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A Mixture of Extracts of Black and Green Teas and Mulberry Leaf Did Not Reduce Weight Gain in Rats Fed a High-fat Diet

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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 green tea extract increases thermogenesis, and extracts of green and black tea and mulberry leaf inhibit the digestion/absorption of carbohydrate and fat. The investigators in this study tested the potential of increasing doses of a mixture of three extracts (50-percent black tea, 20-percent green tea, and 30-percent mulberry) to induce weight loss, steatorrhea, and blood lipid alterations in rats ingesting a high-fat diet, *ad lib*. The mixture was incorporated into chow in quantities of 0.5-, 3.0-, and 6.0 percent by weight; a control group received only chow. Food intake and weight were monitored daily, and quantitative fecal fat measurements were obtained weekly for four weeks. The 3.0- and 6.0-percent chows significantly increased fecal fat excretion to 15 percent of dietary fat intake (controls: 5%); however, no significant reduction in weight gain was observed. After four weeks of treatment, the 3.0- and 6.0-percent dosages were associated with significant reductions in serum triglycerides and increases in high density lipoprotein (HDL) cholesterol. However, these chow concentrations were associated with significant increases in serum ALT, and the 6.0-percent chow markedly increased serum alkaline phosphatase. This study does not provide support for the utility of this combination of black tea, green tea, and mulberry extracts in weight-loss regimens and indicates that high doses of this extract combination may be hepatotoxic. (*Altern Med Rev* 2007;12(4):43-49)

Introduction

At least 402 different dietary supplements for the purpose of weight reduction are sold in the United States,¹ a sizeable portion of which contain various teas or tea extracts. To date, studies designed to demonstrate tea-induced weight loss have yielded variable results.²⁻⁵

The scientific basis for the use of tea for weight control includes reports that green and black tea extracts increase thermogenesis,⁶⁻⁸ black tea inhibits fat digestion,^{9,10} and green tea and mulberry leaf extracts inhibit the digestion and absorption of carbohydrate.¹¹⁻¹⁸ The present study hypothesized that a combination of these extracts would reduce weight gain in rats fed a high-fat diet. Varying dosages of a mixture of extracts of green tea, black tea, and mulberry leaf were added to chow, and potentially beneficial effects on weight loss, fecal fat excretion, and blood lipids were assessed, as were deleterious effects on hepatic and renal function.

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Table 1. Composition of Diets (g/kg)

Nutrient	Controls	0.5% Tea Extract	3.0% Tea Extract	6.0% Tea Extract
Casein	195	195	195	195
DL-Methionine	3.0	3.0	3.0	3.0
Sucrose	341	339	326	311
Corn Starch	150	148	135	120
Anhydrous Milk Fat	210	210	210	210
Cholesterol	1.5	1.5	1.5	1.5
Cellulose	50	50	50	50
Mineral Mix AIN-76	35	35	35	35
Calcium Carbonate	4.0	4.0	4.0	4.0
Vitamin Mix, Teklad	10	10	10	10
Ethoxyquin	0.04	0.04	0.04	0.04
Tea Extract	0	5.0	30	60

Material and Methods

Extracts and Chow

The extract mixture was provided by NatureGen, Inc. (San Diego, CA) and consisted, by weight, of extracts of black tea (50%), green tea (20%) and mulberry (*Morus alba*) leaf (30%). The extracts were produced via repeated extraction of the dried leaves with a water/ethanol mixture, followed by evaporation to dryness. The mixture was incorporated into pellets made from a high-fat chow supplied by Harlan-Teklad Custom Research Diets (Madison, WI) (Table 1). Milk fat comprised about 43 percent of the chow calories, while carbohydrate (primarily corn starch) and protein (casein) provided about 42- and 15 percent of total calories (4.3-4.5 Kcal/g), respectively.

