3 mg/Kg and HFD+ KT 9.9 mg/Kg groups) showed significantly reduced total serum cholesterol and LDL-cholesterol levels as compared to the HFD alone. Serum triglycerides was also significantly reduced (P< 0.05) by all doses of the Khaya tea containing HFD and the levels obtained were significaty the same as those of the SD rats. Additionally, the HFD increased the amount of abdominal lipids as compared to the SD group. Thus the addition of khaya tea reduced (P< 0.05) the abdominal lipids, as compared to those fed on HFD alone. At these doses, the total abdominal lipids decreased significatly (P< 0.05).

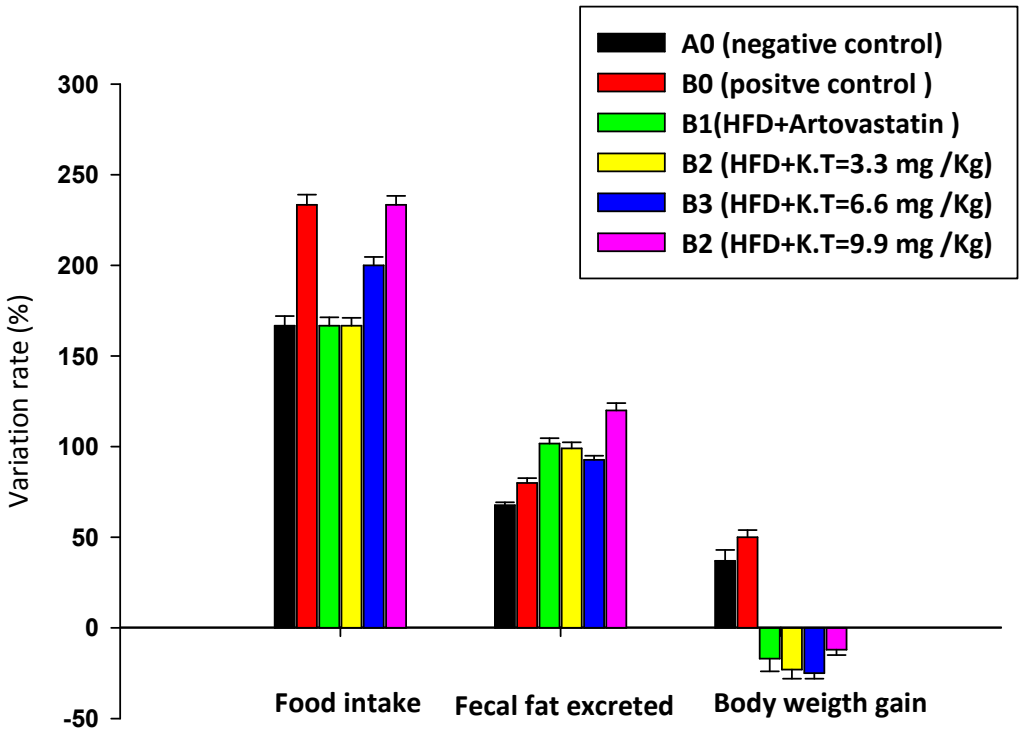

Fig.2. Variation rate of food intakes, fecal fat excreted, and weigth gain of the rats at the end of study (after 90 days)

Table 3. Fecal fat expressed as percent of fat ingested

	A0 : SD	B0 : HFD	B1 : HFD+ Artov.	B2 : HFD+ 3.3mg/Kg (KT)	B3 : HFD+ 6.6mg/Kg (KT)	B4 : HFD+ 9.9mg/Kg (KT)
Fecal lipids (g)	7.59±1.35^{a}	10.15±1.43^{b}	27.73±1.20^{d}	22.84±1.51^{c}	26.35±1.33^{d}	27.35±1.24^{d}
Fecal lipids rate (%)	/	0	17.58	12.69	16.20	17.2

Data are expressed as means ±s.e.m. (n= 3). Means in the same row not sharing a common superscript are significantly different (P< 0.05).

Table 3 shows fecal fat excretion expressed as percentage of fat ingested. All the groups treated by khaya tea (HFD+ KT 3.3 mg/Kg, HFD+ KT 6.6 mg/Kg and HFD+ KT 9.9 mg/Kg groups) reduced significantly more fat than did the control group during time of the study, with fat malabsorption averaging 12.69-17.20 % compared to HFD plus pure water group.

Table 4. Rate of ALT, AST and creatinine of differents rats groups

Parameters	A0	B0	B1	B2	B3	B4
ALT/GPT (U/L)	104±8^{c}	91±4^{b}	81±3^{a}	87±11^{a}	96±5^{b}	80±7^{a}
AST/GOT (U/L)	98±2^{d}	65±5^{b}	61±3^{a}	75± 5^{c}	59±6^{a}	50±6^{a}
Creatinine (mg/L)	8 .40±0.5^{a}	8.67±0.38^{a}	8.75±0.22^{a}	8.40±0.53^{a}	9.57±0.55^{a}	8.73±0.61^{a}

Data are expressed as means ±s.e.m. (n= 3). Means in the same row not sharing a common superscript are significantly different (P< 0.05).