Subjects

Twenty male, Sprague-Dawley (Harlan; Indianapolis, IN) rats with initial weights of approximately 250 g were used. The rats were divided into four groups of five and provided with *ad lib* access to water and high-fat chow into which had been incorporated (by weight) 0.5-, 3.0-, and 6.0 percent of the mixture of extracts; a control group received chow without any of the extract mixture. The black tea dosages were selected to bracket the quantity required to yield an *in vitro* inhibition of pancreatic lipase similar to that of Orlistat[®], a commercially available lipase inhibitor. The green tea

and mulberry dosages were approximately five times greater (per unit body surface area) than the dosages previously shown to inhibit carbohydrate absorption in humans.^{19,20} The study was approved by the Animal Studies Subcommittee of the Minneapolis Veterans Affairs Medical Center.

Measurements

Food intake was assessed daily and individual body weights were obtained weekly. Fecal fat was measured weekly by housing the rats in individual wire screen bottom cages to minimize coprophagy, with measurements after one, two, and three weeks determined on 24-hour fecal collections. A three-day fecal collection was obtained for the measurements at four weeks. Fecal fat was determined using minor modifications of a standard gravimetric technique.²¹ 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

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in a desiccator. The dry weights of the two extracts were added to obtain total fecal fat.

After the final fecal collection, blood was obtained and the following values were determined: hematocrit, ALT, AST, alkaline phosphatase, bilirubin, creatinine, total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides.

Statistical Analysis

Data are expressed as mean \pm standard error of the mean and the significance of differences among groups was calculated by ANOVA.

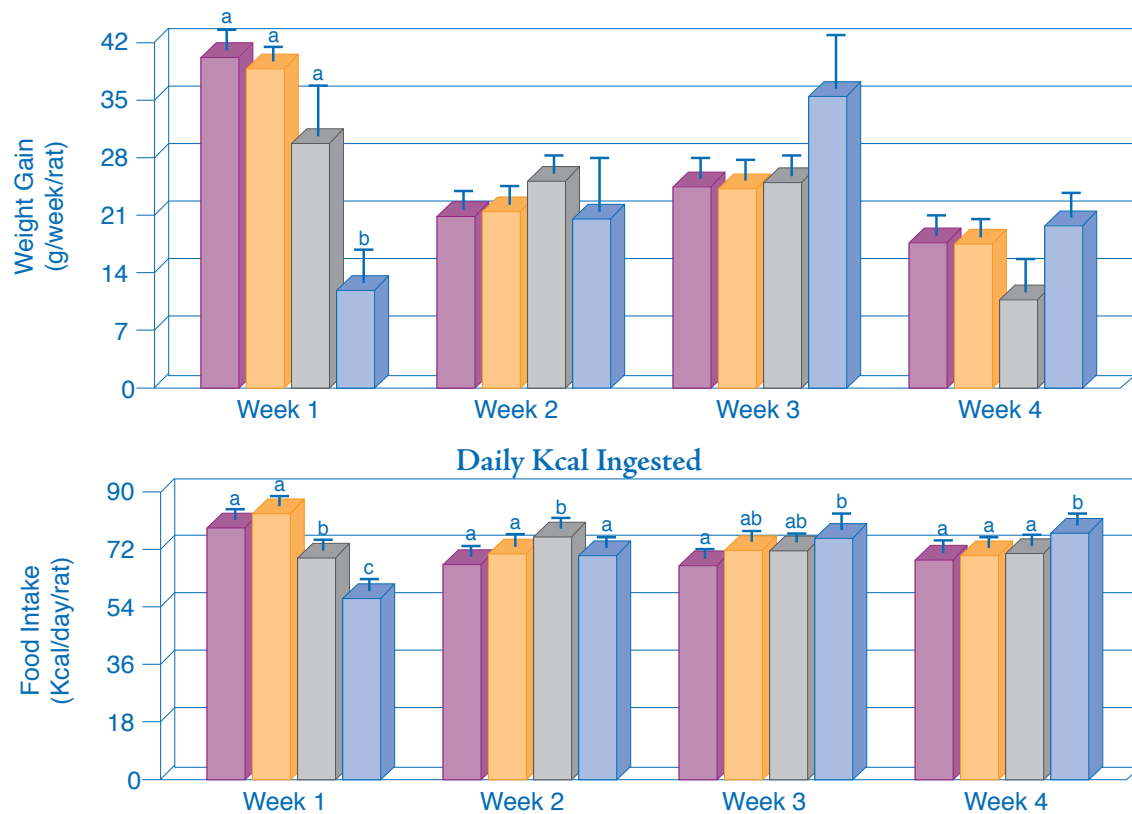
Results

Figure 1 shows the average weekly gain in body weight and the caloric intake for the five rats in each

group. During the first week, rats ingesting 6.0-percent chow gained significantly ($p < 0.001$) less weight than did the other groups (which were not significantly different from each other) (Figure 1, upper panel). In the subsequent three weeks there were no significant differences in weight gain among the four groups, and by the fourth week there were no significant weight differences among the groups (average weight of controls: 362 ± 7 g; 0.5-percent mix: 351 ± 10 g; 3.0-percent mix: 362 ± 8 g; and 6.0-percent mix: 350 ± 5 g). Diarrhea was not observed in any group of animals.

During the first week, caloric intake of rats receiving the 6.0-percent extract chow was appreciably less than the other groups (Figure 1, lower panel). However, during the last three weeks of the study, the calorie ingestion of the 6.0-percent group was greater than that of the controls, with this increase reaching

Figure 1. Mean Weekly Weight Gain

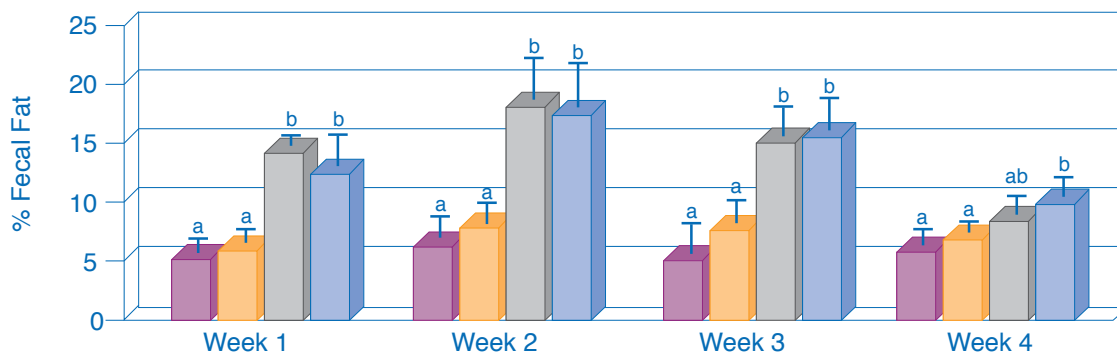


Rats fed a high fat diet containing no tea extract (purple bars), or 0.5% (orange bars), 3% (grey bars) or 6% (blue bars) tea extract. Data are expressed as mean \pm SEM, and different lower case letters indicate significant differences ($p < 0.05$).



Tea and Weight Loss

Figure 2. Fecal Fat Expressed as Percent of Fat Ingested



Rats fed a high fat diet containing no tea extract (purple bars), or 0.5% (orange bars), 3% (grey bars) or 6% (blue bars) of the chow as tea extract. Values represent mean ± SEM of groups. Different lower case letters indicate significant differences (p<0.05) in fecal fat among groups.

statistical significance (p<0.05) during the third and fourth weeks.

Figure 2 shows fecal fat excretion expressed as percentage of fat ingested. The chow containing 0.5-percent of the mixture or extracts had no significant effect on steatorrhea. However, the groups receiving larger doses (3.0% and 6.0%) malabsorbed significantly more fat than did the control group for the first three weeks

of the study, with fat malabsorption averaging 12-17 percent versus five percent in the controls. Steatorrhea diminished by the fourth week, with only the group receiving the 6.0-percent extract showing a significant increase in fecal fat relative to that of the controls.