The results of rats plasma in table 4 above shows, no significant differences (p<0.05) among of creatinine and the significantly decreased (p<0.05) among of ALT and AST of rats treated by Khaya tea comparatively to the controls groups. Similar results was recently reported by Abubakar *et al.* [6] showed that for aqueous stem bark of *KHAYA senegalensis*

inferior to 10 mg/ Kg, there were no sub-chronic toxicity in albino rats during thae last 28 days. This result shows that in the administrated doses, Khaya tea hadn't affected neither liver nor kidney of rats traited by khaya tea.

4 DISCUSSION

The wide spread usage of tea extracts for weight control is supported by rather slim in vivo evidence that includes a Humans study showing better maintenance of weight loss when tea was added to a calorie-restricted regimen [9] and a mouse study showing less weight gain when a tea extract was added to a high-fat diet [10].

Tea could promote weight loss via increased energy expenditure and/or decreased caloric uptake from the gut. Ingestion of green tea extract results in a modest increase in thermogenesis [11], [12], [13]. Although it is not clear to what extent this effect is attributable to the caffeine content versus other tea components such as epigallocatechin. In addition, *in vitro* studies show tea extracts inhibit the activity of enzymes that digest carbohydrate and triglycerides. Green tea inhibits alpha-amylase [14], [15], [16]. Khaya tea like Mulberry extract contains high concentrations of flavonoids that inhibit alpha-glucosidase activity [17], [18], [19].

This is apparently due to the *in vivo* ability of Khaya tea extract to increase thermogenesis and to inhibit pancreatic lipase activity. However, this effect appeared to diminish with time, in that during the last thirty of the study, fat malabsorption diminished with a significant increase in fecal fat observed in rats receiving the khaya tea croissant dosage. It should also be noted that the increase of fecal fat from 12.69 to 17.20 percent observed at the end of the study.Carbohydrate absorption was not measured in the present study, but an extrapolation from human studies [20], [21] suggests the extract should have resulted in malabsorption of sizable amounts of dietary carbohydrate. While the calories of triglyceride not absorbed in the small bowel are lost in feces, non-absorbed carbohydrate can be fermented by colonic bacteria to readily absorbable short-chain fatty acids; thus, most of the calories of malabsorbed carbohydrate are conserved [22]. Given that the rats did not develop diarrhea, the putative carbohydrate malabsorbed as the result of the action of the extract appeared to be removed from the fecal stream by this mechanism. The normal weight gain in rats treated with large doses of three extracts before 60 days, indicates the no-modest increase in food intake (fig. 2) observed in these animals compensated for the extract-induced malabsorption of calories as fat and carbohydrate.The relatively minor degree of steatorrhea induced by extract was associated with reductions in serum triglycerides, but statistically significant increases in HDL-cholesterol concentrations (Table 2). Similar tea-induced alterations in blood lipids, assumed to be beneficial to health, have previously been reported in animal [23], [24]. The Khaya tea prevented the HFD-induced increases in body weight (Fig. 1) and decreased the serum triglyceride, cholesterol, and LDL-cholesterol concentrations after they had been increased by HFD (Table 2). These effects did not depend on decreased food or energy intakes because there were no significant differences between the HFD and HFD plus KT groups. A portion of the administered polyphenol might be absorbed from the intestine, metabolized, and excreted into urine. Probably, higher dose of KT polyphenol might be excreted in the feces without absorption, but the mechanism for this remains to be elucidated. Similar results was recently reported by Paganini Stein *et al.* [25] showed that rats fed the hypercholesterolemic diet for 30 days and which were treated for the last 15 days with *I. paraguariensis* extract (500 mg/kg) showed significantly reduced serum cholesterol (30% reduction) and triglyceride (60.4% reduction). And Pang *et al.* [26] suggesting that the *I. paraguariensis* extract might have a protective effect against HFD-induced obesity in rats through an enhanced expression of uncoupling proteins and elevated AMPK phosphorylation in the visceral adipose tissue.

5 CONCLUSION

This study concludes that "physiological" dosages of khaya tea having the ability to reduce the weight gain in rats and inhibit carbohydrate and lipid absorption. It suggest that KT has an antiobesity function: it prevents the hydrolysis of dietary fat in the small intestine and reduces the subsequent intestinal absorption of dietary fat. The hypolipidemic effects of the Khaya tea shown in this study were in agreement with previous reports showed that Khaya tea efficiently inhibited *in vitro* porcine PL activities [5].

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DISCLOSURE

The authors declared no conflict of interest.

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