The results of serum analyte determinations performed at the conclusion of the study are summarized in Table 2. Total serum cholesterol was not significantly

Table 2. Serum Analytes of Rats after Ingestion of High Fat Diet Containing Various Quantities of the Extract Mixture for Four Weeks*

Analyte	Concentration of Tea Extract in Food			
	0%	0.5%	3%	6%
Cholesterol (mg/dL)	91.0 ± 1.4	99.4 ± 4.5	97.6 ± 4.1	99.6 ± 5.2
HDL (mg/dL)	33.6 ± 0.7 ^a	36.4 ± 0.8 ^a	40.8 ± 1.3 ^b	44.0 ± 1.6 ^b
Triglycerides (mg/dL)	210 ± 37 ^a	248 ± 23 ^a	120 ± 21 ^b	97 ± 32 ^b
Hematocrit (%)	44 ± 1.0	46 ± 0.7	45 ± 0.3	44 ± 0.7
Creatinine (mg/dL)	0.54 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.60 ± 0.32
Alk Phos (IU/L)	206 ± 19 ^a	230 ± 18 ^a	244 ± 12 ^a	381 ± 20 ^b
ALT (IU/L)	37.6 ± 2.7 ^a	34.4 ± 1.4 ^a	46.0 ± 3.0 ^b	47.4 ± 2.8 ^b
AST (IU/L)	92.8 ± 15.1	78.0 ± 5.4	85.0 ± 3.0	88.8 ± 3.1
Bilirubin Total (mg/dL)	<0.1	<0.1	<0.1	>0.1
Creatinine (mg/dL)	0.54 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.60 ± 0.32

*Values represent mean ± SEM of groups. Different lower-case letters indicate differences among groups that were significant at p<0.05.





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altered by the mixture of extracts. Rats receiving 3.0- and 6.0-percent dosages of extract had a modest but significant ($p < 0.05$) increase in serum HDL cholesterol and a dramatic decrease in triglycerides relative to the controls and rats receiving the 0.5-percent dosage. No significant differences among the groups were observed for creatinine and hematocrit. However, the 6.0-percent dosage was associated with an appreciable and highly significant increase in alkaline phosphatase over that observed in the other groups. The ALT of rats receiving the 3.0- or 6.0-percent dietary concentration of extract was significantly increased relative to the controls and the 0.5-percent group. Serum bilirubin and AST were not significantly influenced by the mixture of extracts.

Discussion

The widespread usage of tea extracts for weight control is supported by rather slim *in vivo* evidence that includes a human study showing better maintenance of weight loss when tea was added to a calorie-restricted regimen² and a mouse study showing less weight gain when a tea extract was added to a high-fat diet.³

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,⁶⁻⁸ 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-amylase¹¹⁻¹³ and the sodium-dependent glucose transporter in intestinal mucosa.^{14,15} Mulberry extract contains high concentrations of flavonoids that inhibit alpha-glucosidase activity.¹⁶⁻¹⁸ In humans, a combination of black and green tea and mulberry extracts caused malabsorption of starch but not triglycerides in a rice and butter meal,¹⁹ and mulberry extract reduced the peak blood glucose rise following sucrose ingestion by type 2 diabetics.²⁰ Lastly, black tea is rich in theaflavin, which inhibits pancreatic lipase *in vitro*.^{9,10}

To test the hypothesis that tea/herbal extracts are useful for body-weight reduction, rats were fed a high-fat diet containing a mixture of extracts of black and green teas and mulberry leaves, expected to increase energy expenditure and reduce the availability of

calories from triglycerides and carbohydrate. During the first week of the study, rats receiving the highest dosage of the mixture (6.0-percent of the diet) gained significantly less weight than animals in the control group or the 0.5- or 3.0-percent groups (Figure 1, upper). However, weight gain did not differ significantly among the four groups of rats during the subsequent three weeks, and the average weight of the rats receiving the extracts was not significantly different from that of the controls by the fourth week. This result is contrary to a previous report³ that found black tea extract reduced the weight gain observed in untreated mice ingesting a high-fat diet.

The reduction in weight gain observed during the first study week in rats receiving a diet of 6.0-percent mixture appeared to be largely attributable to an initial aversion to the chow containing this high content of extract (Figure 1, lower). The caloric intake of this group of rats was slightly increased relative to controls during the last three weeks of the study, concurrent with a weight gain that eliminated significant differences among groups at the conclusion of the study.

Controls and rats receiving a 0.5-percent extract diet excreted about five percent of their dietary fat intake in feces; whereas, during the first three weeks of the study, fecal fat was significantly increased to approximately 15 percent in rats receiving 3.0- and 6.0-percent dosages. This is apparently due to the *in vivo* ability of black tea extract to inhibit pancreatic lipase activity. However, this effect appeared to diminish with time, in that during the last week of the study fat malabsorption diminished with a significant increase in fecal fat observed only in rats receiving the 6.0-percent dosage. It should also be noted that the increase of fecal fat from 5- to 15 percent observed during the first three weeks of the study accounted for only about four percent of the caloric intake of the rats.

Carbohydrate absorption was not measured in the present study, but an extrapolation from human studies^{19,20} suggests the extract mixture 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 mixture appeared to be removed from the fecal stream by this mechanism. The normal weight gain in rats treated with large doses of three extracts indicates the modest increase in food intake observed in these animals compensated for the extract-induced malabsorption of calories as fat (about 4 percent of total caloric intake) and carbohydrate.

The relatively minor degree of steatorrhea induced by the two highest extract diets was associated with marked reductions in serum triglycerides and modest, 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} and human^{25,26} studies, although in one human study²⁷ favorable changes in blood lipids observed at day 32 of the study returned to the values observed in controls by day 87.

To assess potential side effects of the study mixture, serum analyses were assessed at the conclusion of the four-week study (Table 2). Hematocrit, serum creatinine, bilirubin, and AST were not altered by any of the study diets. However, rats receiving 3.0- and 6.0 percent of their diet as tea extract had minor but significant increases in ALT, and those receiving the 6.0-percent extract diet had a very appreciable increase in alkaline phosphatase. Given that the rats received a mixture of extracts, it is not possible to identify the components responsible for this toxicity. The quantities of extract administered in the present study were high by human standards in that on a surface-area basis the 3.0- and 6.0-percent dosages in 250 g rats would be equivalent to daily consumption of about 22 and 44 g, respectively, of extracts by an 80-kg human subject. Thus, it is not clear if the consistent alterations in hepatic function observed in this study have any relationship to the rare cases of hepatic necrosis associated with ingestion of large quantities of tea extracts by humans,²⁸⁻³¹ which appear to represent an idiosyncratic reaction.

This study concludes that “super-physiological” dosages of a mixture of tea and mulberry extracts, having the putative ability to increase thermogenesis and inhibit carbohydrate and lipid absorption, had no effect on weight gain in rats allowed *ad lib* access to a high-

fat diet. Presumably, if the rats had been on a calorie-restricted diet, the extract-induced malabsorption might have resulted in reduced weight gain; however, the high dosages of extract required to produce steatorrhea were associated with liver function abnormalities.

Although the extent to which these results are translatable to humans is not clear, this study does not provide support for the widespread use of tea extracts for weight control in subjects not on a caloric-restricted regimen and indicates that very high doses can have a deleterious effect on liver function.

Disclosure

Funding for this project and the tea extracts employed were provided by NatureGen, Inc. Thus, one of the authors, Dr. Zhong, had a potential conflict of interest. None of the other authors had a conflict of interest.

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